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HPLC and ^{1}H-NMR study of chiral recognition in some thromboxane antagonists induced by β-cyclodextrin*

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Abstract: The interaction of β-cyclodextrin with a series of structurally related chiral thromboxane antagonists was investigated using NMR and RP-HPLC. HPLC studies used both a cyclodextrin bonded phase (Cyclobond I), and β-cyclodextrin as a mobile phase additive with an achiral C8 column. Many of the compounds exhibited chiral recognition with β-cyclodextrin in each technique, but only partial correlations between the three data sets were observed. HPLC and ROESY NMR data suggested the possibility of bimodal inclusion.

Keywords: *Chiral recognition; cyclodextrin; chiral HPLC; thromboxane antagonist.*

Introduction

Beta-cyclodextrin has been widely used to achieve chromatographic resolution of enantiomers, both as a mobile phase additive [1] and as a chiral stationary phase [2]. Semi-preparative HPLC chiral separations using β-cyclodextrin have recently been reported [3, 4]. Chiral discrimination has also been observed in NMR experiments with cyclodextrins [5]. The use of such experiments in enantiomeric purity determination has been discussed [6, 7].

NMR experiments have also proved useful in investigation of stoichiometry, stability, and structure of cyclodextrin complexes [8]. In particular, Job plots [9], in which the variation of chemical shifts with substrate: cyclodextrin ratio is used to determine complex stoichiometry, have been widely used [7, 10]. Nuclear Overhauser enhancement (nOe) experiments have been used to demonstrate proximity of cyclodextrin and substrate protons within a complex [11]. The ROESY experiment, which gives positive nuclear Overhauser enhancements over the whole molecular weight range, has been found to be particularly useful for species of moderate molecular weight, such as cyclodextrin complexes [12, 13].

Optimization of separations using cyclodextrin eluents is more complex than when using cyclodextrin stationary phases, owing to additional variables that need to be optimized (notably stationary phase type and additive concentration). Trials for chiral discrimination induced by cyclodextrins, are simple and rapid to carry out using NMR. There might therefore be some value in using NMR and Cyclobond experiments to predict semi-preparative chiral separations obtainable using cyclodextrin eluents.

Sybilska [14] has devised the following equation to describe retention behaviour in RP-HPLC using cyclodextrin-containing eluents,

$$k'_{obs} = (k'_G + (k'_{G.CD} \times K_f \times [CD]_m))/(1 + (K_f \times [CD]_m)), \quad (1)$$

where k'_{obs} = observed capacity factor of solute at cyclodextrin concentration $[CD]_m$ in the mobile phase: k'_G = capacity factor of uncomplexed guest; $k'_{G.CD}$ = capacity factor of complex; and K_f = equilibrium constant for complex formation. Resolution of enantiomeric solutes may therefore occur due to differences in complex retentions ($k'_{G.CD}$ values) and/or in complex stabilities (K_f

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values). Several authors [15, 16] have suggested that the latter factor is generally dominant, as complexes are largely unretained.

Complex stabilities are also thought to be important in determining retention on Cyclobond I stationary phases. Thus, Arnold [17] and Wang [18] have shown correlations between complex stability, determined spectrophotometrically or estimated by computer modelling, and retention on Cyclobond I within structurally related series of compounds.

The variation of NMR chemical shift with cyclodextrin concentration for a complexed solute may be described by equation (2) [19],

$$\Delta\delta/\Delta\delta_\infty = ([CD]_0/[G]_0 + 1/(K_f \times [G]_0) + 1)/2 - (([CD]_0/[G]_0 + 1/(K_f \times [G]_0) + 1)^2/4 - [CD]_0/[G]_0)^{1/2}, \quad (2)$$

where $\Delta\delta$ = measured chemical shift change of guest (at concentration $[G]_0$ on addition of cyclodextrin at concentration $[CD]_0$); $\Delta\delta_\infty$ = limiting chemical shift change at infinite cyclodextrin concentration; K_f = equilibrium constant for complex formation. Resolution of enantiotropic signals, i.e. differences in $\Delta\delta$, may therefore arise due to differences in intrinsic complex chemical shifts ($\Delta\delta_\infty$ values) and/or to differences in complex stabilities (K_f values).

Since complex stabilities may be important in determining chiral discrimination in all three of the above approaches, it seems reasonable to seek correlations between observed enantioselectivities. If such correlations are strong, predictive NMR and Cyclobond experiments may be useful, as discussed above.

