Thermodynamic and Nuclear Magnetic Resonance Study of the Interactions of α- and β-Cyclodextrin with Model Substances: Phenethylamine, Ephedrines, and Related Substances

Mikhail V. Rekharsky,[†,‡] Robert N. Goldberg,*[,†] Frederick P. Schwarz,[†] Yadu B. Tewari,[†] Philip D. Ross,[§] Yuko Yamashoji,[∥] and Yoshihisa Inoue[∥]

Contribution from the Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, Laboratory of Molecular Biology, National Institutes of Health, Bethesda, Maryland 20892, and Department of Molecular Chemistry, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita 565, Japan

Received May 5, 1995[⊗]

Abstract: Titration calorimetry was used to measure equilibrium constants and standard molar enthalpies for the reactions of phenethylamine, ephedrines, and related substances with α- and β-cyclodextrin. Changes in the chemical shifts $\Delta\delta$ of both the ligand and cyclodextrin protons were measured with NMR. The thermodynamic results have been examined in terms of structural features of the ligand that affect these interactions such as the separation of the charge at an amino group and the aromatic ring, steric effects, the presence of additional functional groups (amino, hydroxy, methoxy, and methyl) attached to the aromatic ring, the presence and location of hydroxy group(s) on the ligand, changes in the chirality of the ligand, and the flexibility of the organic molecules attached to the aromatic ring. It was found that the values of thermodynamic quantities for these reactions in phosphate and acetate buffers were different. This difference is attributable to the presence of a hydrophobic alkyl group in the neutral acetic acid molecule and its interaction with the cyclodextrins. Also, there are significant differences in the thermodynamic quantities for the reactions of the chiral isomers of ephedrine and pseudoephedrine in their reactions with β-cyclodextrin. A plot of the standard molar enthalpy vs the standard molar entropy for the reactions of these chiral isomers with α- and β-cyclodextrin is linear; the relative order of the ephedrines and pseudoephedrines in the enthalpy−entropy plot is the same for the reactions of these substances with both α- and β-cyclodextrin. NMR studies demonstrated that the magnitude of the upfield shifts of the cyclodextrin's H3 and H5 protons, $\Delta\delta$(H3) and $\Delta\delta$(H5), and their relative ratio, $\Delta\delta(H5)/\Delta\delta(H3)$, can be used, respectively, as a measure of the complex stability and the depth of inclusion of the ligand into the cavity. The equilibrium constants determined by titration calorimetry correlate well with the changes in chemical shifts $\Delta\delta$ determined by NMR.

Introduction

There have been several systematic thermodynamic studies of cyclodextrin complexes with different classes of organic compounds such as hydrocarbons,[1,2] aliphatic alcohols,[3−8] aliphatic diols,[6,9] phenols,[10,11] cyclohexane derivatives,[12] naphthalene derivatives,[13] and other aromatic compounds.[14−16] Substances related to phenethylamine form an important class of substances that include pharmaceuticals such as the ephedrines and phenylephrines. Surprisingly, the amount of information on the thermodynamics of this class of substances is almost nonexistent. Since studies of this type can yield insight into the nature of the ligand−cyclodextrin complexes formed and can be of value in separation technology,[17−19] in drug delivery systems,[20,21] and as models of enzyme−substrate interactions,[22] we have undertaken this study of the interactions of phenethyl-

[†] National Institute of Standards and Technology.
[‡] Guest researcher from the Department of Chemistry, Moscow State University, Russia.
[§] National Institutes of Health.
[∥] Osaka University.
[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1995.

(1) Wishnia, A.; Lappi, S. J. *J. Mol. Biol.* **1974**, *82*, 77−89.
(2) Tucker, E. E.; Christian, S. D. *J. Am. Chem. Soc.* **1984**, *106*, 1942−1945.
(3) Matsui, Y.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2808−2814.
(4) Barone, G.; Castronuovo, G.; Del Vecchio, P.; Elia, V.; Muscetta, M. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 2089−2101.
(5) Sasaki, K. J.; Christian, S. D.; Tucker, E. E. *Fluid Phase Equilib.* **1989**, *49*, 281−289.
(6) Andini, S.; Castronuovo, G.; Elia, V.; Gallotta, E. *Carbohydr. Res.* **1991**, *217*, 87−97.
(7) Hallén D.; Schön, A.; Shehatta, I.; Wadsö, I. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2859−2863.
(8) Rekharsky, M. V.; Schwarz, F. P.; Tewari, Y. B.; Goldberg, R. N. *J. Phys. Chem.* **1994**, *98*, 10282−10288.
(9) Bastos, M.; Briggner, L.-E.; Shehatta, I.; Wadsö, I. *J. Chem. Thermodyn.* **1990**, *22*, 1181−1190.
(10) Lin, S.-F.; Connors, K. A. *J. Pharm. Sci.* **1983**, *72*, 1333−1338.
(11) Bertrand, G. L.; Faulkner, J. R.; Han, S. M.; Armstrong, D. W. *J. Phys. Chem.* **1989**, *93*, 6863−6867.
(12) Rekharsky, M. V.; Schwarz, F. P.; Tewari, Y. B.; Goldberg, R. N.; Tanaka, M.; Yamashoji, Y. *J. Phys. Chem.* **1994**, *98*, 4098−4103.
(13) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 475−481.
(14) Connors, K. A.; Lin, S.-F.; Wong, A. B. *J. Pharm. Sci.* **1982**, *71*, 217−222.
(15) Wong, A. B.; Lin, S.-F.; Connors, K. A. *J. Pharm. Sci.* **1983**, *72*, 388−390.
(16) Connors, K. A.; Paulson, A.; Toledo-Velasquez, D. *J. Org. Chem.* **1988**, *53*, 2023−2026.
(17) Schurig, V.; Jung, M. In *Recent Advances in Chiral Separations*; Proceedings of the Chromatography Society's International Symposium on Chiral Separation, 2nd Meeting; Stevenson, D., Wilson, I. D., Eds.; Plenum: New York, 1991; pp 117−133.
(18) Berthod, A.; Li, W.; Armstrong D. W. *Anal. Chem.* **1992**, *64*, 873−879.
(19) Yamashoji, Y.; Ariga, T.; Asano, S.; Tanaka, M. *Anal. Chim. Acta* **1992**, *268*, 39−47.
(20) Szejtli, J. *Cyclodextrins Technology*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988.
(21) Amato, M. E.; Djedaini-Pilard, F.; Perly, B.; Scarlata, G. *J. Chem. Soc., Perkin Trans. 2*, **1992**, 2065−2069.
(22) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978.

