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SECOND-SPHERE CO-ORDINATION OF CARBOPLATIN AND RHODIUM COMPLEXES BY CYCLODEXTRINS (CYCLOMALTO-OLIGOSACCHARIDES)

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ABSTRACT

The cyclomalto-oligosaccharides [cyclodextrins, αCD (**1**), βCD (**2**), and γCD (**3**)] and the *pure* methylated derivatives, hexakis(2,6-di-*O*-methyl)-αCD (DM-αCD, **4**) and heptakis(2,6-di-*O*-methyl)-βCD (DM-βCD, **5**), function as second-sphere ligands towards diammine(η^4-cycloocta-1,5-diene)rhodium(I) hexafluorophosphate (**8**), ethylenediammine(η^4-cycloocta-1,5-diene)rhodium(I) hexafluorophosphate (**9**), and diammine(1,1-cyclobutanedicarboxylato)platinum(II) (**11**, carboplatin). The transition metal complexes are included inside the cavities of the CDs both in aqueous solution (^{1}H-n.m.r. data) and in the solid state (X-ray data). In the crystals of the 1:1 adducts **8**–αCD and **11**–αCD, the transition metal complexes have their organic ligands inserted into the cavity of **1** and their ammine ligands hydrogen-bonded to secondary hydroxyl groups on the rim. The ammine ligands of **8** are displaced in the presence of either αCD or DM-αCD, but **9** is stable. Although **8** and **9** also form adducts with βCD, **11** is *specific* in forming a 1:1 adduct with αCD in aqueous solution. The standard enthalpy (−25.3 kJ.mol^{-1}) and entropy (−42 J.K^{-1}.mol^{-1}) changes for the "reaction" between **11** and αCD indicate that adduct formation is an *enthalpy driven* process. The greatly increased solubility (from 50 to 240mM) of **11** in aqueous solutions of αCD, and its encapsulation by αCD in the solid state, could find applications in the use of carboplatin in cancer chemotherapy.

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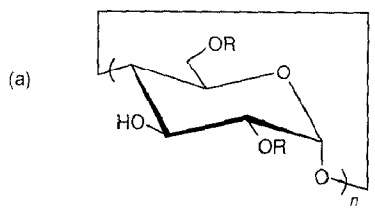
INTRODUCTION

The phenomenon of second-sphere co-ordination[1,2] concerns the non-covalent bonding of chemical entities to the first co-ordination sphere of a transition metal complex. Although the concept[3–5] is not new, there was little research until synthetic molecular receptors of the crown ether type were shown[1,2] to be ideal second-sphere ligands for transition metal complexes bearing aqua and ammine ligands, as well as other neutral protic ligands, *e.g.*, ethylenediamine and aceto-nitrile. The formation of adducts by (benzo) crown ethers is principally the result of electrostatic interactions, including hydrogen bonds and dispersive forces often incorporating both van der Waals and charge transfer interactions[6]. Adduct formation has been observed[1,2] in solution in organic solvents as well as in the solid state. In aqueous solution, protonated polyammonium macrocycles act[7] as efficient second-sphere ligands towards transition metal complexes that carry cyanide anions in their first co-ordination sphere.

Second-sphere co-ordination of transition metal complexes also characterises various biologically important receptors. The acyclic ionophore, Lasalocid A, fulfils[8] the function of a second-sphere ligand for transition metal ammines, and DNA, particularly the Z-form, is stabilised[9,10] by $[Co(NH_3)_6]^{3+}$ and $[Mg(H_2O)_6]^{2+}$ which complex to the surface of the double-stranded helix.

The cyclomalto-oligosaccharides (cyclodextrins), cyclomalto-hexaose (αCD, **1**), -heptaose (βCD, **2**), and -octaose (γCD, **3**) (Fig. 1a) can form[11–13] inclusion complexes (Fig. 1b), both in the solid state and in solution, with a wide range of



1	$n = 6$	R = H	αCD
2	$n = 7$	R = H	βCD
3	$n = 8$	R = H	γCD
4	$n = 6$	R = Me	DM-αCD
5	$n = 7$	R = Me	DM-βCD

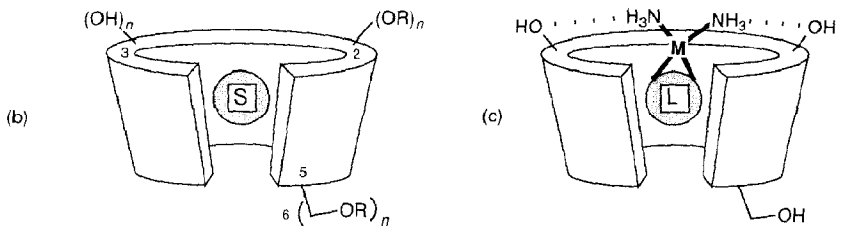
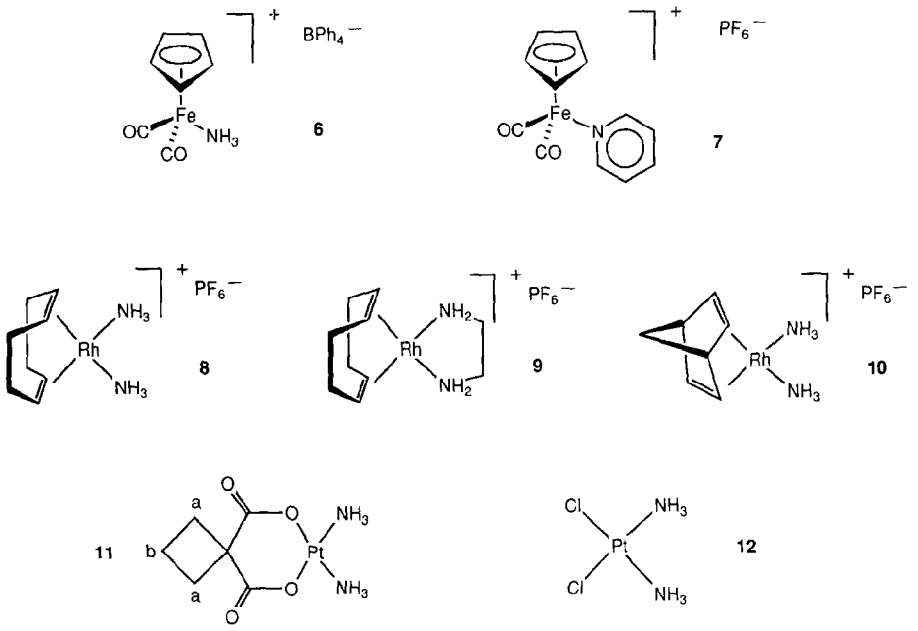


Fig. 1. (a) Structural formulae for αCD (**1**), βCD (**2**), γCD (**3**), DM-αCD (**4**), and DM-βCD (**5**); (b) inclusion of a substrate (S) inside the cavity of a CD; (c) inclusion of the organic ligand (L) of a transition metal complex inside the cavity of a CD, leaving ammine ligands (NH_3) co-ordinated to the transition metal M to form hydrogen bonds to hydroxyl groups on the secondary face of the CD.



substrates. Since the initial observations by Siegel and Breslow[14] in 1975 that ferrocene forms a 1:1 adduct with βCD in both *N,N*-dimethylformamide and methyl sulphoxide, little has been reported on the rôle of CDs as second-sphere ligands for organometallic and metallo-organic complexes. Investigations[15–19] of CDs and their derivatives as artificial esterases[20–24] established that both ferrocene and ruthenocene derivatives form 1:1 adducts with βCD in aqueous methyl sulphoxide.

Crystalline 1:1 adducts between the CDs and ferrocene have been isolated[25–27], and their structures and stabilities have been investigated by o.r.d.[28], solid state ^{2}H-n.m.r. spectroscopy[29], cyclic voltammetry[30], and ^{57}Fe Mössbauer spectroscopy[31]. The formation of adducts of ferrocenecarboxylic acid (and its anion) with βCD[32–34] and a chemically modified derivative[35] has been studied by c.d. spectroscopy. Adducts of modest stabilities (K_a values in the range 60–2200 M^{-1}) are formed in solution and those between the ferrocene nucleus and CDs have been used to resolve racemic derivatives of ferrocene. Thus, (±)-1-ferrocenylethanol, -propanol, and -butanol have been resolved[36,37] by m.p.l.c. on polyamide columns by clution with aqueous αCD. Enantiomeric pairs of ruthenocene, osmocene, and ferrocene derivatives were resolved[38,39] by h.p.l.c. on the commercially available βCD-bonded stationary phase "Cyclobond". Membrane-mediated separations using βCD and γCD as the active transporting agents have been employed[40] to separate the enantiomers of (1-ferrocenylethyl)thiophenol.

