Adamantan-1-ylamine and Adamantan-1-ylamine Hydrochloride Complexes with Cycloamyloses

Robert I. Gelb, Lowell M. Schwartz,* and Daniel A. Laufer
Department of Chemistry, University of Massachusetts, Boston, Massachusetts 02125, U.S.A.

Using both pH potentiometry and spectrophotometry, aqueous complex formation constants have been measured over a range of temperatures for both cyclohexa-amylose (α-cyclodextrin) and cyclohepta-amylose (β-cyclodextrin) with substrates amantadine (adamantan-1-ylamine) and amantadine hydrochloride. Both forms of cycloamylose complex with either amantadine or the hydrochloride with 1 : 1 stoicheiometry and cyclohexa-amylose complexes with amantadine with 2 : 1 stoicheiometry also. The formation constants for the cyclohepta-amylose adducts are unusually strong, *ca.* 1×10^5 and 1×10^4 for amantadine and the hydrochloride, respectively. Enthalpies and entropies of formation are estimated for each complex from the temperature dependence of the equilibrium constants. These $\Delta H°$ and $\Delta S°$ values together with measurements of ^{13}C n.m.r. chemical shift displacements of carbons on both host and substrate species lead to speculation about the structures and binding mechanisms of the complexes. The spectrophotometric experiments also yielded complex formation constants of both cycloamyloses with acidic and basic forms of the indicator Methyl Orange. $\Delta H°$ and $\Delta S°$ values of these complexes are also calculated.

Some derivatives of adamantane have attracted considerable interest in recent years. Amantadine (adamantan-1-ylamine) and the hydrochloride salt appear to have unusual biomedical properties including antiviral activity,[1,2] influenza prophylaxis,[3] membrane permeability,[4] antitumour therapy,[5] and involvement in nerve cell function.[6] Other derivatives, adamantane-1-carboxylic acid and carboxylate ion, have been studied as substrates for cycloamylose complexations [7,8] which have served as models of biological enzyme systems. Because of the possible utility of cycloamylose as a sequestering agent for amantadine and its derivatives we now report a study of cycloamylose complexation of these species. In this paper, we denote amantadine by the symbol ANH_2, protonated amantadine (adamantan-1-ylammonium ion) by ANH_3^+, cycloamylose in general by Cy, cyclohexa-amylose (α-cyclodextrin) by 6-Cy and cyclohepta-amylose (β-cyclodextrin) by 7-Cy.

We sought to measure aqueous complex formation constants of Cy with ANH_2 and/or ANH_3^+ and chose to utilize a pH potentiometric method [9] believed to be particularly well suited to this task. The technique relies on displacement of the ANH_2–ANH_3^+ buffer equilibrium by added portions of Cy and measurement of the consequent pH perturbations. Complex formation constants are determined from pH *versus* added Cy data by a non-linear regression analysis which attempts to fit the data to a set of model equations involving the formation constants as adjustable parameters. While this method has yielded high precision formation constants with a wide variety of acid–base substrate systems [7,9,10] complexing with both 6-Cy and 7-Cy, unsatisfactory results were obtained with amantadine buffer species complexing with either 6-Cy or 7-Cy. The problem with the ANH_2–ANH_3^+–6-Cy system is that cyclohexa-amylose forms binary complexes with both buffer components as well as a ternary complex with the conjugate base. All three complex formation constants must be adjusted to achieve a satisfactory fit of the model equations to the experimental pH *versus* [6-Cy] data. The binary complex 6-Cy·ANH_3^+ is relatively weak, however. Its formation constant cannot be determined accurately because of the low concentrations of 6-Cy·ANH_3^+ in the presence of the other, stronger complexes. Nevertheless, the adjusted values of $K_{6\text{-Cy}\cdot ANH_2}$ and $K_{(6\text{-Cy})_2\cdot ANH_2}$ depended on the adjusted value of the parameter $K_{6\text{-Cy}\cdot ANH_3^+}$. Satisfactory fits could be realized with wide ranges of values for the three formation constants, so that, in effect, the pH potentiometric method was incapable of determining a single set of three parameter values with satisfactory precision. With the system ANH_2–ANH_3^+–7-Cy the difficulty was that 7-Cy forms extremely strong complexes with both ANH_3^+ and ANH_2. It can be shown that in this circumstance, the pH potentiometric method is capable of determining a precise value of the ratio of the two complex formation constants but is not capable of determining either of the two constants independently.

Spectrophotometric Experiments.—The strategy that we adopted for both problems was to use an independent spectrophotometric method [12] to determine the complex formation constant of ANH_3^+ alone. Once $K_{Cy\cdot ANH_3^+}$ is known and introduced as a fixed parameter in the pH potentiometric model equations, the other formation constants are determined with adequate precision. A photometric acid–base indicator system, to be denoted HIn/In^-, is buffered at a pH value near the pK_a of the indicator acid HIn so that appreciable concentrations of both HIn and In^- are present in solution. The absorbance is measured at a wavelength chosen to maximize the difference in absorptivity of HIn and In^-. Cycloamylose forms complexes with both the HIn and In^- species but these complexes are of unequal strength. When Cy is added to the buffered indicator, the HIn/In^- ratio shifts in favour of the stronger complex and the observed solution absorbance changes markedly. By measuring the dependence of absorbance on added Cy, it has been shown [11] that complex formation constants of Cy with both HIn and In^- as well as molar extinction coefficients of both complexed and uncomplexed indicator species are calculable. In the present experiments, we also add varying concentrations of ANH_3^+ as well. Neither ANH_3^+ nor its complex with Cy absorb at the wavelength chosen but the presence of ANH_3^+ in solution perturbs the observed absorbance because the Cy/HIn and Cy/In^- equilibria are shifted. By this perturbation the Cy·ANH_3^+ complex formation constant is determined as well.

