Carbohydrate Research, 192 (1989) 9–15
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE INCLUSION OF THE ENANTIOMERS OF *N*-TRIFLUOROACETYL-4-FLUOROPHENYLALANINE AND *N*-TRIFLUOROACETYLPHENYLALANINE BY CYCLOMALTOHEXAOSE: A ^{2}H- AND ^{19}F-N.M.R. STUDY

NICHOLAS J. SMITH*, THOMAS M. SPOTSWOOD*, AND STEPHEN F. LINCOLN†‡

**Department of Organic Chemistry and †Department of Physical and Inorganic Chemistry, University of Adelaide, South Australia 5001 (Australia)*

(Received December 20th 1988; accepted for publication, April 15th, 1989)

ABSTRACT

^{19}F-N.m.r. studies show that *N*-trifluoroacetyl-D- and -L-4-fluorophenylalanine and *N*-trifluoroacetyl-D- and -L-phenylalanine form 1:1 inclusion complexes with cyclomaltohexaose (α-cyclodextrin) characterised by stability constants of 11.44 ±1.13, 11.40 ±1.09, 6.15 ±0.59, and 6.37 ±0.81 M^{-1}, respectively, in aqueous 0.1M NaCl at pH 6.5 and 25°. Under similar conditions, the correlation time of *N*-trifluoroacetyl-[$^{2}H_{8}$]phenylalanine changed from 65 ±4 ps in the free state to 320 ±40 ps in the complex, consistent with there being little freedom of movement of the guest in the inclusion complex.

INTRODUCTION

The cyclomalto-oligosaccharides (cyclodextrins, CDs)[1–3] form inclusion complexes with a wide range of guest molecules, the stoichiometries and stabilities of which are dependent on the size of the macrocycle and the nature of the guest[1–7]. Since the CDs are chiral, it is anticipated that the separate inclusion of enantiomers should give a pair of diastereoisomers. Such diastereoisomers may have differences in their physical properties which result in chiral discrimination, as shown by the partial resolution of racemic guests by the preferential precipitation of one diastereoisomer of the guest–CD complex[8–10], and by separation of enantiomers on chromatography columns in which the stationary phase consists of CDs bonded to silica[11,12]. Chiral discrimination in D_2O solution has been observed for the enantiomers of propanolol hydrochloride where inclusion by cyclomaltoheptaose (βCD) and cyclomalto-octaose (γCD) yields pairs of diastereoisomers with distinct ^{1}H-n.m.r. spectra[13]. The quantitative aspects of the interactions of CDs and other chiral molecules in solution have been little explored and we now report n.m.r. studies of the interaction of cyclomaltohexaose (αCD) with the enantiomers of *N*-trifluoroacetylphenylalanine and *N*-trifluoroacetyl-4-fluorophenylalanine.

‡Author for correspondence.

0008-6215/89/$ 03.50

EXPERIMENTAL

N-trifluoroacetylphenylalanine, prepared by methods similar to those in the literature[14], had m.p. 116–117.5°. The enantiomers of *N*-trifluoroacetylphenylalanine were obtained by the following enzymic procedure. Carboxypeptidase A (Sigma; 0.0118 g, 3.5×10^{-7} mol) in water (0.5 mL) was added dropwise to a solution of *N*-trifluoroacetylphenylalanine (2.233 g, 0.008 mol) in water (80 mL) at pH 7 at 38° during 4 h with stirring. The pH was readjusted to 7 with LiOH, and the enzyme was denatured by heating at 70° for 30 min. The enzyme was removed and the pH of the filtrate was adjusted to 1 with conc. HCl. The filtrate was extracted with chloroform (9×50 mL), the combined extracts were dried ($MgSO_4$), and the chloroform was removed under vacuum. The residue was recrystallised from water to yield *N*-trifluoroacetyl-D-phenylalanine (0.69 g, 62%), m.p. 118–121°; c.d. (H_2O) $[\Phi]_{269}$ 2940, $[\Phi]_{264}$ 1058, $[\Phi]_{262}$ 2820. The aqueous residues from this preparation were concentrated and the precipitated L-phenylalanine was recrystallised from water and trifluoroacetylated[14] to give *N*-trifluoroacetylated[14] to give *N*-trifluoroacetyl-L-4-fluorophenylalanine (15%), m.p. 133–135°; c.d. (H_2O) $[\Phi]_{270}$ −3100, $[\Phi]_{267}$ −750, $[\Phi]_{264}$ −4100.