Several (η^6-arene)Cr(CO)$_3$ complexes[41] and η^3-allylpalladium complexes[42] form crystalline 1:1 adducts with βCD and γCD. Whereas the diene complexes

$Pt(cod)X_2$(cod =cycloocta-1,5-diene, X = Cl, Br, I) afford[43] crystalline 1:1 adducts with βCD and γCD, the dimers $[Rh(cod)Cl]_2$ and $[Rh(nbd)Cl]_2$ (nbd = norbornadiene) each crystallises[43] with ~2 mol. of βCD. We have investigated[44–47] the (aqueous) solution and solid-state properties of adducts formed by transition metal complexes, including $[Fe(\eta^5\text{-}C_5H_5)(CO)_2(NH_3)][BPh_4]$ (**6**), $[Fe(\eta^5\text{-}C_5H_5)(CO)_2(C_5H_5N)][PF_6]$ (**8**), $[Rh(cod)(H_2NCH_2CH_2NH_2)][PF_6]$ (**9**), and $[Rh(nbd)(NH_3)_2][PF_6]$ (**10**) with αCD (**1**) βCD (**2**), and the chemically-modified[48] CDs, DM-αCD (**4**) and DM-βCD (**5**) (Fig. 1a). ^{1}H-N.m.r. data[44] indicate that **6–9** formed adducts with αCD and βCD in aqueous (D_2O) solutions, whereas *only* βCD included **10**. We now discuss the second-sphere co-ordination of the anti-tumour drug[49–52] carboplatin (**11**), a second generation analogue of cisplatin (**12**), and **8–10** with αCD, βCD, DM-αCD, and DM-βCD in the light of results from X-ray crystallography[53,54], f.a.b.-mass spectrometry[55], ^{1}H-n.m.r. spectroscopy[54], and microcalorimetry[54]. The results have been summarised in four preliminary communications[53–56] and two reviews[1,57].

EXPERIMENTAL

Materials and methods. — αCD and βCD were used as received from Aldrich. The preparation of *pure* DM-βCD (**5**) has been described[58]. The procedure employed[59] in the synthesis of $[Rh(cod)(NH_3)_2][PF_6]$ (**8**) and $[Rh(nbd)(NH_3)_2][PF_6]$ (**10**) was used in the preparation[60] of $[Rh(cod)(H_2NCH_2CH_2NH_2)][PF_6]$ (**9**). A literature procedure[61] was used to prepare carboplatin (**11**). *N,N*-Dimethylformamide, methyl sulphoxide, and benzoyl chloride were distilled before use. Column chromatography was performed on Silica Gel 60 (Merck, 9385). M.p.s were determined with a Reichert hot-stage apparatus and are uncorrected. Microanalyses were carried out by the University of Sheffield Microanalytical Service. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. F.a.b.-m.s. was performed using a primary atom beam of Xe° atoms (8 keV) with a Kratos MS80RF spectrometer coupled to a DS55 data system, with 3-nitrobenzyl alcohol as the matrix. The ^{13}C- and ^{1}H-n.m.r. spectra were recorded with a Bruker AM250 or WH400 spectrometer and the solvent peak was used as reference with respect to the signal of Me_4Si.

Methylation of α-CD. — α-CD (**1**) was methylated using a procedure described[58] for β-CD (**2**). Chromatography (methanol–chloroform, 2:98) of the crude product on silica gel afforded (75%) a mixture of hexakis(2,6-di-*O*-methyl)-α-CD (DM-αCD, **4**), pentakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-α-CD-[(DM+1)-αCD], tetrakis(2,6-di-*O*-methyl)-bis(2,3,6-tri-*O*-methyl)-αCDs [(DM+2)-αCDs], and pentakis(2,6-di-*O*-methyl)-mono(6-*O*-methyl)-α-CD [(DM−1)-αCD]. Negative-ion f.a.b.-m.s. data: *m/z* 1140 (DM-αCD), 1154 [(DM+1)-αCD], 1168 [(DM+2)-αCDs], and 1126 [(DM−1)-αCD].

Benzoylation of methylated α-CDs. — The above mixture (8.8 g) of methyl-

ated α-CDs was treated with benzoyl chloride (60 mL) in dry pyridine (100 mL) as described[58] for methylated βCDs. Chromatography (methanol–chloroform, 5:95) of the crude product on silica gel gave, first, pentakis(3-*O*-benzoyl)-2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)cyclomaltohexaose [(DM+1)-αCD-B_5, 2.8 g], m.p. 128–131°, $[\alpha]_D$ −39° (*c* 0.9, chloroform). Positive-ion f.a.b.-m.s. data: *m/z* 1698 [(M + Na)$^+$].

Anal. Calc. for $C_{84}H_{106}O_{35}$: C, 60.2; H, 6.3. Found: C, 59.5; H, 6.6.

Eluted second was hexakis(3-*O*-benzoyl-2,6-di-*O*-methyl)cyclomaltohexaose (DM-αCD-B_6, 3.2 g), m.p. 132–135°, $[\alpha]_D$ −101° (*c* 1, chloroform). Positive-ion f.a.b.-m.s. data: *m/z* 1789 [(M + Na)$^+$]. N.m.r. data: ^{1}H (250 MHz, CD_3COCD_3), δ 2.52 (s, 18 H, MeO-2), 3.16 (dd, 6 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.0 Hz, H-2), 3.40 (s, 18 H, MeO-6), 3.71 (dd, 6 H, $J_{5,6a}$ 1.0, $J_{6a,6b}$ 11.0 Hz, H-6a), 3.80 (dd, 6 H, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5 Hz, H-4), 3.98 (dd, 6 H, $J_{5,6b}$ 4.5, $J_{6a,6b}$ 11.0 Hz, H-6b), 4.21 (m, 6 H, $J_{4,5}$ 9.5, $J_{5,6a}$ 1.0, $J_{5,6b}$ 4.5 Hz, H-5), 4.95 (d, 6 H, $J_{1,2}$ 3.5 Hz, H-1), 5.71 (dd, 6 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3), 7.32 (dd with second-order perturbation, 12 H, $J_{o,m}$ 7.0, $J_{m,p}$ 7.0 Hz, *m*-H), 7.44 (tt, 6 H, $J_{m,p}$ 7.0 $J_{o,p}$ 1.5 Hz, *p*-H), and 8.05 (d with second-order perturbation, 12 H, $J_{o,m}$ 7.0 Hz, *o*-H); ^{13}C (63 MHz), δ 58.81 (MeO-2), 59.02 (MeO-6), 71.89 (C-5), 72.24 (C-6), 73.91 (C-3), 81.19 (C-4), 81.33 (C-2), 100.85 (C-1), 128.56 (*m*-C), 130.53 (*o*-C), 132.54 (*p*-C), 132.94 (*C*CO), and 165.14 (CO).

Anal. Calc. for $C_{90}H_{108}O_{36}$: C, 61.2; H, 6.1. Found: C, 59.5; H, 5.9.

*Hexakis(2,6-di-*O*-methyl)cyclomaltohexaose (DM-αCD,* **4**). — 6M Potassium hydroxide (10 mL) was added to a solution of DM-αCD-B_6 (1 g, 0.57 mmol) in methanol (100 mL), and the mixture was stirred at room temperature for 18 h, then concentrated. A solution of the residue in water (50 mL) was extracted with ether (2 × 50 mL) and then benzene (2 × 50 mL). The combined benzene extracts were washed with saturated aq. sodium chloride (2 × 50 mL), dried (Na_2SO_4), and concentrated to dryness. Recrystallisation of the residue from cyclohexane afforded **4** (0.52 g, 80%), m.p. >270°, $[\alpha]_D$ +121° (*c* 0.9, chloroform). Negative-ion f.a.b.-m.s. data: *m/z* 1140. N.m.r. data: ^{1}H (250 MHz, C_6H_6), δ 3.15 (dd, 6 H, $J_{1,2}$ 3.4, $J_{2,3}$ 9.6 Hz, H-2), 3.32 (s, 18 H, MeO-6), 3.43 (s, 18 H, MeO-2), 3.58 (dd, 6 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.6 Hz, H-4), 3.70 (dd, 6 H, $J_{5,6a}$ <1, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.80 (dd, 6 H, $J_{5,6b}$ 4.5, $J_{6a,6b}$ 10.7 Hz, H-6b), 4.02 (m, 6 H, $J_{4,5}$ 9.6, $J_{5,6a}$ <1, $J_{5,6b}$ 4.5 Hz, H-5), 4.34 (dd, 6 H, $J_{2,3}$ 9.6, $J_{3,4}$ 9.6 Hz, H-3), 4.84 (d, 6 H, $J_{1,2}$ 3.4 Hz, H-1), and 5.10 (s, 6 H, OH); ^{13}C (63 MHz, $CDCl_3$), δ 59.16 (MeO-6), 60.14 (MeO-2), 70.55 (C-5), 71.24 (C-6), 73.49 (C-3), 81.76 (C-2), 83.51 (C-4), and 100.82 (C-1).