Specific details of these experiments are as follows. We chose the indicator system Methyl Orange [*p*-dimethylaminophenylazobenzene-*p*-sulphonic acid and anion]. Because the acid form has $pK_a < 3.5$, all solutions containing ANH_2/ANH_3^+ (pK_a *ca.* 10) were buffered with HCl to maintain $pH < 2.5$. Consequently, the amantadine was effectively completely protonated. Absorbance measurements were made

Table 1. Parameters obtained from spectrophotometric experiments using Methyl Orange indicator

Temp. (°C)	Solution composition [a]	Fit [b]	10^3 Molar extinction coefficients ($l\ mol^{-1}\ cm^{-1}$) HIn	In^-	Cy·HIn [c]	Cy·In^- [c]	Complex formation constants × 10^{-3} [d] Cy·HIn [c]	Cy·In^- [c]	Cy·ANH_3^+ [c]
18.0	I	0.0007	41.1	5.0	0.3	2.5	0.77 ± 0.01	14.8 ± 0.02 [d]	
18.6	II	0.0003	40.7	5.1	0.1	2.7	0.74 ± 0.01	16.4 ± 0.04	0.0571 ± 0.0008
18.6	III	0.0007	40.7	5.1	(23.2)	(2.5)	(0.21 ± 0.01)	(4.46 ± 0.13)	(10.58 ± 0.29)
25.0	I	0.0010	40.2	5.0	0.4	2.6	0.62 ± 0.01	10.5 ± 0.2	
25.0	II	0.0004	40.0	5.2	0.1	2.8	0.59 ± 0.01	11.8 ± 0.3	0.0498 ± 0.0008
25.0	III	0.0006	40.0	5.1	(21.3)	(2.7)	(0.19 ± 0.01)	(3.81 ± 0.09)	(8.43 ± 0.20)
30.0	II	0.0004	39.4	5.1	0.1	2.8	0.50 ± 0.01	9.06 ± 0.2	0.0461 ± 0.0007
30.0	III	0.0006	39.4	5.1	(20.8)	(2.6)	(0.20 ± 0.01)	(3.30 ± 0.08)	(7.29 ± 0.17)
32.0	I	0.0012	39.2	4.9	0.2	2.7	0.47 ± 0.01	7.19 ± 0.2	
37.0	II	0.0005	38.6	5.1	0.2	2.9	0.39 ± 0.01	6.49 ± 0.2	0.0410 ± 0.0007
37.0	III	0.0005	38.7	5.1	(19.8)	(2.8)	(0.18 ± 0.01)	(2.80 ± 0.06)	(5.72 ± 0.14)
40.0	I	0.0012	38.2	4.8	0.4	2.7	0.35 ± 0.01	4.86 ± 0.10	
47.0	I	0.0012	37.5	4.8	1.0	2.7	0.28 ± 0.01	3.42 ± 0.08	
48.2	II	0.0006	37.3	5.0	1.0	2.9	0.26 ± 0.01	3.55 ± 0.07	0.0315 ± 0.0006
48.2	III	0.0012	37.4	5.0	(17.1)	(2.7)	(0.14 ± 0.02)	(1.97 ± 0.08)	(3.64 ± 0.18)

[a] I, HIn/In^-/6-Cy, 19 different solutions, 7 parameters calculated; II, HIn/In^-/6-Cy/ANH_3^+, 15 solutions, 8 parameters calculated; III, HIn/In^-/7-Cy/ANH_3^+, 16 solutions, 8 parameters calculated. [b] Standard deviation of absorbance value residuals based on *df* degrees of freedom. *df* = no. of solutions less no. of parameters calculated. [c] Entries not in parentheses are for 6-Cy complexes. Entries in parentheses are for 7-Cy complexes. [d] Uncertainties are standard error estimates calculated by non-linear regression and 0.002 absorbance unit standard deviation of recorded measurements.

at a wavelength of 530 nm where HIn and In^- have molar extinction coefficients *ca.* 40×10^3 and 5×10^3 l mol^{-1} cm^{-1}, respectively. Both 6-Cy and 7-Cy form complexes with both indicator species. At 25 °C the 6-Cy·HIn and 6-Cy·In^- complexes have formation constants near 6×10^2 and 1×10^5, respectively, while the molar extinction coefficients of these complexes are $<0.5 \times 10^3$ and 3×10^3 l mol^{-1} cm^{-1}, respectively. These figures show that on a molar basis uncomplexed HIn absorbs about eight times more strongly than uncomplexed In^- and that 6-Cy added to buffered indicator markedly decreases the solution absorbance. The much stronger complex formed with In^- depletes the concentration of strongly absorbing HIn and both complexes are more weakly absorbing species than their respective uncomplexed substrates. Adding 7-Cy has a similar effect. The absorbances of these solutions are thus very sensitive to the Cy/HIn and Cy/In^- equilibria. Added ANH_3^+ complexes with Cy, shifts these equilibria, and changes the solution absorbance. Consequently, this technique is capable of determining the Cy·ANH_3^+ complex formation constant with high precision.