Derivatives of 1,3-dioxane of the type shown in Fig. 1 have demonstrated TXA_2 receptor antagonism [20]. Some of these compounds have also demonstrated selective TXA_2 synthase inhibitory properties. TXA_2 antagonism has been shown to arise largely from one enantiomer of such compounds, and synthase inhibition from the other [21]. There

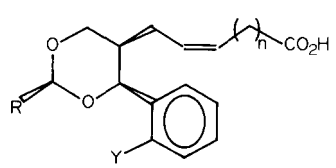

Figure 1
General structure of the thromboxane antagonists studied.

Table 1
Structural features of compounds investigated

Compound	R	Y	n
I	3-pyridyl-CH_2-	-OH	2
II	3-pyridyl-	-OH	2
III	3-pyridyl-$C(CH_3)_2$-	-OH	2
IV	3-pyridyl-CH=CH-	-OH	2
V	3-pyridyl-CH_2-$C(CH_3)_2$-	-OH	2
VI	3-pyridyl-CH_2-CH_2-$C(CH_3)_2$-	-OH	2
VII	N1-imidazoyl-CH_2-	-OH	2
VIII	Phenyl-O-$C(CH_3)_2$-	-OH	2
IX	2-chlorophenyl-	-OH	2
X	*t*-butyl-	-OH	2
XI	CF_3-	-OH	2
XII	CF_3-	-OH	3
XIII	3-pyridyl-	-H	2
XIV	3-pyridyl-CH_2-	-H	3
XV	3-pyridyl-	-OMe	2
XVI	3-pyridyl-CH_2-	-OMe	3
XVII	3-pyridyl-CH_2-CH_2-	-OMe	2

is therefore an interest in the resolution of these racemates.

The enantiomers of compound I (Table 1) have been shown to be resolved effectively using β-cyclodextrin as an HPLC eluent additive [22]. It was therefore considered appropriate to embark upon a full study of the interaction of β-cyclodextrin with a series of similar compounds, using (a) HPLC with β-cyclodextrin in the mobile phase; (b) HPLC with a bonded β-cyclodextrin column; and (c) ^{1}H-NMR experiments. Correlations between the three sets of data were sought. Experiments were also carried out to investigate complex structure and stoichiometry.

Experimental

HPLC studies were carried out on a Varian LC5500 integrated liquid chromatograph fitted with a 20 μl sample injection loop. SGE-100GL4-C8-30/5 (5 μm octyl-silica, 300Å pore size) columns were obtained from Scientific Glass Engineering Ltd (Milton Keynes, UK). A Cyclobond I (250 × 4.6 mm) column was obtained from Technicol Ltd (Stockport, UK). Acetonitrile (HPLC grade), sodium dihydrogenphosphate (AR grade), disodium hydrogenphosphate (AR grade) and anhydrous sodium carbonate (AR grade) were obtained from FSA (Loughborough, UK). Beta-cyclodextrin hydrate was obtained from Aldrich (Gillingham, Dorset, UK) and was used as received. Deuterium oxide (99.9%) was obtained from Fluorochem (Old Glossop, Derbyshire, UK).

Phosphate buffers were prepared by mixing 0.05 M aq. NaH_2PO_4 with 0.05 M aq. Na_2HPO_4 or 0.05 M aq. H_3PO_4 to the required pH. All HPLC mobile phases were filtered through Whatman glass microfibre filters (FSA, Loughborough, UK) and degassed by sparging with helium before use. The mobile phase flow rates employed were 1 ml min^{-1} (through 4.6 mm i.d. columns) or 0.75 ml min^{-1} (through 4 mm i.d. columns). Samples for injection were 0.1 mg ml^{-1} in mobile phase.

^{1}H-NMR experiments were carried out on a Bruker WM400 spectrometer, and on a JEOL GX-270 spectrometer. Water signal suppression was carried out using a homo-gated decoupled method. Sodium salts of the substrates were prepared *in situ* by mixing the acid with a mole equivalent of sodium carbonate. One-dimensional spectra were produced using a sweep width of 4000 Hz, a pulse width of 7 μs, and using the HDO resonance at 4.90 ppm as an internal reference.

Job plots were constructed as previously described [7]. ROESY experiments were carried out using the Z-filter CAMELSPIN experiment devised by Rance [23], using a spin-lock time of 0.8 s.