0002-7863/95/1517-8830$09.00/0

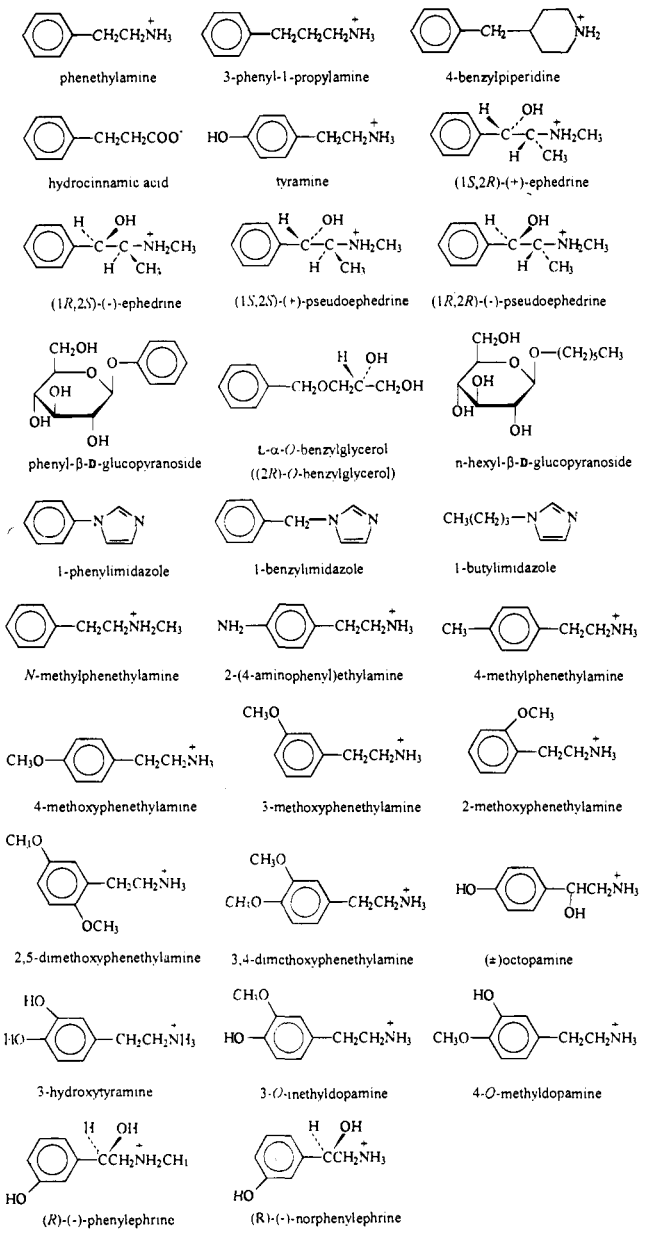

Figure 1. Structures of the ligands used in this study.

amine, ephedrines, and related substances (see Figure 1) with α-, β-, and in a few experiments γ-cyclodextrin. These interactions have been examined with titration calorimetry and NMR.

One item of particular interest is the magnitude of the differences in thermodynamic quantities for the reactions of chiral substances with the cyclodextrins which also contain chiral centers. Information on these differences has come primarily from chromatography,[17,18,23] a method that involves an assumed relationship between the equilibrium constant for the complexation reaction and differences in retention times. Potentiometry,[24] NMR,[25] and calorimetry[8,26,27] have also been used to a limited extent. In our earlier calorimetric study[8] we found that there were no significant differences in any of the thermodynamic quantities for the reactions of aliphatic alcohols with either α- or β-cyclodextrin due to the change in the location of a hydrogen atom on a chiral carbon atom. Since the ephedrines and pseudoephedrines contain two chiral centers, they appeared to be good candidates with which to examine the effects of chiral changes on the thermodynamics of these binding reactions. There are two questions that can be raised: (1) how large are the differences in the thermodynamic quantities due to a change at a chiral center in a substance? and (2) can such differences be correlated with the structural nature of the changes at the chiral centers?