The model equations we employ for data treatment are derived from hypotheses (1) that Beer's law applies to each absorbing species HIn, In^-, Cy·HIn, and Cy·In^- with each species having an independent molar extinction coefficient, (2) that molar thermodynamic equilibria exist for HIn/In^-/H_3O^+ and for the complex formations of Cy·HIn, Cy·In^-, and Cy·ANH_3^+, (3) that because ionic strengths never exceeded 0.1M, ionic activity coefficients could be estimated from the Debye–Hückel correlation using temperature-dependent parameters tabulated by Robinson and Stokes [12] and ion-size parameters of 0.9, 1.0, 0.9, and 1.6 nm for H_3O^+, In^-, ANH_3^+, and Cy complex ions respectively, (4) that activity coefficients of uncharged species have unit value, and (5) that conservation relationships relate analytical concentrations of Cy, Methyl Orange, and ANH_3^+ to their respective species concentrations. The set of equations so derived is solved by a linear multiple regression calculation imbedded in a non-linear regression. The eight unknown parameters we seek are four equilibrium constants (pK_a for HIn and three formation constants for the Cy complexes) and four molar extinction coefficients. Typically we measure absorbances of 16 solutions to find these eight parameters at any given temperature. An interactive digital computer program similar to one previously described [13] proceeds by assuming reasonable values for the equilibrium constants and using these together with known analytical concentrations to calculate molar concentrations of all species in solution. The Beer's law equations, which are 16 linear relationships between solution absorbance and solute concentration, are solved by multiple regression for the four extinction coefficients. The calculated solution absorbances are compared with the observed absorbances and this leads to refined values of the equilibrium constants using a non-linear regression procedure as described.[13] This iteration continues until the sum-of-squares of absorbance deviations ceases to decrease.

An experimental system as complicated as this one requires some validation to be certain that the hypothesized model equations are, indeed, an accurate representation of the physico-chemical phenomena involved. In particular, we must be certain that there is no interactions between cycloamylose, Methyl Orange, or amantadine hydrochloride except the equilibria that are hypothesized. We have two independent means of verification: one is that the calculated solution absorbances be within statistical uncertainty of the experimental absorbances in each series of measurements and the other is that corresponding parameters in solutions of different composition be consistent. The molar extinction coefficients for HIn and In^- must agree in separate experiments using 6-Cy with ANH_3^+. Also complex formation constants for 6-Cy with HIn and In^- must be consistent whether or not ANH_3^+ is present. We are convinced that these criteria cannot be met in the presence of any significant extraneous interactions.

The results of these confirmatory experiments are shown in Table 1. Three distinct types of solutions were measured over a temperature range 18.0—48.2 °C. All solutions contained Methyl Orange but types I and II contained 6-Cy and types II and III contained amantadine hydrochloride. Measurements were made with a Beckman Acta C-III u.v.–visible spectrophotometer which according to the manufacturer's specifications has a photometric precision of ±0.002 absorbance units in the range of our measurements. The fits of our

Table 2. Complex formation constants of amantadine with cyclohexa-amylose and cyclohepta-amylose

Temp. (°C)	Cyclohexa-amylose $K_{6\text{-Cy}\cdot ANH_2}$	Cyclohexa-amylose $K_{(6\text{-Cy})_2\cdot ANH_2}$ [b]	Cyclohepta-amylose $10^4 K_{7\text{-Cy}\cdot ANH_2}$
20.0	315 ± 7 [a]	366 ± 11 [a]	14
25.0	271 ± 8	337 ± 12	10.7, 11.0 [c]
30.0	224 ± 7	299 ± 11	8.5, 8.9, 9.1
37.0			7.1, 7.2, 6.9
40.0	194 ± 5	177 ± 7	
45.0	166 ± 4	129 ± 4	5.5
50.0	127 ± 5	108 ± 5	4.4

[a] Uncertainties are estimated by combining the variance of these parameters calculated by the pH potentiometric nonlinear regression ($K_{Cy\cdot ANH_3^+}$ held fixed) with the variance in these parameters due to the variance in $K_{Cy\cdot ANH_3^+}$ from the spectrophotometric experiments. [b] Refers to the stepwise formation reaction, *i.e.* 6-Cy·ANH_2 + 6-Cy = (6-Cy)$_2$·ANH_2. [c] Multiple entries represent results of replicate experiments each consisting of 8—12 pH measurements at varying concentrations of 7-Cy.

calculations to our experimental absorbance values as shown in Table 1 under the heading Fit were consistently less than this specification. Also the extinction coefficients for HIn and for In^- all fall on a smooth temperature-dependent curve regardless of the type-solution from which they are found. The same is true for 6-Cy·HIn and 6-Cy·In^- complex formation constants. These three checks substantially confirm the validity of our model equations for these experiments.

We performed several other experiments on buffered Methyl Orange solutions in the absence of Cy and amantadine for the purpose of confirming some of the parameters of the indicator. First, we determined extinction coefficients of HIn and In^- at several temperatures by direct measurements of Methyl Orange in 0.2*F** HCl and pH 7 phosphate buffers, respectively. These values agreed with corresponding entries in Table 1 to within 0.3%. Second, we determined the acid dissociation pK_a of HIn both with and without 6-Cy in solution utilizing different wavelengths. At 530 nm we found pK_a 3.404 ± 0.005 in the presence of 6-Cy and 3.404 ± 0.008 in the absence. At 509 nm we observed pK_a 3.408 ± 0.012 in the absence of 6-Cy. These experiments add further credence to our procedures.