N-trifluoroacetyl-4-fluorophenylalanine (Aldrich) was resolved as described above, to give *N*-trifluoroacetyl-D-4-fluorophenylalanine (49%), m.p. 133–135°; c.d. (H_2O) $[\Phi]_{270}$ 3200, $[\Phi]_{267}$ 750, $[\Phi]_{264}$ 3720. L-4-Fluorophenylalanine was trifluoroacetylated[14] to give *N*-trifluoroacetyl-L-4-fluorophenylalanine (15%), m.p. 133–135°; c.d. (h_2O) $[\Phi]_{270}$ −3100, $[\Phi]_{267}$ −750, $[\Phi]_{264}$ −4100.

N-Trifluoroacetyl-[$^2H_8$]phenylalanine was prepared as follows. A mixture of [$^2H_8$]toluene (2.8 g, 0.028 mol) and *N*-bromosuccinimide (9.25 g, 0.052 mol) in CCl_4 (950 mL) was boiled under reflux for 2 h under u.v. irradiation, then cooled, and filtered. The CCl_4 was removed under vacuum and the residue was distilled to give [$^2H_7$]benzyl bromide (4.9 g, 98%), b.p. 92°/18 mmHg; *m/z* 179/177 (M^+). A mixture of [$^2H_7$]benzyl bromide (1.88 g, 0.01 mol) and diethyl acetamidomalonate (2.4 g, 0.011 mol) in dry ethanol (5 mL) was boiled under reflux, and ethanolic sodium ethoxide (20 mL) [from sodium (0.24 g, 0.01 mol) and dry ethanol (5 mL)] was added dropwise. Refluxing was continued for 4 h, and the solution was filtered hot and cooled. The diethyl α-[$^2H_7$]benzylmalonate (47%) was collected and vacuum-dried over P_2O_5; m.p. 104–105.5°; *m/z* 314 (M^+). A solution of the diethyl α-[$^2H_7$]benzylmalonate (2.24 g, 0.008 mol) in ^2H-enriched aqueous 47% HBr (30 mL) was boiled under reflux for 6 h and the pH of the solution was then adjusted to 6.2 with conc. ammonia. The solution was concentrated under vacuum, and the solid product was collected, thoroughly washed with water to remove NH_4Br, recrystallised from aqueous ethanol, and dried under vacuum over P_2O_5 to give [$^2H_8$]phenylalanine (0.23 g, 17%); *m/z* 173 (M^+). *N*-Trifluoroacetyl-[$^2H_8$]phenylalanine, then prepared by *N*-trifluoroacetylation[14], had m.p. 125.5–126°; *m/z* 269 (M^+); ^2H-incorporation, 80%.

αCD (Sigma) was stored over P_2O_5 in a vacuum desiccator prior to use. All

solutions were 0.1M in NaCl and their pH was adjusted to 6.5 ±0.01 with NaOH.

^{2}H-N.m.r. spectra (46.07 MHz) were recorded with a Bruker CXP 300 spectrometer with broad-band ^{1}H decoupling. ^{19}F-N.m.r. spectra (84.63 MHz) were recorded with a Bruker HX 90E spectrometer at 25°. C.d. spectra were recorded on a JASCO J40CS spectropolarimeter.

RESULTS AND DISCUSSION

The plots of the concentration of αCD against the observed chemical (δ_o) shifts of the ^{19}F resonances of the trifluoroacetyl and 4-fluoro groups of *N*-trifluoroacetyl-D- and -L-4-fluorophenylalanine and *N*-trifluoroacetyl-D- and -L-phenylalanine are shown in Fig. 1.