Experimental Section

Materials. The principal substances used in this study, their respective Chemical Abstracts Services registry numbers, empirical formulas, molar masses in kg mol^{-1}, supplier (A = Aldrich, F = Fluka, S = Sigma),[28] and mass fraction moisture content × 100 are as follows: α-cyclodextrin, 10016-20-3, $C_{36}H_{60}O_{30}$, 0.97285, S, 11.0 ± 1.4;[29] β-cyclodextrin, 7585-39-9, $C_{42}H_{70}O_{35}$, 1.1350, S, 12.4 ± 0.4; γ-cyclodextrin, 17465-86-0, $C_{48}H_{80}O_{40}$, 1.2971, S, 8.9 ± 0.7; phenethylamine hydrochloride, 156-28-5, $C_8H_{12}NCl$, 0.15764, A, 0.07 ± 0.04; 3-phenyl-1-propylamine, 2038-57-5, $C_9H_{13}N$, 0.13521, A, 0.65 ± 0.10; 4-benzylpiperidine, 31252-42-3, $C_{12}H_{17}N$, 0.17527, A, 0.46 ± 0.05; hydrocinnamic acid, 501-52-0, $C_9H_{10}O_2$, 0.15018, A, 0.20 ± 0.01; tyramine hydrochloride, 60-19-5, $C_8H_{12}NOCl$, 0.17364, A, 0.08 ± 0.03; (1*S*,2*R*)-(+)-ephedrine hydrochloride, 24221-86-1, $C_{10}H_{16}NOCl$, 0.20170, F, 0.07 ± 0.07; (1*R*,2*S*)-(−)-ephedrine, 299-42-3, $C_{10}H_{15}NO$, 0.16524, F, 0.65 ± 0.03; (1*R*,2*R*)-(−)-pseudoephedrine, 321-97-1, $C_{10}H_{15}NO$, 0.16524, A, 0.14 ± 0.12; (1*S*,2*S*)-(+)-pseudoephedrine, 90-82-4, $C_{10}H_{15}NO$, 0.16524, F, 0.22 ± 0.04; phenyl-β-D-glucopyranoside, 1464-44-4, $C_{12}H_{16}O_6$, 0.25625, A, 0.39 ± 0.10; L-α-*O*-benzylglycerol, 56552-80-8, $C_{10}H_{14}O_3$, 0.18222, S, 0.48 ± 0.01; 1-phenylimidazole, 7164-98-9, $C_9H_8N_2$, 0.14418, A, 0.94 ± 0.07; 1-benzylimidazole, 4238-71-5, $C_{10}H_{10}N_2$, 0.15820, A, 2.96 ± 0.20; 1-butylimidazole, 4316-42-1, $C_7H_{12}N_2$, 0.12419, A, 0.61 ± 0.04; *n*-hexyl-β-D-glucopyranoside, 59080-45-4, $C_{12}H_{24}O_6$, 0.26432, S, 4.1 ± 0.1; *N*-methylphenethylamine, 589-08-2, $C_9H_{13}N$, 0.13521, A, 1.7 ± 0.1; 2-(4-aminophenyl)ethylamine, 13472-00-9, $C_8H_{12}N_2$, 0.13620, A, 11.4 ± 0.4; 4-methylphenethylamine 3261-62-9, $C_9H_{13}N$, 0.13521, A, 1.4 ± 0.2; 4-methoxyphenethylamine, 55-81-2, $C_9H_{13}NO$, 0.15121, A, 4.5 ± 0.1; 3-methoxyphenethylamine, 2039-67-0, $C_9H_{13}NO$, 0.15121, A, 1.3 ± 0.2; 2-methoxyphenethylamine, 2045-79-6, $C_9H_{13}NO$, 0.15121, A, 2.2 ± 0.4; 2,5-dimethoxyphenethylamine, 3600-86-0, $C_{10}H_{15}NO_2$, 0.18123, A, 1.1 ± 0.1; 3,4-dimethoxyphenethylamine, 120-20-7, $C_{10}H_{15}NO_2$, 0.18123, A; (±)-octopamine hydrochloride, 770-05-8, $C_8H_{12}NO_2Cl$, 0.18964, A, 0.14 ± 0.02; 3-hydroxytyramine hydrochloride, 62-31-7, $C_8H_{12}NO_2Cl$, 0.18964, A, 0.08 ± 0.03; 3-*O*-methyldopamine hydrochloride, 1477-68-5, $C_9H_{14}NO_2Cl$, 0.20367, A, 0.18 ± 0.10; 4-*O*-methyldopamine hydrochloride, 645-33-0, $C_9H_{14}NO_2Cl$, 0.20367, A; (*R*)-(−)-phenylephrine hydrochloride, 61-76-7, $C_9H_{14}NO_2Cl$, 0.20367, A, 0.07 ± 0.01; (±)-norphenylephrine hydrochloride, 636-87-3, $C_8H_{12}NO_2Cl$, 0.18964, A, 0.08 ± 0.02.

The vendors used a variety of methods (GC, perchloric acid and NaOH titration, and elemental analysis) to determine the purities of the ligands and stated that the mole fraction purities were all >0.99. The mole fraction purities of the α-, β-, and γ-cyclodextrins were found to be 0.985, 0.980, and 0.983, respectively, using a chromatographic procedure.[12] The vendors also determined angles of optical rotation (all at T = 293.15 K, λ = 589.3 nm, c = 10 g dm^{-3}, and path length = 10 cm) for the following substances: L-α-*O*-benzylglycerol, +6°; (1*R*,2*S*)-(−)-ephedrine, −41.3°; (1*S*,2*R*)-(+)-ephedrine hydrochloride, +33.9°; (*R*)-(−)-phenylephrine hydrochloride, −45.5°; phenyl-β-D-glucopyranoside, −70.4°; DL-octopamine hydrochloride, 0°; (1*R*,2*R*)-

(23) Harada, A.; Saeki, K.; Takahashi, S. *Carbohydr. Res.* **1989**, *192*, 1−7.
(24) Corradini, R.; Impellizzeri, G.; Maccarrone, G.; Marchelli, R.; Rizzarelli, E.; Vecchio, G. *Top. Mol. Organ. Eng.* **1991**, *8*, 209−221.
(25) Smith, N. J.; Spotswood, T. M.; Lincoln, S. F. *Carbohydr. Res.* **1989**, *192*, 9−15.
(26) Cooper, A.; MacNicol, D. D. *J. Chem. Soc., Perkin Trans. 2* **1978**, 760−763.
(27) Barone, G.; Castronuovo, G.; Di Ruocco, V.; Elia, V.; Giancola, C. *Carbohydr. Res.* **1989**, *192*, 331−341.