The last column of Table 1 shows the complex formation constants for Cy with ANH_3^+ calculated in complexing solutions. We fitted van't Hoff lines to these data and from the slopes obtained enthalpies and entropies of complexation as follows: for 6-Cy·ANH_3^+ ΔH° −3.66 ± 0.21 kcal mol^{-1} and ΔS° −4.55 ± 0.71 cal mol^{-1} K^{-1}; for 7-Cy·ANH_3^+, ΔH° −6.65 ± 0.32 kcal mol^{-1} and ΔS° −4.4 ± 1.0 cal mol^{-1} K^{-1}. The uncertainties quoted here are standard error estimates calculated from the scatter of the observed points from the least-squares van't Hoff lines.

pH Potentiometric Experiments.—As described earlier, we made pH potentiometric measures of solutions containing ANH_2/ANH_3^+ and varying concentrations of either 6-Cy or 7-Cy. The model equations describing the systems equilibria and conservation equations were used to calculate pH values from solution composition and certain parameters were adjusted to provide least-squares fits of these calculated to the observed pH values. In the case of 6-Cy, it was found that three complex formation constants, $K_{6\text{-Cy}\cdot ANH_2}$, $K_{6\text{-Cy}\cdot ANH_3^+}$, and $K_{(6\text{-Cy})_2\cdot ANH_2}$, were required to reduce the standard deviation of residuals to the *a priori* known precision of the pH measurements (±0.002) and for the pattern of residuals

* *F* denotes formula weight per litre (analytical concentration).

Table 3. Thermodynamic parameters ΔH° and ΔS° for complexations of adamantane derivatives [a] with 6-Cy and 7-Cy

	ΔH°/ kcal mol^{-1}	ΔS°/ cal mol^{-1} K^{-1}
6-Cy Binary complexes		
6-Cy·ANH_2	−4.84 ± 0.34 [b]	−5.1 ± 1.1 [b]
6-Cy·ANH_3^+	−3.66 ± 0.21	−4.5 ± 0.7
6-Cy·ACO_2^- [c]	−3.4 ± 0.6	−1.3 ± 1.9
6-Cy·ACO_2H [c]	−5.6 ± 0.7	−9.0 ± 2.0
7-Cy Binary complexes		
7-Cy·ANH_2	−6.92 ± 0.19	+0.1 ± 0.6
7-Cy·ANH_3^+	−6.65 ± 0.32	−4.4 ± 1.1
6-Cy Ternary complexes [d]		
(6-Cy)$_2$·ANH_2	−8.18 ± 0.74	−16.1 ± 2.4
(6-Cy)$_2$·ACO_2H [c]	−9.5 ± 0.6	−19.0 ± 2.0

[a] ANH_2 = amantadine, ACO_2H = adamantanecarboxylic acid, ANH_3^+ = amantadinium ion, ACO_2^- = adamantanecarboxylate ion. [b] Uncertainties are standard error estimates calculated from the scatter of points about least-squares van't Hoff lines. [c] From ref. 7. [d] Stepwise formation constants, *e.g.* 6-Cy·ANH_2 + 6-Cy = (6-Cy)$_2$·ANH_2.

to be random with respect to 6-Cy concentration. The formation constant for the ternary complex is here defined in stepwise fashion, *i.e.* the formation reaction is 6-Cy·ANH_2 + 6-Cy = (6-Cy)$_2$·ANH_2. Furthermore, we found it necessary to fix the value of $K_{6\text{-Cy}\cdot ANH_3^+}$ in order to determine unique values of the other two constants. At each temperature we utilized the value of $K_{6\text{-Cy}\cdot ANH_3^+}$ as read from the van't Hoff plot described above. This practice served as smoothing and interpolation procedures for $K_{6\text{-Cy}\cdot ANH_3^+}$ *versus* temperature. The results obtained are shown in Table 2 under the heading Cyclohexa-amylose. The uncertainties quoted here are standard error estimates calculated by combining the parameter variance from the pH potentiometric non-linear regression with the variance in these parameters due to the variance in $K_{6\text{-Cy}\cdot ANH_3^+}$ from the spectrophotometric experiments. This latter variance is propagated through the pH potentiometric calculation to the ANH_2 complexes.

In the case of 7-Cy, the calculations contrasted with 6-Cy in that we found it impossible to achieve standard deviation of pH residuals as low as 0.002 even with ternary complexes included in the model equations. The best fits we could achieve varied from 0.003 to 0.01 pH as the temperature decreased. Associated with the poor fits were non-random patterns of residuals with respect to 7-Cy concentration. Furthermore, we noted a certain erratic behaviour of the pH meter system when measuring solutions of ANH_2/ANH_3^+/7-Cy. The pH reading often drifted up and down by nearly 0.01 pH unit over a time span of 1 or 2 min. A similar but smaller effect was observed with ANH_2/ANH_3^+ solutions and in 6-Cy experiments but we were able to account for the fluctuation by time-averaging the pH readings for a period of a few minutes. Such averaging in 7-Cy experiments still resulted in substantially decreased precision and, therefore, we conclude that some systematic error is involved in the 7-Cy results, probably due to a chemisorption process at one of the electrodes. The non-linear regression calculations were performed with fixed values of $K_{7\text{-Cy}\cdot ANH_3^+}$ taken from the van't Hoff plots of spectrophotometric results and time-averaged pH data typically obtained over 5 min. We estimated statistical uncertainties in $K_{7\text{-Cy}\cdot ANH_2}$ parameters by making replicate experiments at several temperatures as indicated in Table 2. Finally, Table 3 shows the ΔH° and ΔS° values for

Table 4. Results of ^{13}C n.m.r. measurements of solutions containing ANH_2/ANH_3^+ and 6-Cy or 7-Cy

Adamantane derivative resonances [a]