Results and Discussion

Chromatographic studies

The results of chromatographic studies employing β-cyclodextrin (a) as an eluent additive and (b) bonded to silica are summarized in Table 2. The conditions were chosen to provide an effective comparison between the two approaches, and are not necessarily optimal for resolution of a given racemate. The mobile phase organic modifier content was chosen to give adequate retention on the Cyclobond column, and to solubilize cyclodextrin in the mobile phase. The chiral eluent was almost saturated with β-cyclodextrin in order to maximize enantioselectivity. The C8 phase employed with the chiral eluent was found to give adequate retention of all the solutes with the mobile phase used. Those compounds not eluted from the C8 phase were eluted in reasonable time from a less hydrophobic phase (Zorbax CN), but were not resolved.

A high degree of enantioselectivity was observed over a wide range of structural variations. Eight of the 17 compounds were baseline-resolved by at least one of the chromatographic methods, and only one compound was completely unresolved by both techniques.

A positive correlation ($r = 0.89$) was obtained between the selectivities (α values) observed using the two chromatographic systems. However, this correlation was far from linear. All the compounds resolved using the chiral eluent were also resolved on the Cyclobond column. The Cyclobond data would therefore be of qualitative but not quantitative utility in predicting the results of experiments using a β-cyclodextrin eluent. Differences between the data sets are most probably a reflection of changes in the complexing ability of the cyclodextrin on bonding to silica, and also to the possibility that selectivity using the cyclodextrin eluent may be influenced by differential adsorption of complexes on the stationary phase as well as to differences in complex stabilities.

The effect of eluent pH on enantioselectivity for compounds I (R = $-CH_2$-pyridyl) and XII (R = $-CF_3$) is illustrated in Fig. 2. There is little change in selectivity with pH for compound XII, indicating that the influence of carboxyl group ionization is small. Compound I, however, shows a marked increase in enantioselectivity as eluent pH is increased from 3 to 7. This probably indicates that ionization of the pyridyl group disfavours enantioselective inclusion.

Examination of the data in Table 2 for compounds XI and XII suggests that the effect of chain length, n, on selectivity is small. The nature of the R group, however, has a substantial effect. Although it has been shown above that pyridyl ionization is a major determinant of enantioselectivity, it is clear from Table 2 that this functionality need not be present for resolution to be observed. Selectivity is noticeably low in those cases where there is a substantial alkyl group between the pyridyl group and the dioxane ring. This may be because the site of inclusion is too far removed from the chiral centres in the molecule in these cases for enantioselective interactions to be strong.

It is clear from the data for compounds XIII to XVII that the nature of the group Y has a marked effect on enantioselectivity. In particular, when Y is a methoxyl group low resolution is observed. The methoxyl group may be hindering inclusion by steric hindrance.

Table 2
Resolution of thromboxane antagonists using β-cyclodextrin

Compound	CD mobile phase*			CD bonded phase†			NMR chiral discrimination‡
	k'_1	α	R_s	k'_1	α	R_s	
I	7.4	1.62	5.2	7.0	1.35	3.1	Yes
II	6.0	1.13	1.0	7.3	1.10	1.2	Yes
III	33.5	1.58	5.7	9.6	1.24	2.2	Yes
IV	28.9	1.21	2.1	10.6	1.11	1.4	Yes
V	56.0	Not resolved		11.8	1.06	0.6	No
VI	96.0	Not resolved		33.7	1.08	0.8	Yes
VII	5.6	1.21	0.8	4.1	1.10	1.1	Yes
VIII	Not eluted			11.8	1.06	0.6	Yes
IX	18.6	1.19	1.5	10.0	1.11	0.8	Yes
X	13.6	1.06	0.7	18.0	1.06	0.8	Yes
XI	9.1	1.22	2.3	4.0	1.23	2.4	No
XII	20.2	1.22	2.3	5.9	1.16	1.7	No
XIII	5.7	1.28	2.1	14.2	1.25	2.5	Yes
XIV	10.5	1.66	4.8	30.1	1.34	4.8	Yes
XV	45.9	Not resolved		8.4	Not resolved		Yes
XVI	Not eluted			11.4	1.07	0.7	No
XVII	Not eluted			15.9	1.11	1.0	Yes

*Column: SGE100GLC4-C8-30/5; eluent: acetonitrile–sodium phosphate (50 mM, pH 7.0) (10:90, v/v) containing 29 mg ml^{-1} β-cyclodextrin.
†Column: Cyclobond I, 250 × 4.6 mm; eluent: acetonitrile–sodium phosphate (50 mM, pH 7.0) (10:90, v/v).
‡270 MHz ^{1}H-NMR spectrum of 1:1 mixture of racemate sodium salt with β-cyclodextrin.