Anal. Calc. for $C_{48}H_{84}O_{30}$: C, 50.5; H, 7.4. Found: C, 49.8; H, 7.6.

*Pentakis(2,6-di-*O*-methyl)mono(2,3,6-tri-*O*-methyl)cyclomaltohexaose [(DM+1)-αCD].* — *O*-Debenzoylation of (DM+1)-αCD-B_5 as described above for DMαCD-B_6 afforded (DM+1)-αCD (77%), m.p. 249–252°, $[\alpha]_D$ +117° (*c* 1, chloroform). Positive-ion f.a.b.-m.s. data: *m/z* 1177 [(M + Na)$^+$].

Anal. Calc. for $C_{49}H_{86}O_{30}$: C, 51.0; H, 7.5. Found: C, 52.6; H, 8.1.

Diammine (1,1-cyclobutanedicarboxylato)platinum (II) – cyclomaltohexaose pentahydrate. — A solution of αCD (**1**; 105 mg, 0.1 mmol) in water (1 mL) at 80°

was used to dissolve carboplatin (**11**; 34.7 mg, 0.09 mmol). The solution was filtered and allowed to cool during 8 h to room temperature. After slow concentration during several weeks, a small amount of the title 1:1 adduct (11 mg), m.p. 235–255° (dec.), was obtained. The X-ray crystal structure data have been reported[54].

Anal. Calc. for $C_{42}H_{82}N_2O_{39}Pt$: C, 35.2; H, 5.8; N, 1.95. Found: C, 35.0; H, 5.9; N, 1.8.

Diammine(η⁴-cyclo-octa-1,5-diene)rhodium(I) hexafluorophosphate–cyclomaltohexaose hexahydrate. — The complex $[Rh(cod)(NH_3)_2][PF_6]$ (**8**; 80 mg, 0.20 mmol) was dissolved in water (1 mL) containing αCD (**1**; 240 mg, 0.25 mmol) with gentle warming. On cooling, a white precipitate formed, followed by yellow crystals. On warming gently, the yellow crystals dissolved, and the white precipitate was collected. *Slow cooling* of the filtrate during 6 h afforded yellow crystals of the title 1:1 adduct, m.p. >200° (dec.). The X-ray crystal structure data have been reported[55]. A satisfactory elemental analysis could not be obtained for this adduct.

Quantitative ^{1}H-n.m.r. spectroscopy. — CDs or their methylated derivatives were dried under reduced pressure at 70° for at least 3 h prior to the preparation of solutions of known concentrations in D_2O. By starting with stock solutions of the CDs and the complexes, stepwise dilutions allowed a series of 6–8 solutions of known concentrations to be prepared. Spectra were recorded with a Bruker AM-250 spectrometer [external 3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionic acid sodium salt, TSP]. In order to reduce the intensity of the HDO signal, the CDs were lyophilised with D_2O prior to being used, or the HDO signal was pre-irradiated. The concentration dependencies of the ^{1}H-n.m.r. signals for the CD and the free transition metal complex, established over a range 0.003–0.05 mol.kg^{-1}, were linear and were used for calibration. After recording the spectrum of equimolal solutions of the CD and the 1:1 adduct, the chemical shift (δ_0) observed for the resonance of the probe proton was ascertained from the output data of the spectrometer. Similarly, the chemical shifts (δ_p) for the resonances of protons in the free components at comparable concentrations were deduced from the calibration plots. This produced a spread of chemical shift changes or Δ values ($\delta_o - \delta_p$) at various concentrations, c, of the 1:1 adducts (see Appendix).

Microcalorimetry. — The general methodology has been described[62–64]. An aqueous solution (0.0237 mol.kg^{-1}) of carboplatin (**11**) was mixed in separate experiments with aqueous solutions of αCD (**1**) of various concentrations (0.01–0.1 mol.kg^{-1}) in a modified LKB Batch Microcalorimeter operating at 25 ±0.01°. It was established that the enthalpies of dilution of **11** were negligible compared to the changes in enthalpy during the associative "reactions". The "reaction" vessel was charged with solutions of **1** and **11**, and the reference cell contained aqueous **1** of the same concentration as in the "reaction" cell and pure water. In this manner, the contribution to the change in enthalpy arising from the enthalpy of dilution of **1** was annulled. From the enthalpies of "reaction", the change in enthalpy per mole of **11** was readily calculated for each concentration of **1** (see the Appendix).

RESULTS AND DISCUSSION

The rhodium complex **8** dissolved in water when either αCD or βCD was present. Moreover, the addition of 1 mol of **8** to a solution of αCD in D_2O caused significant shifts (*e.g.*, 20.8 Hz at 0.021 mol.kg^{-1}) in the ^{1}H-n.m.r. spectrum of the signal for H-3 to higher frequencies; H-3 is oriented in a belt-like manner around

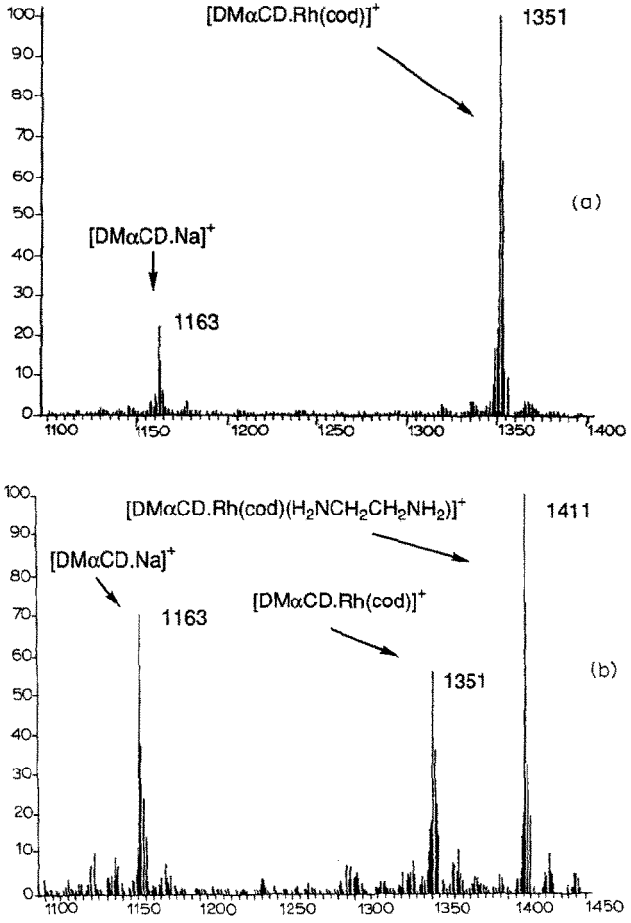


Fig. 2. F.a.b.-mass spectra (positive-ion detection) of adducts of DM-αCD with (a) $[Rh(cod)(NH_3)_2][PF_6]$ and (b) $[Rh(cod)(H_2NCH_2CH_2NH_2)][PF_6]$.

the inside of the CD cavity. Simultaneously, the signal for H-5, the other inwardly directed circle of protons in the cavity, was shifted to lower frequencies. Attempts to quantify these changes were frustated by the slow "drift" of the signals. Since the pD of the D_2O solution increased with time, it was concluded that displacement of

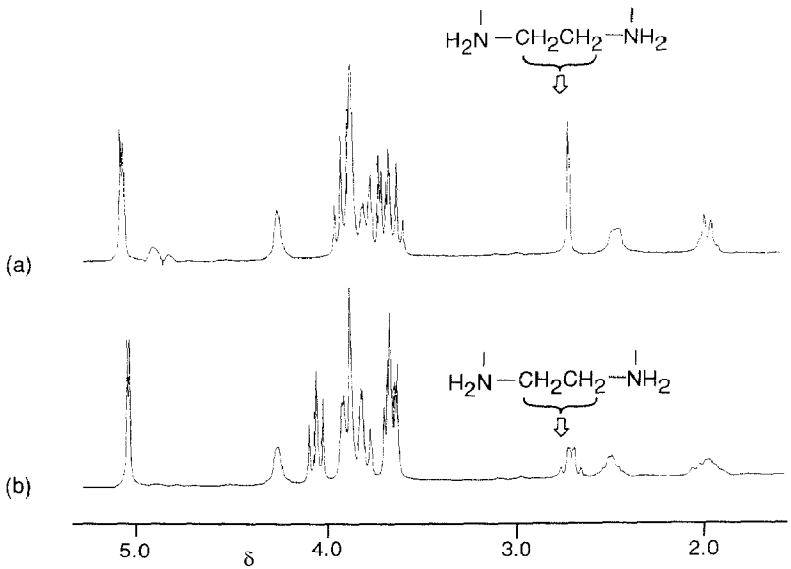


Fig. 3. Partial ^{1}H-n.m.r. spectra (250 MHz) of [Rh(cod)($H_2NCH_2CH_2NH_2$)][PF_6] in D_2O in the presence of 1 mol of (a) αCD and (b) βCD.

the ligand from **8** occurred in the presence of αCD, releasing ammonia from the first co-ordination sphere. This observation was not surprising since the ammine ligands in **8** are known to be labile in dichloromethane solution in the presence of a diaza-18-crown-6 derivative[65]. The question arises as to whether or not αCD enters the first co-ordination sphere of a modified rhodium complex.