Carbon no.	Cyclohexa-amylose solutions: Intrinsic resonances ANH_2	Cyclohexa-amylose solutions: Intrinsic resonances ANH_3^+	Displacements in complexes [b]: 6-Cy·ANH_2	6-Cy·ANH_3^+	$(6\text{-Cy})_2$·ANH_2	6-Cy·ACO_2^- [d]	Cyclohepta-amylose solutions: Intrinsic resonances ANH_2	Intrinsic resonances ANH_3^+	Displacements in complexes [b]: 7-Cy·ANH_2	7-Cy·ANH_3^+
1	47.71	53.61	0.2	−0.20	1.6	−0.03	47.59	53.57	0.9	−0.14
2	45.67	41.14	0.1	−0.02	1.4	0.01	45.60	41.20	0.3	0.21
3	30.41	29.84	0.2	−0.01	−1.1	−0.02	30.46	29.84	−0.3	−0.01
4	36.75	35.88	0.4	0.64	0.3	0.43	36.67	35.94	0.6	0.44
SE [c]	0.04	0.01	0.3	0.02	0.5	0.05	0.05	0.01	0.2	0.03

Cycloamylose resonances [a]

Carbon no.	Cyclohexa-amylose solutions: Intrinsic 6-Cy resonance	Displacements in complexes [b]: 6-Cy·ANH_2	6-Cy·ANH_3^+	$(6\text{-Cy})_2ANH_2$	6-Cy·ACO_2^- [d]	Cyclohepta-amylose solutions: Intrinsic 7-Cy resonance	Displacements in complexes [b]: 7-Cy·ANH_2	7-Cy·ANH_3^+
1	102.39	0.28	0.22	0.74	0.25	102.87	0.58	0.57
2	72.85	−0.05	−0.14	0.17	0.11	72.23	0.07	−0.01
3	74.46	0.18	0.34	0.31	0.08	74.23	0.20	0.39
4	82.25	0.23	0.29	−0.07	0.12	82.16	0.41	0.59
5	73.07	0.15	0.12	0.15	0.16	72.89	0.41	0.34
6	61.58	−0.05	−0.04	−0.15	−0.10	61.44	−0.14	0.01
SE [c]	0.01	0.06	0.04	0.06	0.04	0.01	0.02	0.02

[a] All resonances and displacements are in p.p.m. Resonances are measured downfield from external Me_4Si. [b] Displacements are defined as intrinsic resonance in complex minus resonance of uncomplexed species. [c] Standard error estimates based on ±0.01 p.p.m. uncertainty in observed resonance lines. [d] From ref. 7.

these complexation reactions as calculated by least-squares van't Hoff lines.

^{13}C *N.m.r. Experiments.*—The ^{13}C n.m.r. spectra of 5% D_2O (v/v aqueous) solutions containing 6-Cy (0—0.1*F*) or 7-Cy (0—0.02*F*) in combination with ANH_3^+/ANH_2 mixtures (0—0.09*F*/0—0.01*F*, respectively) consist of six resonance lines from non-equivalent Cy carbons and four lines corresponding to ANH_3^+/ANH_2 carbons. Assignments of ANH_3^+/ANH_2 peaks were made consistent with symmetry factors, relative relaxation rates, and observed multiplicities in gated $^{13}C(^1H)$ decoupling experiments. The intrinsic chemical shifts of all free and bound species were extracted from the observed resonances by means of an interactive computer program which (1) accepts independently measured estimates of the ANH_3^+ acid dissociation and relevant complex formation constants as fixed parameters along with analytical concentrations of reactant species, (2) computes concentrations of all species present in solution in accordance with equilibria, mass–charge conservation, and activity coefficient relations, and (3) partitions the observed chemical shifts among concentration-weighted intrinsic resonances as assumed at the rapid exchange limit. In Table 4 we display the results of these calculations as intrinsic resonances of uncomplexed reactants Cy, ANH_2, or ANH_3^+ and the displacement of these resonances upon complexation. Two columns of displacements headed 6-Cy·ACO_2^- are the corresponding resonances in adamantane-1-carboxylate–6-Cy solutions as reported previously.[7] We will discuss the ramifications of these results as follows.

Cyclohexa-amylose Resonances.—In Table 4 we note that the C-5 and C-6 displacements of the 6-Cy·ANH_2, 6-Cy·ANH_3^+, and 6-Cy·ACO_2^- binary complexes are equal within the listed uncertainties. This suggests that the primary hydroxy-substituted 6-Cy rim is perturbed to the same extent by the group common to all three substrates, *i.e.* the adamantyl residue. The relatively small magnitudes of these perturbations are consistent with a shallow insertion of the bulky adamantyl moiety into the 6-Cy cavity at the secondary hydroxy-rim. The displacements of the corresponding C-2 and C-3 resonances are not equal, however, and we attribute these disparities to differing electric field effects of the NH_2, NH_3^+, or CO_2^- substituents of included substrates. The ternary $(6\text{-Cy})_2$·ANH_2 displacements seen in Table 4 are very similar to the corresponding small displacement values of the binary 6-Cy·ANH_2 complex at C-5 and C-6 but have larger positive values at C-2 and C-3. These trends are consistent with an orientation where the wider secondary hydroxy-rims face each other in the ternary complex.

Adamantane Derivative Resonances in 6-Cy *Solutions.*—Here C-4 of the substrates are substantially and approximately equally perturbed in all three binary complexes. This is in agreement with the common orientation suggested above, namely shallow insertion of the adamantyl residue into the Cy cavity. With the exception of C-4, the substrate carbons of the $(6\text{-Cy})_2$·ANH_2 complex are perturbed much more than the corresponding binary carbons. This trend seems to confirm the ternary orientation suggested above where the two 6-Cy molecules completely occlude the ANH_2 substrate in the wider portion of the double cavity.