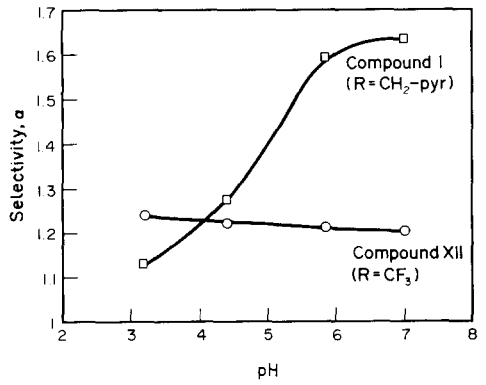

Figure 2
Variation of chromatographic enantioselectivity with pH for compounds I and XII. Column: SGE-100GL4-C8-30/5. Mobile phases: acetonitrile–phosphate buffer (50 mM) (10:90, v/v) containing 20 mg ml^{-1} β-cyclodextrin.

NMR studies

For 13 of the compounds chiral discrimination was exhibited (i.e. duplication of signals) in their proton NMR spectra on addition of a mole equivalent of β-cyclodextrin, as shown in Table 2. This is exemplified in Fig. 3, which shows duplication of aromatic proton signals in spectra of compound I. In all 17 cases, addition of racemate to β-cyclodextrin caused up-field shifts in cavity proton signals, indicating that these protons are shielded by included substrate aromatic groups. Thus, all 17 compounds are complexed by β-cyclodextrin to some degree.

Table 2 shows that nine of the 17 compounds were resolved by all three approaches. This indicates that there is some correlation between the degrees of chiral discrimination in each technique. However, correlations are far from perfect.

Thus, the one compound (XV) which was unresolved by either chromatographic technique showed clear chiral discrimination in NMR. Furthermore, compounds XI and XII, which were baseline resolved by both chromatographic approaches, showed no enantiotropic splittings in NMR signals in the presence of β-cyclodextrin. The lack of correlation in such cases may be due to the poorly resolved nature of some of the NMR signals. It may also reflect the fact that factors other than complex stability are operative in determining enantioselectivity in each technique. The predictive role of NMR with respect to HPLC experiments may therefore be restricted to indicating whether or not inclusion is occurring in a given case. This is of value, since inclusion is a prerequisite for chiral discrimination by β-cyclodextrin in aqueous solution, and it is not always easy to predict whether a given solute will include by examination of its structure.

Six of the racemates exhibited baseline resolution of enantiotropic signals in NMR