Further evidence that a reaction had occurred came from f.a.b.-m.s. of a mixture of **8** with the more labile DM-αCD (**4**), which can be detected (see Experimental) in the negative-ion detection mode in a *pure* sample, following the "benzoylation–debenzoylation" procedure developed[58] in the purification of DM-βCD (**5**). A strong peak at *m/z* 1351, corresponding to [DM-αCD.Rh(cod)]$^+$, was observed (Fig. 2a) together with a much weaker one at *m/z* 1163 for [DM-αCD.Na]$^+$. This result is in sharp contrast to that obtained when DM-αCD was mixed with **9**, which contains the much less labile (bidentate) ethylenediamine ligand. Although a peak was present (Fig. 2b) with *m/z* 1351 (which was weaker than that for the Na^+ ion complex), the major peak, corresponding to the second-sphere co-ordination adduct, [DM-αCD.Rh(cod)-($H_2NCH_2CH_2NH_2$)]$^+$, was at *m/z* 1411. In view of the quantitative nature of the information provided[66,67] by f.a.b.-m.s. of Group 1A and IIA metal cationic complexes of crown ethers, it is reasonable to assume that DM-αCD forms a strong adduct with the [Rh(cod)($H_2NCH_2CH_2NH_2$)]$^+$ ion. It is concluded that the ligands of **9**, unlike **8**, should resist ligand displacement by αCD, as well as by DM-αCD, in aqueous solution.

This expectation was confirmed by the ^{1}H-n.m.r. spectrum (Fig. 3a) of a solu-

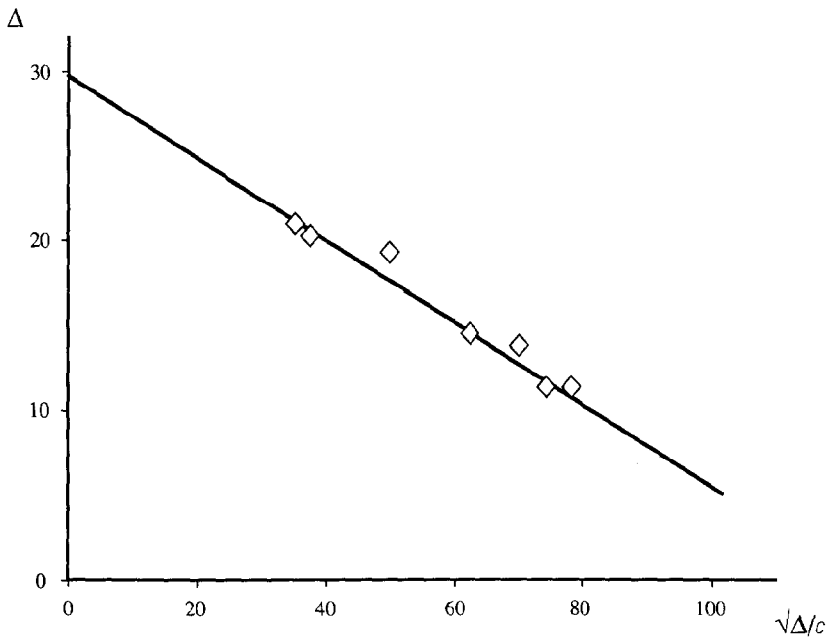


Fig. 4. Plot of Δ for the H-3 probe in αCD *versus* $(\Delta/c)^{1/2}$ for the formation of an adduct between αCD and $[Rh(cod)(H_2NCH_2CH_2NH_2)][PF_6]$.

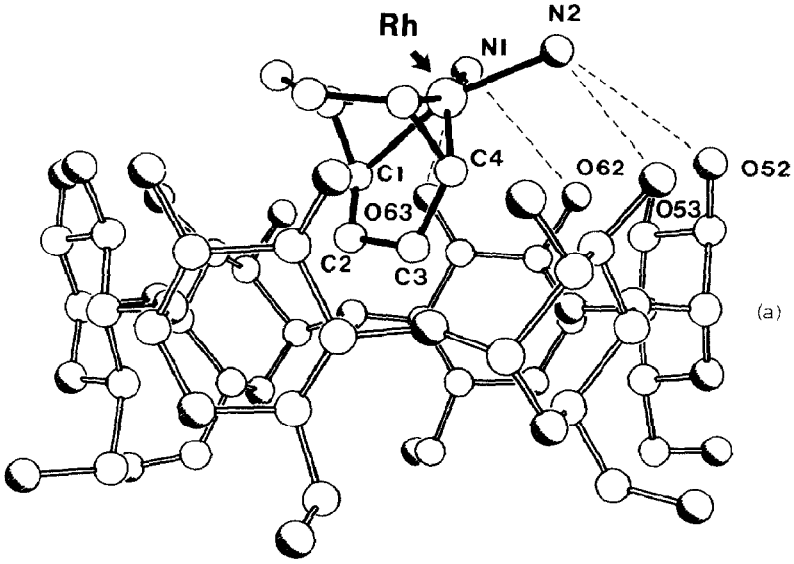
tion of **9** in D_2O in the presence of αCD, which did not change with time. Moreover, the *N*-methylene protons were diastereotopic, resonating as an AA'BB' system at δ 2.64–2.78. The *N*-methylene protons in the 1:1 adduct of **9** and βCD resonated as a singlet in D_2O (Fig. 3b).

The ^{1}H-n.m.r. spectrum of αCD in solution in D_2O in the presence of 1 mol of **9** revealed shifts (*e.g.*, 21.1 Hz at a concentration of 0.017 mol.kg^{-1}) to higher frequencies of the signals for H-2 and H-3, and to lower frequency of the signal for H-5. The differences in chemical shifts observed on changing the concentration *c* [in mol.kg^{-1} (D_2O)] of the "1:1 adduct" were analysed using equation *1* (see Appendix),

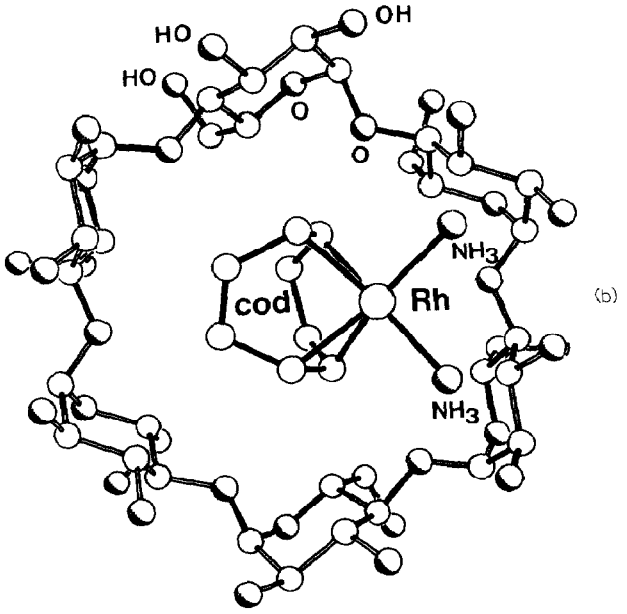
$$\Delta = \Delta_0 - (\Delta/c)^{1/2} \cdot (\Delta_0/K_a)^{1/2} \qquad (1)$$

where Δ is the difference between the observed chemical shift (δ_0) for the resonance of the probe proton (H-3 in αCD) in the solution of the "1:1 adduct" and that (δ_p) for the resonance of H-3 in αCD, Δ_0 is the difference between the limiting chemical shift (δ_A) of the 1:1 adduct (assumed to be fully formed) and δ_p, and K_a is the equilibrium constant given by [A]/[CD][C], where A is the adduct, CD is the cyclodextrin, and C is the transition metal complex. Over the range of concentrations 0.003–0.048 mol.kg^{-1}, the chemical shift (δ_p) of the signal for H-3 in αCD varied by up to 5 Hz. These shifts could not be neglected relative to that of 30–40 Hz

associated with the formation of the adduct. The dependence of δ_p on the CD concentration reflects the marked tendency of αCD to self-associate in aqueous solution[68]. When 1 mol of **9** was present with αCD in solution in D_2O, and using H-3 as the probe proton over the concentration range of 0.002–0.017 mol.kg^{-1}, a plot of Δ against $(\Delta/c)^{1/2}$ gave (Fig. 4) a straight line indicating that a 1:1 adduct was indeed formed. A K_a value of 520 mol^{-1}.kg was deduced from the slope $(-\Delta_0/K_a)^{1/2}$, which was -0.238 Hz$^{1/2}$.mol$^{1/2}$.kg$^{1/2}$, and the intercept Δ_0 which was 29.7 Hz.