For the formation of a 1:1 complex (S.αCD) between a guest molecule (S) and αCD,

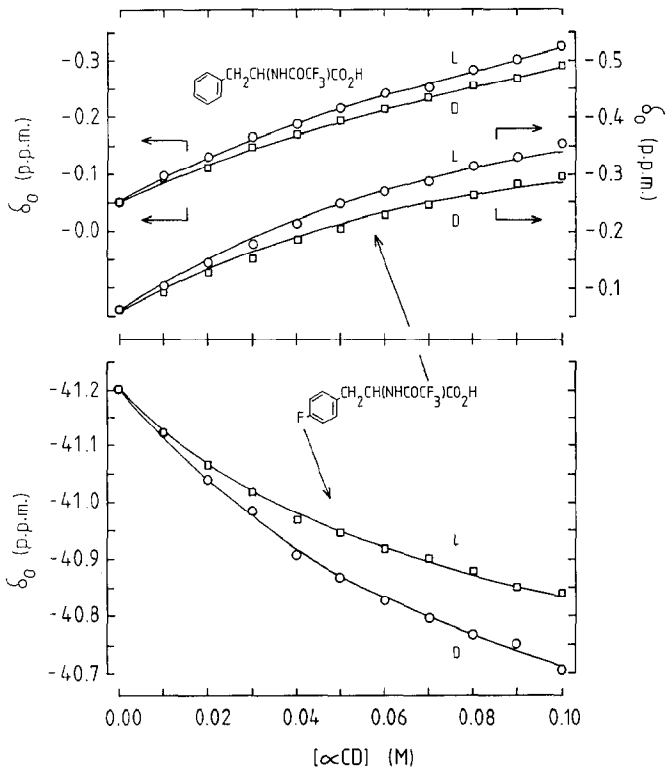

Fig. 1. The variation of the ^{19}F chemical shifts (δ_o) of 5mM *N*-trifluoroacetyl-DL-phenylalanine and *N*-trifluoroacetyl-DL-4-fluorophenylalanine with the concentration of αCD in aqueous 0.1M at pH 6.5 and 25°. The solid curves represent the best-fits of the δ_{obs} data to equation *3*. For *N*-trifluoroacetyl-DL-4-fluorophenylalanine, the CF_3CONH and 4-F data for each enantiomer were simultaneously fitted to equation *3* (see Discussion).

$$S + \alpha CD \underset{}{\overset{K_1}{\rightleftharpoons}} S.\alpha CD \qquad 1$$

the stability constant

$$K_1 = [S.\alpha CD]/[S][\alpha CD] \qquad 2$$

may be derived from the observed chemical shift of the guest resonances (δ_o) under conditions of fast exchange on the n.m.r. time-scale through

$$\delta_o = (\delta_i [S.\alpha CD] + \delta_f [S])/([S] + [S.\alpha CD]), \qquad 3$$

where δ_i and δ_f are the chemical shifts of the resonances of S in the included and free states, respectively. Further,

$$2[S.\alpha CD] = A + B + 1/K_1 - \{(A + B + 1/K_1)^2 - 4AB\}^{1/2}, \qquad 4$$

where $A = [S.\alpha CD] + [\alpha CD]$ and $B = [S.\alpha CD] + [S]$. When the variation of δ_o for each system was fitted to equation *3* using a non-linear least-squares analysis, the best-fit values of K_1 and δ_i given in Table I were obtained. There is a significant difference in δ_i for included *N*-trifluoroacetyl-D- and -L-4-fluorophenylalanine indicative of differing host–guest interactions in the diastereoisomers of S.αCD. However, there were no significant variations in K_1 for the diastereoisomers. This finding suggests that, in the preponderant form of the inclusion complex, the chiral centre of the guest may be too distant from the annulus of αCD to cause a

TABLE I

STABILITY CONSTANTS AND ^{19}F CHEMICAL SHIFTS THAT CHARACTERISE THE INCLUSION OF *N*-TRIFLUOROACETYL-D- AND -L-4-FLUOROPHENYLALANINE, *N*-TRIFLUOROACETYL-D- AND -L-PHENYLALANINE BY αCD IN AQUEOUS 0.1M SODIUM CHLORIDE AT pH 6.5 AND 25°

Guest	K_1 (M^{-1})	δ_c (*p.p.m.*)[a]
N-Trifluoroacetyl-4-fluorophenylalanine		
D enantiomer	11.44 ±1.13	−0.485 ±0.025[b]
		−40.280 ±0.051[c]
L enantiomer	11.40 ±1.09	−0.597 ±0.040[b]
		−40.500 ±0.040[c]
N-Trifluoroacetylphenylalanine		
D enantiomer	6.15 ±0.59	−0.676 ±0.041[b]
L enantiomer	6.37 ±0.81	−0.740 ±0.060[b]

[a]External reference: 2% CF_3COONa in D_2O assigned $\delta = 0$. The ^{19}F resonances of the CF_3CONH and 4-F substituents of free *N*-trifluoroacetyl-DL-4-fluorophenylalanine were at −0.06 and −41.2 p.p.m., respectively, and that of the CF_3CONH substituent of free *N*-trifluoroacetyl-DL-phenylalanine was at −0.05 p.p.m. [b]CF_3CONH resonance. [c]4-F resonance.

significant difference in the free energy changes that accompany the formation of the diastereomeric inclusion complexes, and that the aromatic ring is inserted into the annulus whereas the amino acid substituent protrudes into the solvent. This situation is consistent with δ_i for 4-F being substantially greater than δ_i for trifluoroacetyl, and for the difference in δ_i for the included enantiomers being greater for 4-F than for the trifluoroacetyl moiety (Table I). For *N*-trifluoroacetyl-L-4-fluorophenylalanine, δ_i is at a higher field than for the D enantiomer, and the resonances of 4-F and the trifluoroacetyl moiety are shifted downfield and upfield, respectively, on inclusion. Although the values of δ_0 are different for *N*-trifluoroacetyl-D- and -L-phenylalanine in the presence of identical concentrations of αCD (Fig. 1), the derived δ_i and K_1 values for the S.αCD diastereoisomers do not differ significantly (Table I).