(28) Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.
(29) All uncertainties given in this paper are, unless indicated otherwise, based on two estimated standard deviations of the mean.

(−)-pseudoephedrine, −49.8°; (1*S*,2*S*)-(+)-pseudoephedrine, +52.0°. All substances were used without further purification.

Moisture contents were determined by Karl Fisher analysis for all substances except the hydrochlorides. For these substances, the moisture contents were determined by loss of mass following drying in a vacuum desiccator over phosphorous pentoxide. The moisture content of 3,4-dimethoxyphenethylamine was not determined because the thermodynamic results were very uncertain. The moisture content of 4-*O*-methyldopamine hydrochloride was not determined because of the very high cost of this substance and the need for at least 0.1 g of this substance for its proper analysis. Thus, the moisture contents of these two substances were assumed to be zero.

Measurements. A Microcal Omega isothermal titration calorimeter[30] was used for all of the measurements. The calorimetric and computational procedures have been described previously.[12,31]

Proton NMR spectra were measured at $T = 298.15$ K with a 600 MHz Bruker AM-600 instrument; D_2O solutions buffered at pD = 7.0 with {D_3PO_4 (0.05 mol dm^{-3}) + NaOD} were used for all experiments. All chemical shifts were relative to the DOH signal at 4.75 ppm. The concentration of cyclodextrin was kept fixed at 0.015 mol dm^{-3} throughout the experiment, and the ligand was added in portions to this solution. This was done because the gradual addition of cyclodextrin to a ligand solution of a fixed concentration caused large changes in the viscosity of the solution, which in turn sometimes led to extra chemical shifts that were not associated with complex formation. The ratio of the concentration of the ligand to the concentration of the cyclodextrin in solution was determined from the relative intensities of the signals of the protons in the cyclodextrins and in the ligands. The NMR measurements were performed at two to six different ratios of ligand concentration to cyclodextrin concentration.

Results

Calorimetry. The treatment of the data obtained with the titration calorimeter has been described previously.[12] All equilibrium constants and standard molar enthalpies of reaction reported in this paper are based on a 1:1 binding model and a single binding site:

$$\text{cyclodextrin(sln)} + \text{ligand(sln)} = \text{cyclodextrin·ligand(sln)} \quad (1)$$

The equilibrium constant for this reaction is:

$$K = a(\text{cyclodextrin·ligand})/\{a(\text{cyclodextrin})\cdot a(\text{ligand})\} \quad (2)$$

where a is the activity of the indicated substance. Nonideality corrections are assumed to be negligible for both the measured equilibrium constants and the standard molar enthalpies of the reaction. This approximation should hold reasonably well even when dealing with a charged ligand (e.g., phenethylamine$^+$) since the reaction is charge symmetric and the activity coefficients in the numerator and denominator should largely cancel at low and moderate ionic strengths. The term "sln" is used in eq 1 to denote the fact that the reaction has occurred in a solution which may or may not be equivalent to a dilute aqueous solution which would be designated as "aq". This point will be amplified later.

In addition to the 1:1 binding model, calculations were also performed in which the complex cyclodextrin·(ligand)$_2$ was assumed present. It was found that the additional parameters obtained from these calculations had uncertainties that were comparable to the parameters themselves and that the quality of the overall fit was not improved. Thus, the assumption of the 1:1 binding model and a single binding site is the simplest choice and more complex models are not justified at this time.

It was also found that the values of $\Delta_rH°$ and K obtained from the titration curves were not significantly affected by the random deletion of up to around one-third of the data points in the titration curves. This shows that the individual titration curves are well defined, which in turn helps to fix the values of $\Delta_rH°$ and K.

The assignment of the charge number z to the predominant ligand species in solution requires either a knowledge of the ionization behavior (i.e., its pK's) of that substance or a knowledge of structurally similar substances. We have obtained the following pK's from two computerized databases:[32,33] for hydrocinnamic acid, pK = 4.44 at T = 298.15 K and I = 0.1 mol dm^{-3};[32] for tyramine, pK_1 = 9.45 and pK_2 = 10.56, both at T = 298.15 K and I = 0.1 mol dm^{-3};[33] for (±)-octopamine, pK = 9.91 at T = 298.15 K and I = 0.1 mol dm^{-3};[33] for 3-phenyl-1-propylamine, pK = 10.32 at T = 298.15 K and I = 0.1 mol dm^{-3};[33] for 3,4-dimethoxyphenethylamine, pK = 9.78 at T = 298.15 K and I = 0.1 mol dm^{-3};[33] for 3-*O*-methyldopamine, pK = 10.47 at T = 298.15 K and I = 0.1 mol dm^{-3};[33] for (1*R*,2*S*)-(−)-ephedrine, pK = 9.39 at T = 298.15 K and I = 0;[34] for (1*S*,2*S*)-(+)-pseudoephedrine, pK = 9.53 at T = 298.15 K and I = 0;[34] and for 3-hydroxytyramine, pK_1 = 8.86, pK_2 = 10.31, and pK_3 = 13.1 at T = 298.15 K and I = 0.1 mol dm^{-3}.[32] Upon the basis of these data and the structures of these substances, we have assigned the following charge numbers to the predominant ligand species in solution at the indicated pHs: −1 for hydrocinnamic acid at pH = 6.9; +1 for tyramine at pH = 5.0 and pH = 6.9; +1 for (±)-octopamine at pH = 5.0 and pH = 6.9; +1 for 3-phenyl-1-propylamine at pH = 5.0 and pH = 6.9; +1 for 3,4-dimethoxyphenethylamine at pH = 5.0 and pH = 6.9; +1 for 3-*O*-methyldopamine at pH = 5.0 and pH = 6.9; +1 for (1*R*,2*S*)-(−)-ephedrine and (1*S*,2*R*)-(+)-ephedrine at pH = 5.0 and pH = 6.9; +1 for (1*R*,2*R*)-(−)-pseudoephedrine and (1*S*,2*S*)-(+)-pseudoephedrine at pH = 5.0 and pH = 6.9; +1 for 3-hydroxytyramine at pH = 5.0 and pH = 6.9. Upon the basis of structural similarities of these substances with other substances found in the two computerized databases,[32,33] charge numbers have also been assigned to the other predominant species occurring at the pHs used in the experiments (see Tables 1 and 2). In all cases, the pHs used for the experiments were well removed (|pH − pK| ≥ 2) from the pK's at which ionizations occur. Thus, the complication of having more than one species present in these solutions in any significant amount (>1%) is avoided.