(a)



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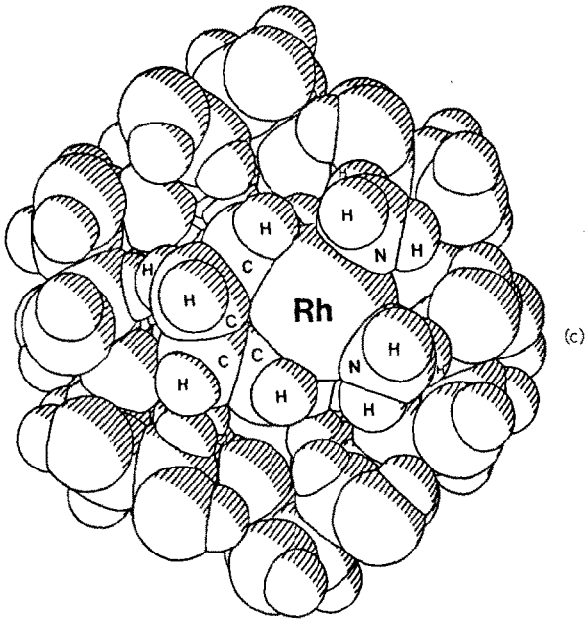


Fig. 5. Crystal structure of the 1:1 adduct of αCD and $[Rh(cod)(H_2NCH_2CH_2NH_2)][PF_6]$. Skeletal representations viewed from (a) the side, (b) the top, and (c) a space-filling representation viewed from the top.

Despite the instability of the rhodium complex **8** in aqueous solutions of αCD, single crystals of the 1:1 adduct, suitable for X-ray crystallography, were obtained as a hexahydrate. Side-on (Fig. 5a) and plan (Fig. 5b) views of the skeletal representation are shown in Fig. 5 together with a space-filling representation (Fig. 5c) of $[\alpha CD.Rh(cod)(NH_3)_2]^+$. The cod ligand is positioned almost exactly over the centre of the CD torus. One of the bismethylene units of the cod ligand is inserted into the αCD cavity with C-2 and C-3 lying 0.88 and 1.08 Å, respectively, below the mean plane of the oxygen atoms associated with the twelve secondary hydroxyl groups. The olefinic C-1 and C-4 on the cod ligand lie 0.22 and 0.21 Å, respectively, above this plane. The two ammine ligands on the metal are positioned over two of the glucopyranosyl residues. There are four intra-adduct N···O contacts of <3.5 Å, namely, two between N-1 and O-62 and O-63 [3.46 and 3.25 Å, respectively] on one of the glucopyranosyl residues, and two between N-2 and O-52 and O-53 [3.31 and 3.24 Å, respectively] on the other glucopyranosyl residue. There is an extensive array of hydrogen bonds, some involving the water molecules and others linking symmetry-related αCD molecules. Although there are six water molecules in the asymmetric unit, they occupy seven discrete sites (five are fully occupied and two half-occupied). These results, which indicate the binding of the cationic rhodium complexes **8** and **9** to αCD as well as to βCD, contrast with the selectivities exhibited[43] by the neutral dimeric complexes, $[Rh(cod)Cl]_2$ and $[Rh(nbd)Cl]_2$, which are included by βCD but not by αCD.

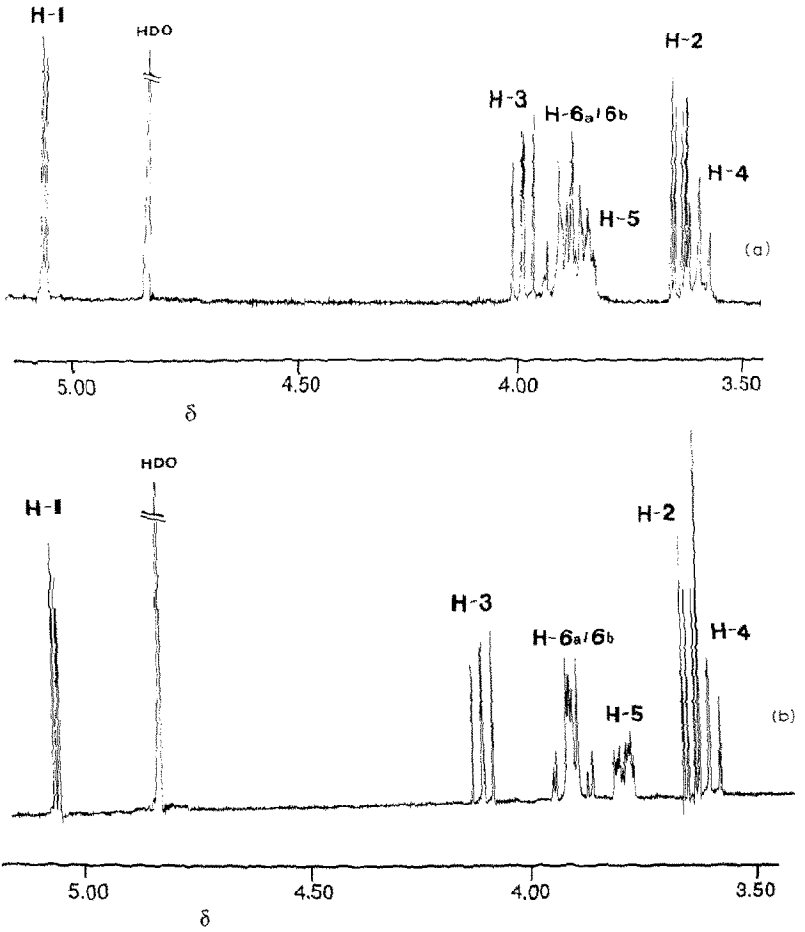


Fig. 6. The ^{1}H-n.m.r. spectra (400 MHz) of (a) αCD and (b) the 1:1 adduct of αCD and carboplatin in D_2O at ~0.013 mol.kg^{-1}.

Generally speaking, transition metal complexes, such as **8–10**, which carry organic ligands in their first spheres, even though they are salts, are rather insoluble in water. The neutral platinum complex, carboplatin (**11**), is no exception. The use of **11** as an anti-cancer drug prompted an investigation of its binding by CDs in aqueous solutions. αCD readily formed a 1:1 adduct with **11** in aqueous solution, but βCD and γCD failed to bind **11**. The resonances of the proton probes in **11** and the CDs were not shifted on addition of **11** to solutions of βCD and γCD in D_2O. This finding suggests that the tight fit of the relatively small cyclobutane ring in the organic ligand on **11**, necessary for (inclusion) adduct formation, is possible only with αCD. Moreover, concentrations of **11** of the order of 90 mg.mL^{-1} (240mM) can be achieved at room temperature in an aqueous solution of αCD compared

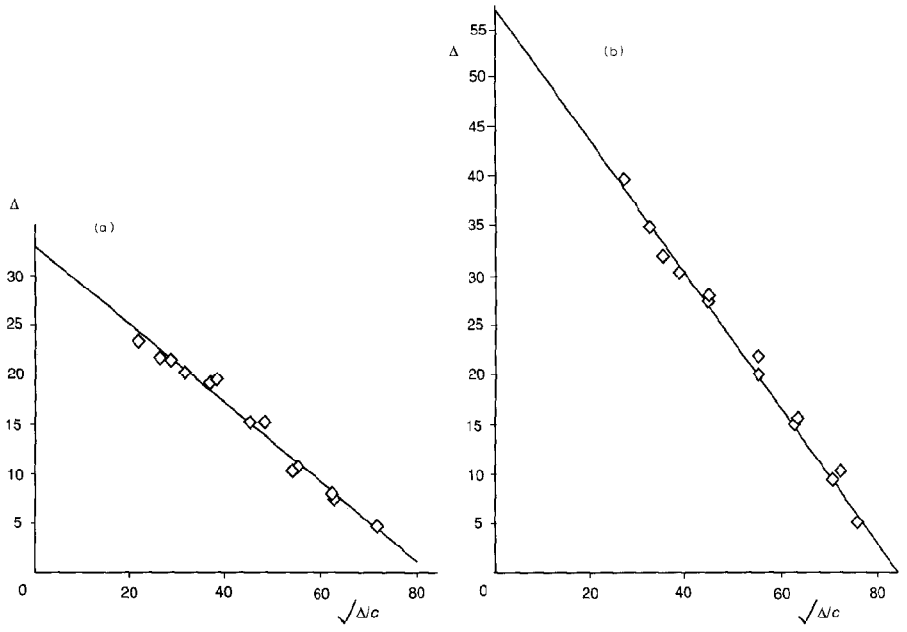


Fig. 7. Plots of Δ *versus* $(\Delta/c)^{1/2}$ for the formation of an adduct of αCD and carboplatin, using (a) H-3 in αCD and (b) H-a in carboplatin as the probes.