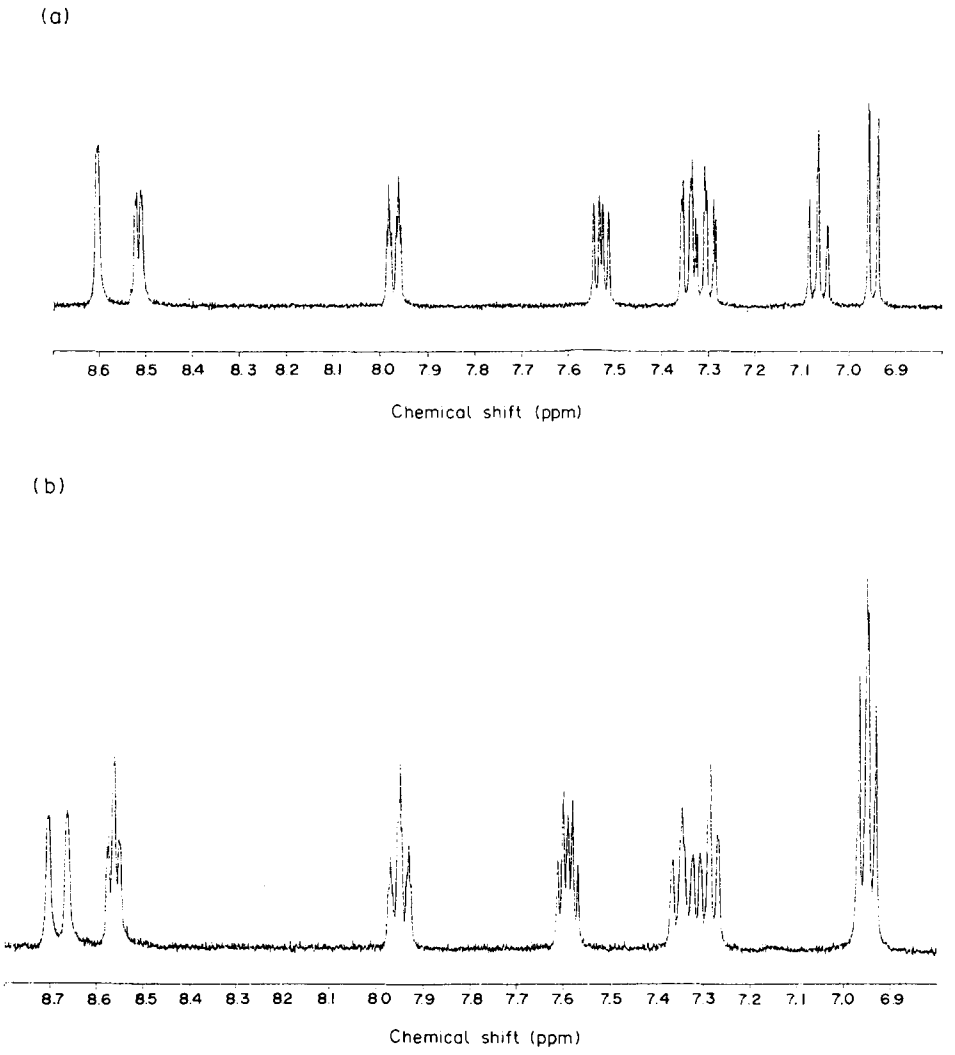

Figure 3
Aromatic proton regions of ^{1}H-NMR spectra of ±-compound I (4.6 mg ml^{-1}) + Na_2CO_3 (1.3 mg ml^{-1}) in D_2O at 400 MHz, (a) initially (b) on addition of 1 mol. equivalent (15 mg ml^{-1}) β-cyclodextrin.

under the conditions employed. In three of these cases (compounds II, X and XV), chromatographic resolution was less than baseline. It is clear, therefore that the NMR technique may have utility in optical purity analysis of these compounds.

In cases where resolution was not baseline, signals were of complex multiplicity, or poorly resolved from substrate or cyclodextrin signals. The conditions employed were not necessarily optimal for determination of enantiomeric purity. Higher degrees of enantiotropic splitting might be achieved by increasing the cyclodextrin: substrate mole ratio, although cyclodextrin solubility limitations would then necessitate reductions in substrate concentrations with consequent loss of sensitivity or increase in experiment time. Better resolution of signals would be achieved on a spectrometer of higher field than that employed in these studies (270 MHz).

A ROESY (CAMELSPIN) experiment on the compound I–β-cyclodextrin system was carried out in order to gain further information on the nature of the complex formed. Cross-peaks were observed between aromatic (phenol and pyridyl) signals of the substrate and cyclodextrin proton signals, indicating that both aromatic moieties of compound I may be included in the cyclodextrin cavity [24].

In order to investigate this possibility further, experiments with 3-ethylpyridine and 2-isopropylphenol (which were taken to be reasonable models for the two ends of the compound I structure) were carried out. Evidence of aromatic inclusion on β-cyclo-

dextrin signals were observed in both cases. This confirmed that both aromatic moieties of compound I might be capable of inclusion.

A Job (continuous variation) plot on compound I with β-cyclodextrin showed a maximum at 0.5, indicating 1:1 complex stoichiometry. Thus, the aromatic groups of compound I clearly do not both include in cyclodextrin in the same complex. It seems likely that two types of 1:1 complex are present, one with the pyridyl group included, and the other with the phenol group included. Such 'bimodal' inclusion has been reported for other systems [25].

The fact that both aromatic moieties of compound I may interact with β-cyclodextrin would account for the fact that such a wide range of these compounds may be resolved chromatographically, and that both R and Y groups have an influence on selectivity.

Conclusions

A high degree of enantioselectivity has been observed in NMR and HPLC experiments involving structurally related thromboxane antagonists with β-cyclodextrin. There is evidence that this enantioselectivity is enhanced by the ability of more than one group in these structures to complex with the cyclodextrin.

There is no clear correlation between the selectivity observed in NMR experiments and in HPLC studies using β-cyclodextrin bonded to the stationary phase or dissolved in the mobile phase. The predictive value of one technique with respect to the others is therefore limited. However, the two HPLC approaches may be complementary in application. NMR experiments such as CAMELSPIN can provide useful information regarding cyclodextrin–substrate complex structure.

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