Thermodynamic quantities for the reactions of the various ligands used in this study with α- and β-cyclodextrin are presented in Tables 1 and 2, respectively. The standard molar Gibbs energies of reaction $\Delta_rG°$ and standard molar entropies of reaction $\Delta_rS°$ given in Tables 1 and 2 were calculated from the measured equilibrium constants and standard molar enthalpies of reaction $\Delta_rH°$.

The computer program Origin,[31] used to calculate the equilibrium constant and the standard molar enthalpy of reaction from a titration experiment, also returns a standard deviation based on the scatter of the data in a single titration experiment. To test whether these standard deviations were reasonable, four to six titration experiments were also performed on three different ligand−cyclodextrin pairs. The standard deviations

(30) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131−137.

(31) Yang, C. P. *Omega Data Analysis in Origin*; Microcal: Northampton, MA, 1990.

(32) Murray, K.; May, P. M. *Joint Expert Speciation System*; Murdoch University: Murdoch, Western Australia, 1992.

(33) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critical Stability Constants of Metal Complexes Database: NIST Standard Reference Database 46*; National Institute of Standards and Technology: Gaithersburg, MD, 1993.

(34) Christensen, J. J.; Hansen, L. D.; Izatt, R. M. *Handbook of Proton Ionization Heats and Related Thermodynamic Quantities*; John Wiley & Sons: New York, 1976.

Table 1. Thermodynamic quantities K, $\Delta_r H°$, $\Delta_r G°$, and $\Delta_r S°$ for the Reaction α-Cyclodextrin(sln) + Ligand(sln) = α-Cyclodextrin·Ligand(sln) at $T = 298.15$ K[a]