with only 18 mg.mL^{-1} (50mM) for a saturated aqueous solution. The ^{1}H-n.m.r. spectrum (400 MHz) of αCD in solution in D_2O is shown in Fig. 6a together with the relevant proton assignments[69]. Addition of 1 mol of **11** to the solution resulted in significant shifts of the signal for H-3 to higher frequency and that for H-5 to lower frequency. The signals for the H-a and H-b of **11** were also shifted to higher frequency in the presence of 1 mol of αCD. The partial spectrum for the "1:1 adduct" at 0.013 mol.kg^{-1} is shown in Fig. 6b. The nature and magnitude of the changes in chemical shifts of the resonances of the CD and the complex suggest that the cyclobutane ring is bound within the αCD cavity in aqueous solution. This proposal is supported by the fact that the chemical shifts of the resonances of H-1, H-2, and H-4 on the outer surface of the CD torus are unaffected by the presence of **11** in the cavity. Moreover, the vicinal coupling constants ($J_{1,2}$ 3.5, $J_{2,3}$ 9.0, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0, $J_{5,6a}$ 2.0, and $J_{5,6b}$ 4.0 Hz) deduced for αCD from Fig. 6b compare favourably with the literature[69] data ($J_{1,2}$ 3.7, $J_{2,3}$ 9.8, $J_{3,4}$ 8.8, $J_{4,5}$ 10.0, $J_{5,6a}$ 1.8, $J_{5,6b}$ 3.7 Hz) for αCD. This observation implies that the conformation of the gluco-pyranosyl rings of αCD are not changed substantially on formation of an adduct with **11**.

The chemical shift differences for the resonances of H-3 in αCD and H-a in **11**, observed on changing the concentration c of the "1:1 adduct", were analysed using equation *1* with corrections for the concentration dependencies of the H-3 resonance on [αCD] and of the H-a resonance on [**11**]. Plots of Δ against $(\Delta/c)^{1/2}$

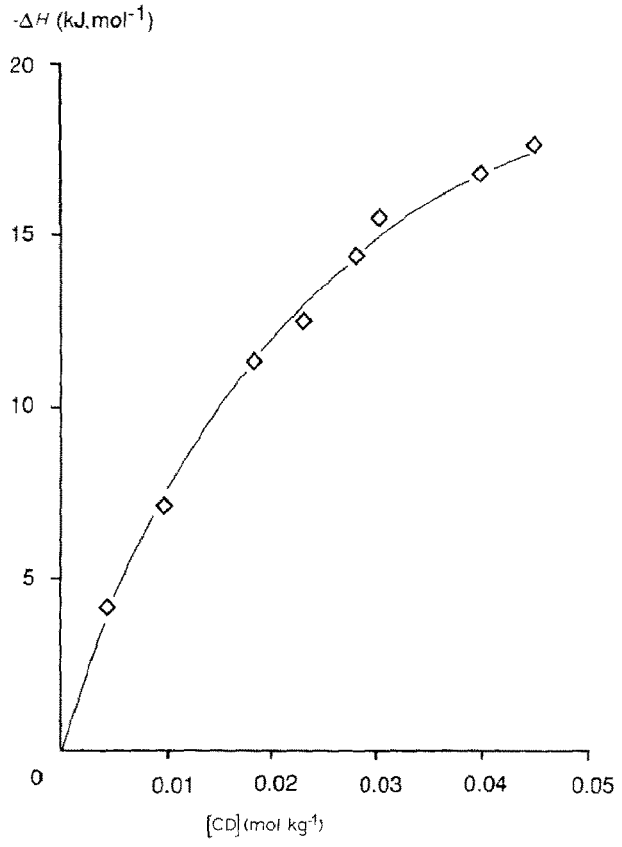


Fig. 8. Plot (◇) of the molar enthalpy change ($-\Delta H$) *versus* the concentration ([CD]) of αCD, togethe with the curve calculated on the basis of $K_a = 60$ mol^{-1}.kg and $\Delta H^0 = -25.3$ kJ.mol^{-1}.

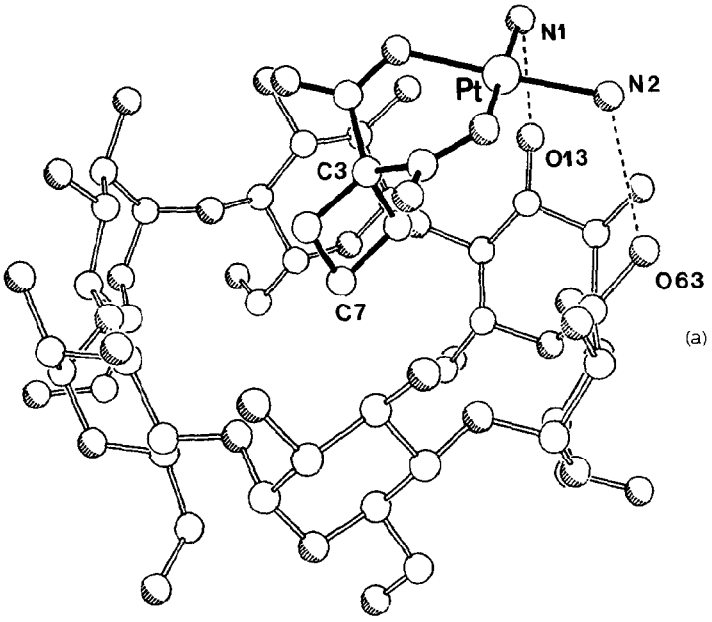
for H-3 and H-a gave (Fig. 7) straight lines as required for the formation of a 1:1 adduct, and K_a values of 130 and 200 mol^{-1}.kg, respectively, corresponding to standard free energy changes ($-\Delta G^0$) of 11.9 and 13.0 kJ.mol^{-1}, were calculated. The changes in standard enthalpy (ΔH^0) and entropy (ΔS^0) were determined also by microcalorimetry. An aqueous solution of **11** (0.0237 mol.kg^{-1}) was mixed with a range of various concentrations (0.01–0.1 mol.kg^{-1}) of αCD in a batch calorimeter operating at 25°. Assuming 1:1 adduct formation, then the stoichiometric concentration ($[CD]_0$) of αCD, which is varied, and the concentration ($[C]_0$) of **11**, which is kept constant, are related by equation *2* to K_a, ΔH^0, and ΔH, the change in molar enthalpy (see Appendix). From the values of ΔH and $[CD]_0$, equation *2* was solved, using a standard non-linear least squares fitting routine, to give $K_a = 60$ mol^{-1}.kg and $\Delta H^0 = -25.3$ kJ.mol^{-1}. The values determined experimentally, together with the theoretical curve calculated on the basis of the values

for K_a and ΔH^0, are shown in Fig. 8. The K_a value corresponds to a ΔG^0 value of -10.4 kJ.mol^{-1} and, together with the derived value for ΔH^0, allows a value of -42 J.K^{-1}.mol^{-1} to be determined for ΔS^0. Comparison of these thermodynamic parameters indicates that the formation of an adduct between αCD and **11** is an enthalpy-driven rather than an entropy-driven process.

$$[CD]_0 = \frac{\Delta H \Delta H^0 + \Delta H \Delta H^0 K_a[C]_0 - (\Delta H)^2 K_a[C]_0}{K_a(\Delta H^0)^2 - K_a \Delta H \Delta H^0} \qquad (2)$$

The spectroscopic and thermodynamic evidence suggested that the cyclobutane ring of **11** is directed inside the cavity of αCD and this was confirmed by X-ray crystallography of the penta-aquated form of the crystalline 1:1 adduct.