In principle, the direction of change of the chemical shift should reflect changes in the environment that arise on inclusion of the guest. However, several factors affect changes in the ^{19}F chemical shifts exhibited by guests included by CDs, and deduction of the orientation of the included guest from such changes depends on a quantitative analysis of these factors which is not achievable at present.

The ^{19}F chemical shift data shown in Fig. 1 were also fitted to a model that involved a 1:2 complex:

$$\alpha\text{CD} + \text{S}.\alpha\text{CD} \overset{K_2}{\rightleftharpoons} \text{S}.(\alpha\text{CD})_2, \qquad 5$$

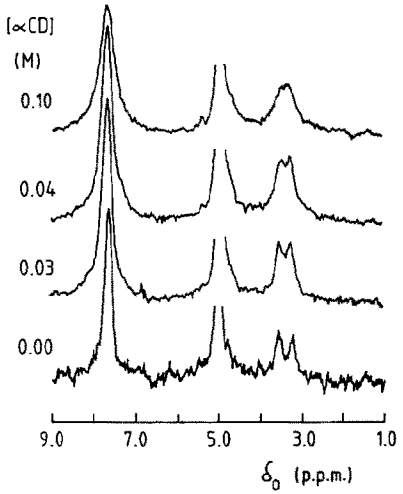

Fig. 2. 46.05-MHz ^{2}H-N.m.r. spectra of 5mM *N*-trifluoroacetyl-[$^2H_8$]phenylalanine observed in the presence of an increasing concentration of αCD in aqueous 0.1M NaCl at pH 6.5 and 25°. The benzylic ^{2}H resonances appear at 3.2 and 3.5 p.p.m., the α^2H resonance at 4.75 p.p.m. [partially obscured by ^{2}HHO (δ 5.0 p.p.m.)], and the aromatic ^{2}H resonance at 7.5 p.p.m. The cut-off resonance is that of ^{2}HHO.

TABLE II

VARIATION OF THE WIDTH AT HALF AMPLITUDE ($W_{1/2}$) OF THE AROMATIC ^2H RESONANCE OF *N*-TRIFLUOROACETYL-[$^2H_8$]PHENYLALANINE WITH CONCENTRATION OF αCD IN AQUEOUS 0.1M SODIUM CHLORIDE AT pH 6.5 AND 25°

[αCD] (M)	$W_{1/2}$ (Hz)[a]	T_1^{-1} (s^{-1})
0	9.7	30.3
0.010	11.0	34.4
0.020	11.5	36.1
0.030	12.1	37.9
0.040	13.3	41.8
0.050	14.2	44.5
0.100	17.5	54.9

[a]Error is ±0.5 Hz.

as has been observed in the fluorocinnamate–αCD system[4]. However, the magnitudes of the derived stability constants and chemical shifts characterising S.αCD and S.$(\alpha CD)_2$ were substantially exceeded by their standard deviations, and it was concluded that the experimental data were inconsistent with the formation of S.$(\alpha CD)_2$ in significant proportion.

In the presence of the αCD, two ^{19}F resonances were observed for each type of fluorine substituent in *N*-trifluoroacetyl-DL-phenylalanine and *N*-trifluoroacetyl-DL-4-fluorophenylalanine, and the variations of their chemical shifts with change in the concentration of αCD were the same as those observed for the resolved D and L enantiomers.

The ^2H resonances of *N*-trifluoroacetyl-[$^2H_8$]phenylalanine broaden with increasing concentration of αCD as shown in Fig. 2. The two benzylic deuterons are non-equivalent and their resonances appeared at 3.2 and 3.5 p.p.m. No ^2H–^2H splitting was observed as expected[15]. The variation in broadening was measured from the width at half amplitude ($W_{1/2}$) of the aromatic resonance at 7.5 p.p.m., and these data appear in Table II.