ligand	m (mol kg^{-1})	pH	N	K	$\Delta_r H°$ (kJ mol^{-1})	$\Delta_r G°$ (kJ mol^{-1})	$\Delta_r S°$ (J K^{-1} mol^{-1})
phenethylamine$^+$	0.200	6.9[b]	2	13.2 ± 1.2	−13.3 ± 0.9	−6.40 ± 0.24	−23 ± 3
phenethylamine$^+$	0.224	5.0[c]	2	11.4 ± 1.0	−13.2 ± 1.0	−6.03 ± 0.23	−24 ± 3
3-phenyl-1-propylamine$^+$	0.200	6.9[b]	2	23.7 ± 0.7	−16.8 ± 0.4	−7.85 ± 0.08	−30.0 ± 1.4
3-phenyl-1-propylamine$^+$	0.246	5.0[c]	2	21.7 ± 0.7	−15.9 ± 0.4	−7.63 ± 0.08	−27.7 ± 1.4
4-benzylpiperidine$^+$	0.115	6.9[b]	2	44.9 ± 2.6	−19.9 ± 0.9	−9.43 ± 0.15	−35 ± 3
hydrocinnamate$^-$	0.191	6.9[b]	2	31.3 ± 0.6	−15.5 ± 0.2	−8.54 ± 0.05	−23.3 ± 0.7
tyramine$^+$	0.222	6.9[b]	2	9.4 ± 0.7	−21.5 ± 1.3	−5.55 ± 0.19	−54 ± 5
tyramine$^+$	0.203	5.0[c]	2	7.4 ± 0.9	−18.4 ± 2.0	−4.96 ± 0.32	−45 ± 7
(1*S*,2*R*)-(+)-ephedrine$^+$	0.213	6.9[b]	2	18.0 ± 0.9	−15.0 ± 0.6	−7.17 ± 0.13	−26 ± 2
(1*S*,2*R*)-(+)-ephedrine$^+$	0.208	5.0[c]	1	8.9 ± 1.4	−16.2 ± 2.3	−5.42 ± 0.42	−36 ± 8
(1*R*,2*S*)-(−)-ephedrine$^+$	0.201	6.9[b]	3	17.0 ± 0.9	−15.6 ± 0.7	−7.02 ± 0.13	−29 ± 3
(1*R*,2*S*)-(−)-ephedrine$^+$	0.188	5.0[c]	2	11.7 ± 1.4	−12.0 ± 1.2	−6.10 ± 0.32	−20 ± 4
(1*R*,2*R*)-(−)-pseudoephedrine$^+$	0.204	6.9[b]	2	18.6 ± 0.6	−17.8 ± 0.4	−7.25 ± 0.08	−35.4 ± 1.4
(1*S*,2*S*)-(+)-pseudoephedrine$^+$	0.210	6.9[b]	3	19.9 ± 0.6	−18.4 ± 0.4	−7.41 ± 0.07	−36.9 ± 1.4
(1*S*,2*S*)-(+)-pseudoephedrine$^+$	0.230	5.0[c]	2	14.7 ± 0.9	−13.7 ± 0.7	−6.66 ± 0.16	−24 ± 2
phenyl-β-D-glucopyranoside^0	0.076	6.9[b]	2	9.5 ± 7.6	−21 ± 15	−5.6 ± 4.0	−52 ± 52
L-α-*O*-benzylglycerol^0	0.169	6.9[b]	2	14.7 ± 2.6	−14.8 ± 2.2	−6.66 ± 0.48	−27 ± 8
n-hexyl-β-D-glucopyranoside^0	0.101	6.9[b]	2	839 ± 35	−18.5 ± 0.3	−16.69 ± 0.11	−6.1 ± 1.1
1-phenylimidazole^0	0.045	10.0[d]	2	37 ± 12	−20.4 ± 5.5	−9.0 ± 1.0	−38 ± 19
1-benzylimidazole^0	0.062	10.0[d]	2	60 ± 5	−28.9 ± 1.9	−10.15 ± 0.22	−63 ± 7
1-butylimidazole^0	0.102	10.0[d]	2	173 ± 5	−20.4 ± 0.2	−12.77 ± 0.07	−25.6 ± 0.7
N-methylphenethylamine$^+$	0.153	6.9[b]	2	13.6 ± 1.8	−14.8 ± 1.7	−6.47 ± 0.35	−28 ± 6
N-methylphenethylamine$^+$	0.235	5.0[c]	2	13.9 ± 1.0	−11.8 ± 0.7	−6.52 ± 0.19	−18 ± 2
2-(4-aminophenyl)ethylamine$^+$	0.211	6.9[b]	2	[e]			
4-methylphenethylamine$^+$	0.121	6.9[b]	2	60.6 ± 1.5	−16.5 ± 0.3	−10.17 ± 0.06	−21.2 ± 1.0
4-methylphenethylamine$^+$	0.186	5.0[c]	2	45.0 ± 1.3	−13.9 ± 0.3	−9.44 ± 0.07	−15.0 ± 1.0
4-methoxyphenethylamine$^+$	0.148	6.9[b]	1	44.2 ± 0.4	−19.8 ± 0.2	−9.39 ± 0.02	−34.9 ± 0.7
4-methoxyphenethylamine$^+$	0.220	5.0[c]	2	30.1 ± 0.6	−15.0 ± 0.2	−8.44 ± 0.05	−22.0 ± 0.7
3-methoxyphenethylamine$^+$	0.199	6.9[b]	2	22.6 ± 0.7	−19.0 ± 0.4	−7.73 ± 0.08	−37.8 ± 1.4
3-methoxyphenethylamine$^+$	0.222	5.0[c]	2	17.1 ± 0.5	−14.6 ± 0.3	−7.04 ± 0.07	−25.4 ± 1.0
2-methoxyphenethylamine$^+$	0.221	6.9[b]	1	[f]			
2-methoxyphenethylamine$^+$	0.236	5.0[c]	2	13.9 ± 0.9	−15.8 ± 0.8	−6.52 ± 0.17	−31 ± 3
2,5-dimethoxyphenethylamine$^+$	0.190	6.9[b]	2	34.9 ± 0.6	−18.8 ± 0.2	−8.81 ± 0.04	−33.5 ± 0.7
2,5-dimethoxyphenethylamine$^+$	0.212	5.0[c]	2	26.9 ± 1.0	−13.6 ± 0.4	−8.16 ± 0.09	−18.2 ± 1.4
3,4-dimethoxyphenethylamine$^+$	0.191	6.9[b]	2	7.9 ± 2.9	−9.0 ± 2.9	−5.1 ± 1.1	−13 ± 10
3,4-dimethoxyphenethylamine$^+$	0.202	5.0[c]	2	[e]			
(±)-octopamine$^+$	0.196	6.9[b]	2	12.3 ± 0.6	−28.1 ± 1.2	−6.22 ± 0.12	−73 ± 4
(±)-octopamine$^+$	0.213	5.0[c]	2	9.0 ± 0.6	−23.1 ± 1.3	−5.45 ± 0.17	−59 ± 4
3-hydroxytyramine$^+$	0.192	6.9[b]	2	[e]			
3-*O*-methyldopamine$^+$	0.191	6.9[b]	2	[e]			
3-*O*-methyldopamine$^+$	0.217	5.0[c]	2	[e]			
4-*O*-methyldopamine$^+$	0.193	6.9[b]	2	5.2 ± 3.6	−10 ± 6	−4.1 ± 2.9	−20 ± 22
4-*O*-methyldopamine$^+$	0.201	5.0[c]	1	≈13	≈ −3.0		
(*R*)-(−)-phenylephrine$^+$	0.175	6.9[b]	2	4.1 ± 1.6	−34 ± 12	−3.5 ± 1.2	−102 ± 40
(*R*)-(−)-phenylephrine$^+$	0.191	5.0[c]	2	3.6 ± 1.2	−25 ± 8	−3.2 ± 1.0	−73 ± 27
(±)-norphenylephrine$^+$	0.171	6.9[b]	2	7.0 ± 2.1	−16 ± 4	−4.8 ± 0.9	−38 ± 14
(±)-norphenylephrine$^+$	0.182	5.0[c]	2	4.5 ± 1.9	−17 ± 7	−3.7 ± 1.4	−45 ± 24