Side-on and plan views of skeletal representations of the X-ray crystal structure for [αCD–carboplatin] are shown in Figs. 9a and 9b, respectively. αCD adopts its characteristic toroidal conformation with local pseudo-C_6 symmetry. The bound platinum complex is positioned over the secondary face of the αCD with a geometry which is essentially that[70] for the free complex. The cyclobutane ring is inserted into the centre of the αCD torus with the "axis" joining C-3 and C-7 in the ring almost coincident with the 6-fold axis of the αCD. C-7 lies 1.03 Å below the



(a)

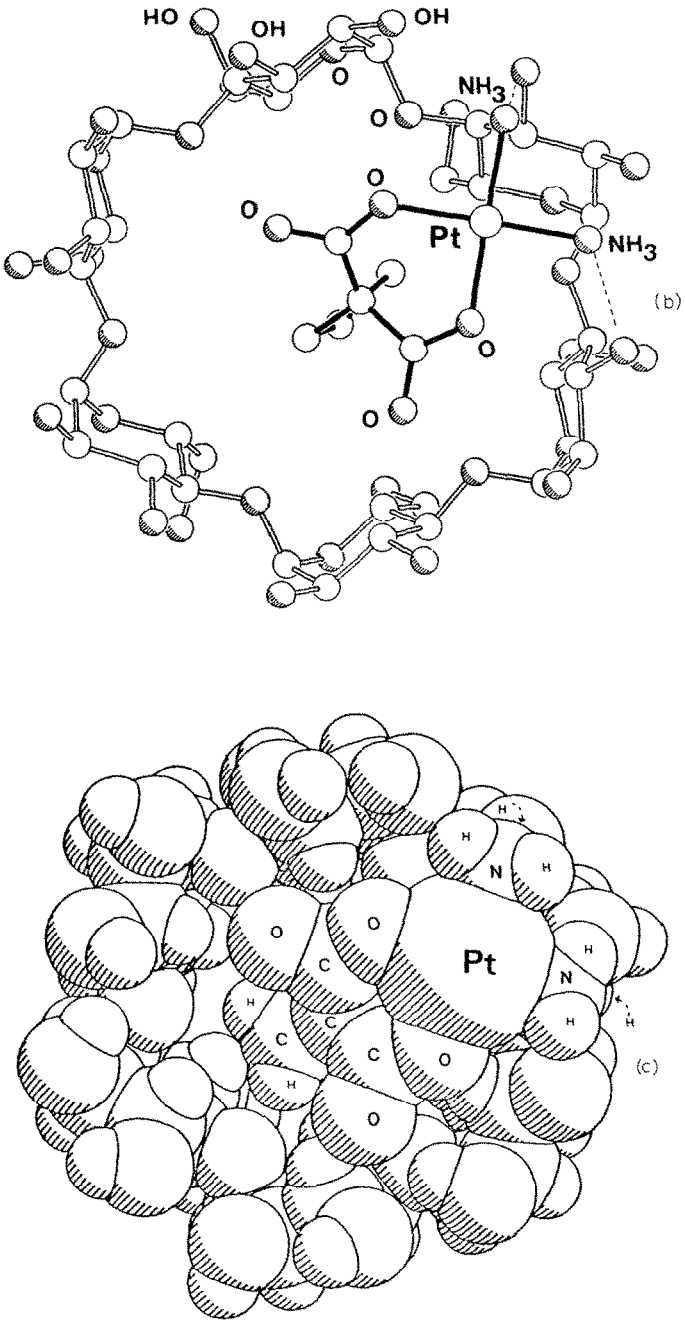


Fig. 9. Crystal structure of the 1:1 adduct of αCD and carboplatin (**11**). Skeletal representations viewed from (a) the side and (b) the top, and (c) a space-filling representation viewed from the top.

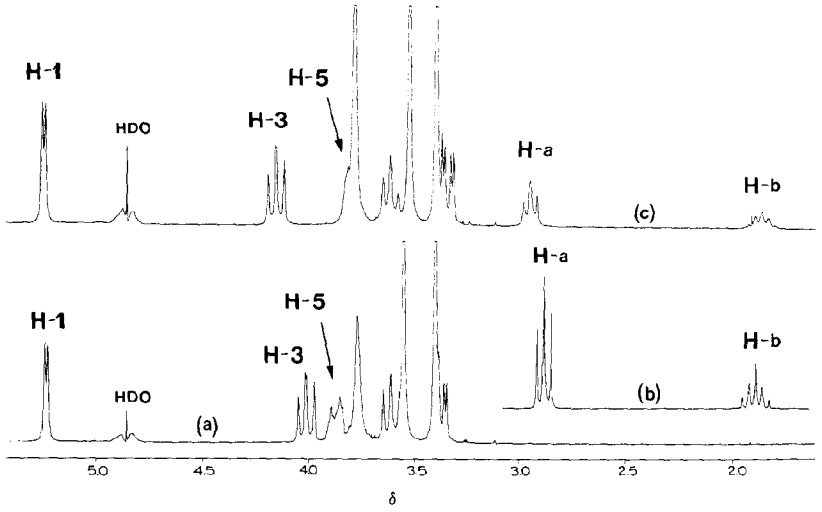


Fig. 10. Partial ^{1}H-n.m.r. spectra (250 MHz) of (a) DM-αCD, (b) carboplatin, and (c) a 1:1 molar ratio of DM-αCD–carboplatin in D_2O.

mean plane of the oxygen atoms associated with the twelve secondary hydroxyl groups, and 0.94 Å above the mean plane of the six glycosidic oxygen atoms which encircle the interior of the αCD cavity. For the most part, the ammine ligands are oriented over one of the glucopyranosyl residues which, in turn, is tilted more significantly with respect to the local pseudo-C_6 axis of the αCD receptor. There are two [N–H···O]hydrogen bonds (3.14 Å between N-1 and O-13, and 2.94 Å between N-2 and O-63) involving the ammine ligands on the platinum and secondary hydroxyl groups at the 3-positions of neighbouring glucopyranosyl residues of the αCD. There are no hydrogen bonds between the carbonyl oxygen atoms in **11** and the secondary hydroxyl groups on the rim of αCD. However, there are hydrogen bonds from these carbonyl oxygen atoms and the ammine hydrogen atoms to symmetry-related αCD molecules, and between the five water molecules and the αCD hydroxyl groups and the carbonyl oxygen atoms and ammine hydrogen atoms in **11**.

Since DM-αCD (**4**) is more soluble[11–13] in water than αCD, **4** was investigated as a second-sphere ligand for **11**. Addition of 1 mol of **11** to 20mM DM-αCD in D_2O resulted in (Fig. 10) a significant shift (37 Hz at 250 MHz) to higher frequency of the signal for H-3. Although the signal for H-5 was shifted significantly (19 Hz at 250 MHz) to lower frequency, those of the other glucopyranosyl ring protons were almost unchanged. The signal for H-a on the cyclobutane ring of **11** was also shifted significantly (18 Hz at 250 MHz) to higher frequency, whereas that of H-b was shifted (7 Hz displacement at 250 MHz) to lower frequency. Since H-3 and H-5 are oriented within the cavity of DM-αCD, it was concluded that formation of the adduct involves insertion of the cyclobutane ring of **11** into the cavity (*cf.* the

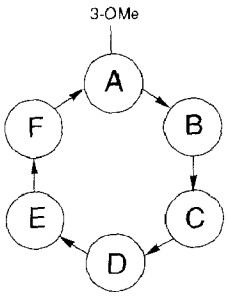


Fig. 11. Schematic diagram of (DM+1)-αCD viewed from the secondary face, illustrating the ring-labelling sequence A to F. The arrows correspond to α-(1→4) linkages between 2,3,6-tri-*O*-methyl-D-glucopyranosyl (A) and 2,6-di-*O*-methyl-D-glucopyranosyl (B, C, D, E, and F) residues.

supramolecular structure of αCD–carboplatin). The resonances for H-a and H-b were broadened considerably in the presence of DM-αCD, which implies restricted molecular motion of **11** as expected on inclusion. Quantitative ^{1}H-n.m.r. spectroscopy on solutions in D_2O, using H-3 and H-a in the adduct as probes, led to values of 289 and 298 $mol^{-1}.kg$ for K_a. The average K_a value of 294 $mol^{-1}.kg$, which corresponds to a ΔG^0 value of -13 $kJ.mol^{-1}$, indicates that binding of **11** to DM-