Cyclohepta-amylose Resonances.—The displacements listed in Table 4 indicate substantial deshielding perturbations at Cy C-1, C-3, C-4, and C-5 in both complexes. This suggests that most of the 7-Cy cavity interacts with the same substrate residue, presumably the adamantyl group. These large 7-Cy displacements contrast with the smaller displacements at these same sites for the corresponding 6-Cy complexes and suggest

complete insertion of the substrate into the 7-Cy cavity. We attribute the differences in the displacements at 7-Cy C-3 to differing interactions between the NH_2 and NH_3^+ groups with the secondary hydroxy-groups of 7-Cy as noted above for the 6-Cy system.

Adamantane Derivative Resonances in 7-Cy *Solutions.*—C-2, C-3, and C-4 of both substrates are perturbed to about the same extent in the 7-Cy·ANH_2 and 7-Cy·ANH_3^+ complexes, suggesting similar inclusion orientations. The large displacements at C-4, which are similar in sign and magnitude to the corresponding displacements in the 6-Cy system, imply that the adamantanyl residue enters the cavity in both complexes. The substantial disparity in 7-Cy·ANH_2 *versus* 7-Cy·ANH_3^+ displacements at C-1 of the adamantanyl group reflects differing interactions between the amine and ammonium substituents with the secondary hydroxy-groups.

Discussion

Cyclohexa-amylose Complexes.—We begin to interpret the thermodynamic and ^{13}C n.m.r. spectrometric results above by noting a recently reported [14] correlation (1) between ΔH° and ΔS° values for 6-Cy complexations. This correlation is based

$$\Delta H^\circ = (406 \pm 15)\Delta S^\circ - (1.2 \pm 0.2) \times 10^3 \quad (1)$$
ΔH° in cal mol^{-1}, ΔS° in cal mol^{-1} K^{-1}

on thermodynamic data for 31 6-Cy complexations with substrate species including aliphatic and aromatic carboxylic acids and their anions, substituted phenols, alcohols, inorganic ions, and small organic solvent molecules such as dioxane and acetonitrile. The values of ΔH° and ΔS° for the 6-Cy binary complexes of ANH_2 and ANH_3^+ both appear to fit this correlation to within the estimated values of their uncertainties. We have also reported [10] an apparent correlation between ΔH° and $\Delta\delta^{Cyl}$, the displacement upon complexation of the conformationally sensitive C-1 of 6-Cy. The correlation equation (2) was based on ^{13}C n.m.r. spectrometric and

$$\Delta\delta^{Cyl} = (-0.038 \pm 0.004)\Delta H^\circ + (0.09 \pm 0.03) \quad (2)$$

thermodynamic data for 20 complexes. In recent work [15] we have found complexes which deviate from this correlation. These are 6-Cy complexes of I^-, ClO_4^-, and SCN^- where the charged substrate could interact directly with C-1 of the macrocycle. Direct interaction of this sort does not occur in the 6-Cy complexes with ANH_2 and ANH_3^+ and, indeed, both binary complexes fit this correlation to within the estimated uncertainties of $\Delta\delta^{Cyl}$ and ΔH°. We attribute these correlations to the properties of 6-Cy rather than to the substrates which vary markedly in chemical nature. Furthermore, we interpret the substantially negative ΔS° values which accompany strong substrate binding as reflecting constraint in the 6-Cy macrocycle. In other words, strong binding interactions between 6-Cy and the substrate lead to conformational changes in 6-Cy which results in loss of internal degrees of freedom and leads to ordering in the complex, *i.e.* negative ΔS°. Consequently, these complexes tend to form because of the favourably negative ΔH° of complexation and in spite of the unfavourably positive entropic ($-T\Delta S^\circ$) contribution to the free energy of formation.

While the correlations cited above seem to reflect certain thermodynamic and structural features of 6-Cy complexations they do not directly reveal the nature of the mechanism of the binding forces. The identities of these forces must be surmised by inference and among those most frequently cited are hydrophobic mechanisms which rely on release of trapped solvent either near the hydrophobic substrate or in the 6-Cy cavity. These mechanisms require large positive ΔS° values and clearly cannot play an important role here. In this connection we note that the adamantanyl group, which appears to bond to 6-Cy in both ANH_2 and ANH_3^+ complexes, is too bulky to enter the 6-Cy cavity fully. It is thus unlikely to release any water held there and by the same token is unable to 'free' much water held in the vicinity of the hydrocarbon skeleton.

The thermodynamic data for ternary complex formation provide further evidence against hydrophobic mechanisms. ΔH° and ΔS° for addition of a second 6-Cy complexon to 6-Cy·ANH_2 are -8.2 kcal mol^{-1} and -16.1 cal mol^{-1} K^{-1}, respectively. These values conform to the correlation between ΔH° and ΔS° for binary 6-Cy complexes. While interactions between the macrocyclic complexons may be partly responsible for binding in the ternary complex, the fact that ΔH° and ΔS° conform to the same correlation as the binary complex, where such interactions cannot exist, strongly suggests that the same binding mechanism operates in both cases. This reasoning is also applicable to the binary and ternary 6-Cy complexes of adamantane-1-carboxylic acid (ACO_2H). ΔH° and ΔS° values previously measured [7] and shown in Table 3 also conform to the ΔH°, ΔS° correlation. Because neither the amine nor the carboxylic acid substituent is hydrophobic, it seems unreasonable to associate the strongly exothermic ternary complex formations with hydrophobic forces. Instead we conclude that short-range dipolar and induced dipolar interactions provide the binding force in these complexations. We note that no ternary complex is formed with the strongly solvated ANH_3^+ species and that only a very weak ternary complex (stepwise formation constant *K ca.* 3) is detectable [7] with adamantane-1-carboxylate ion (ACO_2^-) which is presumably solvated at the carboxylate site. This behaviour is in marked contrast to the strong bonding forces which lead to ternary 6-Cy complexes of ANH_2 and ACO_2H which substrates are solvated to a lesser extent than their ionic counterparts. Thus, the substrates' interaction with the second 6-Cy cavity differ substantially between the neutral and ionic species. The ionic sites are imbedded in a network of strongly bound and not very polarizable water molecules and these sites form a very weak or no ternary complex. On the other hand, the neutral substituents are relatively unsolvated and polarizable and are able to interact effectively with the 6-Cy cavity to form strong ternary complexes.