[a] The molality (m) of the ligand for which the measurements were performed, the pH, and N the number of sets of titration experiments performed are given in columns 2, 3, and 4, respectively. In all cases the molality of the α-cyclodextrin was 0.0015−0.0035 mol kg^{-1}. The basis of the uncertainties is discussed in the text. [b] Phosphate buffer {(NaH_2PO_4, 0.025 mol kg^{-1}) + (Na_2HPO_4, 0.025 mol kg^{-1}), pH = 6.9}. [c] Acetate buffer {($NaC_2H_3O_2$, 0.1 mol kg^{-1}) + $C_2H_4O_2$, pH = 5.0}. [d] Glycine buffer {($C_2H_5NO_2$, 0.1 mol kg^{-1}) + HCl, pH = 10.0}. [e] K and/or $\Delta_r H°$ values for this reaction were too small to measure with the titration calorimeter. [f] Thermodynamic quantities for this reaction could not be determined since a precipitate was formed when this buffer was used.

both for the equilibrium constants and for the standard molar enthalpies of reaction were then calculated directly from the results of these experiments. The standard deviations that were calculated directly were either less than or comparable to the standard deviations obtained from the Origin program, except for the case when the standard deviations were <1−2% of the thermodynamic quantity. In this case, the calorimetry is more precise than our ability to prepare solutions. Accordingly, we have set a minimum uncertainty of ±1% for the various thermodynamic quantities obtained from these experiments. In all other cases, the results and uncertainties for the equilibrium constants and standard molar enthalpies of reaction in Tables 1 and 2 were obtained as weighted averages[35] of the results of the titration experiments done for each ligand−cyclodextrin pair. These uncertainties refer to two estimated standard deviations of the mean. In a few cases the standard deviations were comparable or larger than the quantities determined. We consider these to be approximate results and have denoted them as such in Tables 1 and 2. Also, in several cases, it was not possible to measure either K or $\Delta_r H°$ for a binding reaction. This implies that K and/or $\Delta_r H°$ values are too small to measure with the titration calorimeter.

(35) Rossini, F. D. *Experimental Thermochemistry*; Interscience: New York, 1956.

Effect of Buffer. Many of the reactions were studied with both phosphate and acetate buffers having respective pHs of 6.9 and 5.0. In many cases (see Tables 1 and 2), there are significant differences in the thermodynamic quantities for the reaction of a given ligand with α- and β-cyclodextrin with these two buffers. The reason for these differences was understood with a series of control experiments which are now described. In the first two control experiments, an aqueous solution of

Table 2. Thermodynamic Quantities K, $\Delta_r H°$, $\Delta_r G°$, and $\Delta_r S°$ for the Reaction β-Cyclodextrin(sln) + Ligand(sln) = β-Cyclodextrin·Ligand(sln) at $T = 298.15$ K[a]