For a small molecule tumbling isotropically in solution where, for a nucleus such as ^2H, nuclear relaxation is overwhelmingly dominated by a quadrupolar interaction, the following equality holds

$$\pi W_{1/2} = 1/T_2 = 1/T_1 = 4.3 \times 10^{11}\, t_c, \qquad 6$$

where T_2, T_1, and t_c are the transverse and longitudinal relaxation times and the molecular correlation time, respectively[16,17]. The last equality in equation 6 is usually written $1/T_{1q} = 4.3 \times 10^{11}\, t_q$ in order to indicate that relaxation occurs as a consequence of quadrupolar interaction and that the correlation time, t_q, pertains to those molecular motions that influence quadrupolar-induced relaxation. Here, $1/T_{1q}$ is written as $1/T_1$ since, for ^2H, quadrupolar-induced relaxation is completely dominant, and t_q is replaced by t_c, a correlation time for molecular tumbling, since

this factor controls the rate of quadrupolar relaxation in the CD inclusion complex (see below). For the inclusion of *N*-trifluoroacetyl-[$^2H_8$]phenylalanine by αCD to form a 1:1 complex, T_1 varies according to

$$T_1^{-1} = x_f T_{1f}^{-1} + x_i T_{1i}^{-1}, \qquad 7$$

where x_f and x_i are the mole fractions of free and included guest and T_{1f} and T_{1i} are the ^2H longitudinal relaxation times pertaining to these two states. A linear regression of the aromatic ^2H T_1 data in Table II, employing x_c and x_f calculated from $K_1 = 11.4$ M^{-1} (Table I), yields $T_{1f} = 35 \pm 1.5$ ms and $T_{1i} = 7.0 \pm 0.7$ ms, from which $t_{cf} = 65 \pm 4$ ps and $t_{ci} = 320 \pm 40$ ps are calculated through equation *6*. This treatment assumes that the differences in chemical shift between the non-equivalent aromatic ^2H are small by comparison with the quadrupole-induced line broadening. Thus the correlation time for *N*-trifluoroacetyl-[$^2H_8$]phenylalanine increases five-fold to 320 ps on inclusion, which is similar to t_c (340 ps) calculated for αCD, and indicates that the guest has little freedom of motion and tumbles as one with the αCD complex. A similar conclusion has been drawn from a similar lengthening of t_c observed for cinnamate on inclusion by αCD[17].

REFERENCES

1 J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
2 W. Saenger, in J. L. Atwood, J. E. D. Davis, and D. D. MacNicol (Eds.), *Inclusion Compounds*, Vol. 2, Academic Press, New York, 1984, pp. 231–259.
3 R. J. Clarke, J. H. Coates, and S. F. Lincoln, *Adv. Carbohydr. Chem. Biochem.*, 46 (1988) 205–250.
4 I. M. Brereton, T. M. Spotswood, S. F. Lincoln, and E. H. Williams, *J. Chem. Soc., Faraday Trans. 1*, 80 (1984) 3147–3156.
5 R. L. Schiller, S. F. Lincoln, and J. H. Coates, *J. Chem. Soc., Faraday Trans. 1*, 82 (1986) 2123–2132 (in this reference, "annular radii" should read "annular diameters").
6 S. F. Lincoln, A. M. Hounslow, J. H. Coates, and B. G. Doddridge, *J. Chem. Soc., Faraday Trans. 1*, 83 (1987) 2697–2703.
7 S. F. Lincoln, A. M. Hounslow, J. H. Coates, R. P. Villani, and R. L. Schiller, *J. Inclusion Phenom.*, 6 (1988) 183–191.
8 F. Cramer and W. Dietsche, *Chem. Ber.*, 92 (1959) 378–384.
9 M. Mikolajczyk, J. Drabowicz, and F. Cramer, *J. Chem. Soc., Chem. Commun.*, (1971) 317–318.
10 M. Mikolajczyk and J. Drabowicz, *J. Am. Chem. Soc.*, 100 (1978) 2510–2515.
11 W. L. Hinze, T. E. Rieh, D. W. Armstrong, W. DeMond, A. Alak, and T. Ward, *Anal. Chem.*, 57 (1985) 237–242.
12 D. W. Armstrong, T. Ward, R. D. Armstrong, and T. E. Beesley, *Science*, 232 (1986) 1132–1135.
13 D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 25 (1987) 208–215.
14 F. Weygand and E. Liesing, *Chem. Ber.*, 87 (1954) 248–256.
15 H. H. Mantsch, H. Saito, and I. C. P. Smith, *Prog. Nucl. Magn. Reson. Spectrosc.*, 11 (1977) 211–272.
16 C. Brevard, J. P. Kintzinger, and J.-M. Lehn, *Tetrahedron*, 28 (1972) 2429–2445.
17 J. P. Behr and J.-M. Lehn, *J. Am. Chem. Soc.*, 98 (1976) 1743–1747.