Cyclohepta-amylose Complexes.—As can be seen in Tables 1 and 2, the complexes of ANH_2 and ANH_3^+ with 7-Cy are much stronger than the corresponding complexes with 6-Cy. In an attempt to explain this behaviour, we compare the ΔH° and ΔS° values in Table 3 and note that 7-Cy·ANH_2 is stronger than 6-Cy·ANH_2 both because the enthalpic contribution to the free energy of formation is more favourable (-6.9 *versus* -4.8 kcal mol^{-1}) and because the entropic contribution ($-T\Delta S^\circ$) is less unfavourable ($+0.3$ *versus* $+1.5$ kcal mol^{-1}). In the case of ANH_3^+, however, the greater strength of the 7-Cy *versus* the 6-Cy complex is due entirely to the more favourable enthalpic contribution of -6.7 *versus* -3.7 kcal mol^{-1}, the entropy changes being essentially equal. To help further in seeking interpretations of these effects, we constructed space-filling models of the four complexes, 6-Cy and 7-Cy with ANH_2 and ANH_3^+. These models show clearly that the adamantyl skeleton can penetrate the 7-Cy cavity at the wide secondary hydroxy-rim but cannot fully penetrate the 6-Cy cavity. The amine or ammonium group comes in close proximity to this rim in 7-Cy but not in 6-Cy. Thus we hypothesize that the more favourable enthalpic

Table 5. Thermodynamic parameters ΔH° and ΔS° for complexation of Methyl Orange acid–base species with 6-Cy and 7-Cy

	Solution type [a]	ΔH°/ kcal mol^{-1}	ΔS°/ cal mol^{-1} K^{-1}
6-Cy·HIn	I	-6.6 ± 0.2 [b]	-9.4 ± 0.6 [b]
	II	-6.6 ± 0.2	-9.4 ± 0.7
6-Cy·In$^-$	I	-9.4 ± 0.1	-13.2 ± 0.5
	II	-9.6 ± 0.2	-13.7 ± 0.8
7-Cy·HIn	III	-2.5 ± 0.6	$+1.9 \pm 2.0$
7-Cy·In$^-$	III	-5.1 ± 0.3	-0.8 ± 0.8

[a] See Table 1 for definitions. [b] Uncertainties are standard error estimates calculated from the scatter of the observed points about the least-squares van't Hoff lines.

contributions to 7-Cy complexation with both ANH_2 and ANH_3^+ substrates are due to a combination of two factors: (1) more effective close-range interactions between the adamantyl group and the 7-Cy cavity and (2) dipolar or hydrogen bonding interactions between the secondary hydroxy-rim and the amino-group or its solvation sphere. This hypothesis seems to be supported by the ^{13}C n.m.r. results shown in Table 4. We note that C-1, which is bound directly to the amine function, exhibits substantial downfield displacement on complexation in 7-Cy·ANH_2. This indicates strong interactions of the amine function with 7-Cy. In 6-Cy complexation, however, displacements of the amantadine C-1 are smaller in the binary complex but quite large in the ternary (6-Cy)$_2$·ANH_2 where the principal interaction occurs between 6-Cy and NH_2.

The comparative ΔS° data indicate that 7-Cy complexes with ANH_2 with no entropy change whereas 7-Cy complexes with ANH_3^+ with an entropy change of -4.4 cal mol^{-1} K^{-1}. If the principal difference between these substrates is the extensive solvation of the ion, then it appears that the secondary hydroxy-rim of 7-Cy and/or the secondary or outer solvation sphere of the NH_3^+ group suffer some constraint to internal motion upon complexation. However, ANH_2 is not as strongly solvated as ANH_3^+ and so cannot interact with 7-Cy in this way and the complex forms virtually without conformational restraints.

Cycloamylose Complexes with Methyl Orange Substrates.— As by-products of the spectrophotometric experiments described earlier, we obtained values of complex formation constants of 6-Cy and 7-Cy with acidic and basic forms of Methyl Orange indicator which were denoted by HIn and In$^-$, respectively. These formation constants at various temperatures are given in Table 1 and van't Hoff plots of these data lead to complexation ΔH° and ΔS° values shown in Table 5. First, we note the agreement between corresponding 6-Cy parameters calculated from solutions with and without ANH_3^+ present. This agreement further substantiates the hypothesized non-interaction between ANH_3^+ and either 6-Cy or HIn/In$^-$. Second, we note that both 6-Cy·HIn and 6-Cy·In$^-$ deviate somewhat from the ΔH°, ΔS° correlation equation (1) in the direction that the observed ΔS° is less negative (more positive) than the correlation predicts for the observed ΔH°. Our interpretation is that those substrates whose ΔH°, ΔS° values are correlated by equation (1) form complexes with 6-Cy by a common mechanism involving dipolar or induced dipolar interaction and accompanied by a corresponding restraint of internal motion of the macrocyclic 6-Cy ring leading to the negative ΔS° values of complexation. Since ΔS° for 6-Cy complexation of both acidic and anionic forms of Methyl Orange are anomalous in the sense of being less negative than expected, we conclude that the macrocyclic ring suffers less conformational restraint in these complexes.