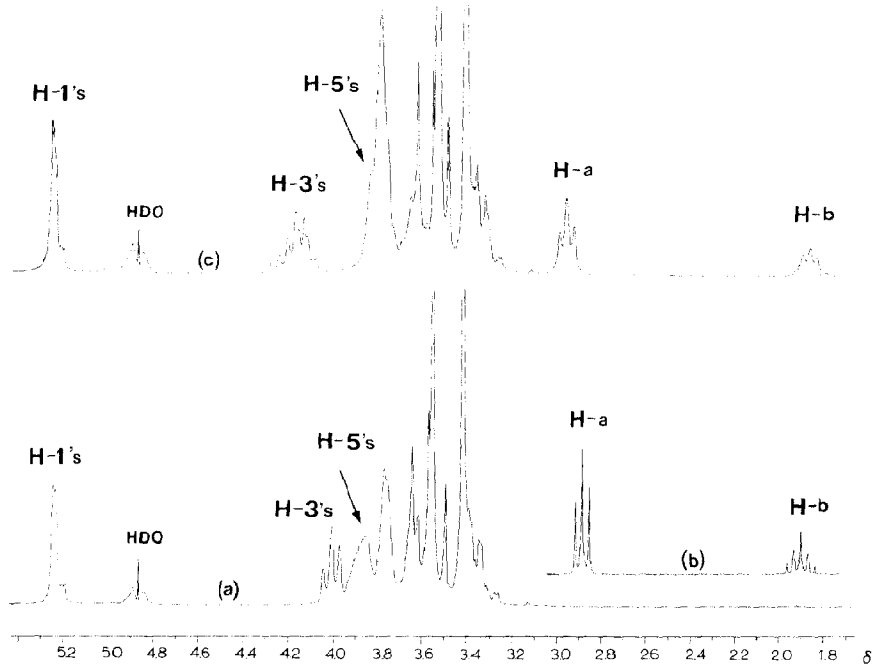


Fig. 12. Partial ^{1}H-n.m.r. spectra (250 MHz) of (a) (DM+1)-αCD, (b) carboplatin, and (c) a 1:1 molar ratio of (DM+1)-αCD–carboplatin in D_2O.

αCD is only slightly stronger than its binding to αCD. The ability of 2-hydroxyethylated CDs to form adducts with **11** is being investigated.

Because of their high molecular symmetry (C_n), the ^{1}H-n.m.r. spectra of constitutionally symmetrical CDs are deceptively simple. Chemically modified derivatives can be labelled[58] so that they become asymmetric. In this way, every substituted glucopyranosyl residue (*i.e.*, B, C, D, E, and F), not just the one (A) which carries the "label", becomes constitutionally different (Fig. 11) and should have different chemical shift data. The methylated αCD, (DM+1)-αCD, which contains one 2,3,6-tri-*O*-methyl-D-glucopyranosyl residue, can be regarded as an MeO-3-labelled DM-αCD.

The signals for the heterotopic H-3 in the ^{1}H-n.m.r. spectrum (250 MHz) of 48mM (DM+1)-αCD in D_2O were virtually coincident (Fig. 12a) apart from the resonance for H-3 on the labelled residue, which is obscured by a group of other signals at lower frequency. On the addition of 1 mol of **11**, not only were the signals for H-3 shifted to higher frequency but they were dispersed (Fig. 12b), indicating a preferential geometry of binding of **11** inside the now asymmetrical cavity. Crystals of the adducts of **11** with DM-αCD or (DM+1)-αCD, suitable for X-ray crystallography, have not yet been obtained. The crystal structure[71] of DM-αCD will be reported elsewhere.

CONCLUSIONS

In this paper, we have evaluated the potential of CDs and their methylated derivatives to act as second-sphere ligands towards (*i*) cationic rhodium complexes (*e.g.*, **8** and **9**) and (*ii*) a neutral platinum complex (carboplatin) with important anti-tumour properties. The following results stand out as the highlights from the research findings.

The preparative procedure developed previously[58] to obtain *pure* DM-βCD **5** can also be used to prepare *pure* DM-αCD **4**.

In the presence of αCD and DM-αCD, $[Rh(cod)(NH_3)_2]^+$ loses its ammine ligands and the CD probably enters the first co-ordination sphere of the metal. If this ligand exchange has indeed occurred, then we could have created[57] a system which (*i*) is potentially a water-soluble hydrogenation catalyst that (*ii*) could bind organic substrates within (*iii*) a chiral receptor that (*iv*) is readily available.

Both $[Rh(cod)(H_2NCH_2CH_2NH_2)]^+$ and carboplatin form 1:1 adducts with αCD in aqueous solution with small, but significant, changes in the standard free energies for the adduct formations being deduced from quantitative ^{1}H-n.m.r. spectroscopic data.

Although $[Rh(cod)(NH_3)_2]^+$ and $[Rh(cod)(H_2NCH_2CH_2NH_2)]^+$ form 1:1 adducts with βCD, as well as with αCD, carboplatin is highly receptor-specific for αCD and does not form adducts with βCD or γCD.

Microcalorimetry has revealed that the greatly enhanced solubility of carboplatin in aqueous solutions containing αCD is enthalpy driven.

Water-soluble chemically modified CDs, such as DM-αCD, form even stronger 1:1 adducts with carboplatin in aqueous solution.

The X-ray crystal structures of the 1:1 adducts $[\alpha CD.Rh(cod)(NH_3)_2]^+$ and [αCD.carboplatin] put the concept of second-sphere co-ordination of organometallic and metallo-organic complexes by CDs on a firm structural footing.

The present investigation has resulted in a patent[72].

ACKNOWLEDGMENTS

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APPENDIX

Quantitative ^1H-n.m.r. spectroscopy. — For an aqueous solution (D_2O) containing equimolar amounts of CD and C, if the total concentration of the CD (bound and unbound) is c, then it follows that the total concentration of C (bound and unbound) is also c (equations *3* and *4*).

$$[CD] + [A] = c \tag{3}$$

$$[C] + [A] = c \tag{4}$$

If x is the equilibrium concentration ([A]) of A, then $K_a = [A]/[CD][C] = x(c - x)^2$.

For a given proton in either C or CD, the observed chemical shift (δ_0) is related to the corresponding chemical shift (δ_p) in free C or CD and the chemical shift (δ_A) of the 1:1 adduct by equation *5*, and rearrangement gives equation *6*.

$$\delta_0 = \frac{c - x}{c}\delta_p + \frac{x}{c}\delta_A \tag{5}$$

$$\frac{x}{c} = \frac{\delta_0 - \delta_p}{\delta_A - \delta_p} \tag{6}$$

If Δ and Δ_0 are defined by equations *7* and *8*, respectively, then x in equation *6* can be rewritten in terms of Δ and Δ_0 as shown in equation *9*.

$$\Delta = \delta_0 - \delta_p \tag{7}$$

$$\Delta_0 = \delta_A - \delta_p \tag{8}$$

$$x = c(\Delta/\Delta_0) \tag{9}$$

Although Δ can be determined directly from the ^{1}H-n.m.r. spectra, Δ_0 is unknown since δ_A cannot be determined by experiment. Substituting x from equation *9* into $K_a = x(c - x)^2$ affords equation *10*, which relates K_a to c, Δ, and Δ_0.

$$K_a[c - c(\Delta/\Delta_0)]^2 = c(\Delta/\Delta_0) \qquad (10)$$

Rearrangement of equation *10* gives equation *1* (see RESULTS AND DISCUSSION).

$$\Delta = \Delta_0 - (\Delta/c)^{1/2}(\Delta_0/K_a)^{1/2} \qquad (1)$$

Microcalorimetry. — If the stoichiometric concentration ($[C]_0$) of carboplatin is kept constant and the concentration of αCD ($[CD]_0$) is varied, then the change in enthalpy per mole (ΔH) is related to the standard enthalpy change (ΔH^0) for the formation of a 1:1 adduct by equation *11*.

$$\Delta H = \Delta H^0[A]/[C]_0 \qquad (11)$$

The equilibrium concentrations of carboplatin, αCD, and the adduct are related by equations *12* and *13*.

$$[CD] = [CD]_0 - [A] \qquad (12)$$

$$[C] = [C]_0 - [A] \qquad (13)$$

Combining equations *11–13* allows [CD] and [C] to be defined in terms of ΔH and ΔH^0 as in equations *14* and *15*.

$$[CD] = [CD]_0 - (\Delta H/\Delta H^0)\,[C]_0 \qquad (14)$$

$$[C] = [C]_0 - (\Delta H/\Delta H^0)\,[C]_0 \qquad (15)$$

Incorporating equations *11*, *14*, and *15* into $K_a = [A]/[CD][C]$ generates equation *16*,

$$K_a = \frac{(\Delta H/\Delta H^0)[C]_0}{\{[CD]_0 - (\Delta H/\Delta H^0)[C]_0\}\,\{(1 - (\Delta H/\Delta H^0)[C]_0\}} \qquad (16)$$

rearrangement of which leads to equation *2* (see RESULTS AND DISCUSSION).

$$[CD]_0 = \frac{\Delta H\Delta H^0 + \Delta H\Delta H^0 K_a[C]_0 - (\Delta H)^2 K_a[C]_0}{K_a(\Delta H^0)^2 - K_a\Delta H\Delta H^0} \qquad (2)$$

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