The complexes of 7-Cy with HIn and In$^-$ are both weaker than their corresponding 6-Cy complexes. Table 5 shows that ΔH° for both 7-Cy complexes is *ca.* 4 kcal mol^{-1} more positive than the corresponding 6-Cy complex and ΔS° is within statistical uncertainty of zero for both 7-Cy complexes. It appears that the larger 7-Cy ring forms weaker bonds and is essentially unconstrained by these substrates.

Experimental

Amantadine hydrochloride (Gold Label) and amantadine were obtained from the Aldrich Chemical Co. and were used without further purification. Cyclohexa-amylose and cyclohepta-amylose were obtained from the same source and treated as follows. 6-Cy samples were allowed to aerate for at least one week before use. To determine the hydration state of the aerated solid 6-Cy, we dried samples at 100 °C *in vacuo* until constant weight was achieved and then calculated weight losses corresponding to 6 moles of water per mole 6-Cy dried. Thus, we used aerated solid in our experiments and calculated solution formalities based on a hexahydrate formula weight. ^{13}C N.m.r. measurements under conditions of high signal-to-noise ratio (>200) yielded only six resonances corresponding to the six non-equivalent carbons of 6-Cy and indicating negligible organic impurity in the samples. Furthermore, pH potentiometric measurements involving addition of small quantities of acid or base to concentrated 6-Cy solutions indicated no detectable concentrations of acidic or basic impurities in the pH range from 2 to 10.

7-Cy samples were twice recystallized from water and air-dried for one week. The hydration state of the crystals was somewhat dependent on atmospheric humidity and varied between the normal octahydrate to an apparent decahydrate under conditions of high humidity. Thus, in order to know the formula weight of the 7-Cy reagent used to prepare a particular solution, it was necessary to determine the current hydration state by drying to constant weight a sample of the reagent taken simultaneously with the solution preparation. No acidic or basic impurities were detected in the 7-Cy.

pH Potentiometric measurements employed an Orion 801 pH meter equipped with conventional glass and Ag–AgCl reference electrodes which were allowed ample time for thermal equilibration before use. Meter standardization was checked frequently to assure stability. Nevertheless, measurements with ANH_2/ANH_3^+ buffers gave unusually large fluctuations. ANH_3^+ concentrations employed in pH potentiometric experiments were *ca.* 3m*F* while 6-Cy and 7-Cy concentrations were varied between 0 and 10m*F*.

Spectrophotometric measurements employed a Beckman Acta CIII u.v.–visible spectrophotometer equipped with 1.00 cm thermostatted quartz cuvettes. Particular care was taken to assure thermal equilibration. The concentrations of 6-Cy and 7-Cy varied up to *ca.* 5 m*F* and those of ANH_3^+ ranged up to 6m*F* in these experiments. ^{13}C N.m.r. spectrometric measurement used a Bruker HX-270 spectrometer equipped with 10 mm sample tubes thermostatted at 30 ± 1 °C. The instrument operated at 67.89 MHz for ^{13}C observation and typically employed 10 K transients. Repeated measurements with replicate samples indicated a reproducibility of ±0.01 p.p.m.

Acknowledgements

We acknowledge the support of the National Institute of General Medical Sciences, U.S. Public Health Service (Grant GM 26004). The Bruker HX-270 experiments were

performed at the n.m.r. facilities of The Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology.

References

1 C. W. Potter, C. Z. Teh, L. Harvey, and R. Jennings, *J. Antimicrob. Chemother.*, 1981, **7**, 575.
2 D. D. Richmond, P. Yazaki, and K. Y. Hosteler, *Virology*, 1981, **112**, 81.
3 M. Hamzawi, R. Jennings, and C. W. Potter, *Med. Microbiol. Immunol.*, 1981, **169**, 250.
4 H. Sapper, W. Jordan, and W. Lohmann, *Stud. Biophys.*, 1980, **80**, 105.
5 E. Busse, H. Rose, M. Helmholz, and B. Kriueger, *Radiobiol., Radiother.*, 1980, **21**, 788.
6 C. VanderVende and M. T. Spoerlein, *Neuropharmacology*, 1979, **18**, 633.
7 R I. Gelb, L. M. Schwartz, and D. A. Laufer, *Bio-org. Chem.*, 1980, **9**, 450.
8 K. Fujita, T. Ueda, T. Imoto, I. Tabushi, N. Toh, and T. Koga, *Bio-org. Chem.*, 1982, **11**, 108.
9 R. I. Gelb, L. M. Schwartz, R. F. Johnson, and D. A. Laufer, *J. Am. Chem. Soc.*, 1979, **101**, 1869.
10 R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Furhman, R. F. Johnson, and D. A. Laufer, *J. Am. Chem. Soc.*, 1981, **103**, 1750.
11 R. I. Gelb, L. M. Schwartz, B. Cardelino, and D. A. Laufer, *Anal. Biochem.*, 1980, **103**, 362.
12 R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions,' Butterworths, London, 2nd. rev. edn., 1965.
13 L. M. Schwartz and R. I. Gelb, *Anal. Chem.*, 1978, **50**, 1571.
14 R. I. Gelb, L. M. Schwartz, and D. A. Laufer, *Carbohydr. Res.*, 1983, **118**, 111.
15 R. I. Gelb, L. M. Schwartz, M. Radeos, and D. A. Laufer, *J. Phys. Chem.*, 1983, **87**, 3349.

Received 11*th February* 1983; *Paper* 3/217