ligand	m (mol kg^{-1})	pH	N	K	$\Delta_r H°$ (kJ mol^{-1})	$\Delta_r G°$ kJ mol^{-1})	$\Delta_r S°$ (J K^{-1} mol^{-1})
phenethylamine$^+$	0.200	6.9[b]	2	24.4 ± 1.6	−6.4 ± 0.3	−7.92 ± 0.17	5.1 ± 1.2
phenethylamine$^+$	0.224	5.0[c]	2	20.0 ± 1.9	−6.9 ± 0.5	−7.43 ± 0.25	2 ± 2
3-phenyl-1-propylamine$^+$	0.200	6.9[b]	2	108 ± 3	−9.2 ± 0.1	−11.61 ± 0.07	8.1 ± 0.4
3-phenyl-1-propylamine$^+$	0.246	5.0[c]	2	95 ± 3	−9.44 ± 0.15	−11.29 ± 0.08	6.2 ± 0.6
4-benzylpiperidine$^+$	0.115	6.9[b]	2	1987 ± 219	−13.8 ± 0.4	−18.83 ± 0.29	17 ± 2
hydrocinnamate$^-$	0.191	6.9[b]	2	141 ± 3	−7.60 ± 0.08	−12.27 ± 0.06	15.7 ± 0.3
tyramine$^+$	0.222	6.9[b]	2	71.3 ± 0.7	−13.8 ± 0.2	−10.58 ± 0.03	−10.8 ± 0.7
tyramine$^+$	0.203	5.0[c]	2	63.0 ± 0.7	−13.79 ± 0.14	−10.27 ± 0.03	−11.8 ± 0.5
(1*S*,2*R*)-(+)-ephedrine$^+$	0.213	6.9[b]	2	71.3 ± 1.2	−8.71 ± 0.09	−10.58 ± 0.05	6.3 ± 0.3
(1*S*,2*R*)-(+)-ephedrine$^+$	0.208	5.0[c]	1	55.3 ± 1.4	−8.79 ± 0.13	−9.95 ± 0.07	3.9 ± 0.5
(1*R*,2*S*)-(−)-ephedrine$^+$	0.201	6.9[b]	3	79.2 ± 1.6	−9.7 ± 0.1	−10.84 ± 0.05	3.8 ± 0.4
(1*R*,2*S*)-(−)-ephedrine$^+$	0.188	5.0[c]	2	59.3 ± 1.3	−9.31 ± 0.12	−10.12 ± 0.06	2.7 ± 0.5
(1*R*,2*R*)-(−)-pseudoephedrine$^+$	0.204	6.9[b]	2	68.9 ± 0.8	−9.99 ± 0.10	−10.49 ± 0.03	1.7 ± 0.4
(1*S*,2*S*)-(+)-pseudoephedrine$^+$	0.210	6.9[b]	3	96.7 ± 1.0	−12.54 ± 0.13	−11.33 ± 0.03	−4.1 ± 0.5
(1*S*,2*S*)-(+)-pseudoephedrine$^+$	0.230	5.0[c]	2	78.1 ± 0.8	−12.23 ± 0.13	−10.80 ± 0.03	−4.8 ± 0.5
phenyl-β-D-glucopyranoside^0	0.076	6.9[b]	2	≈10	≈−14		
L-α-*O*-benzylglycerol^0	0.169	6.9[b]	2	128 ± 3	−9.2 ± 0.1	−12.03 ± 0.06	9.5 ± 0.4
n-hexyl-β-D-glucopyranoside^0	0.101	6.9[b]	2	≈750	≈0.14		
1-phenylimidazole^0	0.045	10.0[d]	2	25 ± 14	−39 ± 19	−8.0 ± 2.0	−104 ± 64
1-benzylimidazole^0	0.062	10.0[d]	2	411 ± 13	−15.9 ± 0.2	−14.92 ± 0.08	−3.3 ± 0.7
1-butylimidazole^0	0.102	10.0[d]	2	155 ± 8	−10.7 ± 0.3	−12.50 ± 0.13	6.0 ± 1.1
N-methylphenethylamine$^+$	0.153	6.9[b]	2	28.5 ± 2.7	−6.33 ± 0.47	−8.30 ± 0.25	6.6 ± 1.8
N-methylphenethylamine$^+$	0.235	5.0[c]	2	21.4 ± 1.2	−7.3 ± 0.3	−7.59 ± 0.14	1.0 ± 1.1
2-(4-aminophenyl)ethylamine$^+$	0.211	6.9[b]	2	31.3 ± 2.0	−8.72 ± 0.38	−8.54 ± 0.16	−0.6 ± 1.4
4-methylphenethylamine$^+$	0.138	6.9[b]	2	88.3 ± 3.1	−6.84 ± 0.14	−11.11 ± 0.09	14.3 ± 0.6
4-methylphenethylamine$^+$	0.186	5.0[c]	2	74.9 ± 2.8	−7.23 ± 0.14	−10.70 ± 0.10	11.6 ± 0.6
4-methoxyphenethylamine$^+$	0.148	6.9[b]	1	92.8 ± 1.3	−8.08 ± 0.08	−11.23 ± 0.04	10.6 ± 0.3
4-methoxyphenethylamine$^+$	0.220	5.0[c]	2	77.3 ± 1.5	−8.21 ± 0.08	−10.78 ± 0.05	8.6 ± 0.3
3-methoxyphenethylamine$^+$	0.199	6.9[c]	2	76.2 ± 1.1	−11.7 ± 0.1	−10.74 ± 0.04	−3.2 ± 0.4
3-methoxyphenethylamine$^+$	0.222	5.0[c]	2	66.1 ± 0.85	−13.32 ± 0.13	−10.39 ± 0.04	−9.8 ± 0.5
2-methoxyphenethylamine$^+$	0.221	6.9[b]	1	*e*			
2-methoxyphenethylamine$^+$	0.236	5.0[c]	2	8.0 ± 1.0	−13.5 ± 1.6	−5.15 ± 0.33	−28 ± 6
2,5-dimethoxyphenethylamine$^+$	0.190	6.9[b]	2	38.9 ± 1.0	−9.39 ± 0.17	−9.08 ± 0.07	−1.0 ± 0.6
2,5-dimethoxyphenethylamine$^+$	0.212	5.0[c]	2	36.8 ± 1.0	−10.2 ± 0.2	−8.94 ± 0.07	−4.2 ± 0.7
3,4-dimethoxyphenethylamine$^+$	0.191	6.9[b]	2	13.9 ± 6.1	−2.0 ± 0.7	−6.52 ± 1.4	15 ± 5
3,4-dimethoxyphenethylamine$^+$	0.202	5.0[c]	2	*f*			
(±)-octopamine$^+$	0.196	6.9[b]	2	52.7 ± 0.7	−13.68 ± 0.14	−9.83 ± 0.03	−12.9 ± 0.5
(±)-octopamine$^+$	0.213	5.0[c]	2	44.3 ± 0.6	−15.86 ± 0.17	−9.40 ± 0.04	−21.7 ± 0.6
3-hydroxytyramine$^+$	0.193	6.9[b]	2	31.8 ± 0.5	−16.52 ± 0.17	−8.58 ± 0.04	−26.6 ± 0.6
3-hydroxytyramine$^+$	0.202	5.0[c]	3	30.3 ± 0.4	−18.3 ± 0.2	−8.46 ± 0.03	−33.0 ± 0.7
3-*O*-methyldopamine$^+$	0.191	6.9[b]	2	10.7 ± 3.8	−5.7 ± 1.8	−5.88 ± 1.1	1 ± 7
3-*O*-methyldopamine$^+$	0.217	5.0[c]	2	4.3 ± 1.8	−13.4 ± 5.5	−3.62 ± 1.3	−33 ± 19
4-*O*-methyldopamine$^+$	0.193	6.9[b]	2	60.4 ± 0.6	−13.41 ± 0.07	−10.17 ± 0.07	−10.9 ± 0.3
4-*O*-methyldopamine$^+$	0.201	5.0[c]	2	51.7 ± 1.5	−15.3 ± 0.3	−9.78 ± 0.07	−18.5 ± 1.0
(*R*)-(−)-phenylephrine$^+$	0.175	6.9[b]	2	46.6 ± 0.5	−19.44 ± 0.13	−9.52 ± 0.03	−33.3 ± 0.5
(*R*)-(−)-phenylephrine$^+$	0.191	5.0[c]	2	39.3 ± 0.4	−21.90 ± 0.22	−9.10 ± 0.03	−42.9 ± 0.8
(±)-norphenylephrine$^+$	0.171	6.9[b]	2	38.6 ± 0.6	−18.16 ± 0.18	−9.06 ± 0.04	−30.5 ± 0.6
(±)-norphenylephrine$^+$	0.182	5.0[c]	2	32.7 ± 0.6	−20.7 ± 0.3	−8.65 ± 0.05	−40.4 ± 1.0

[a] The molality (m) of the ligand for which the measurements were performed, the pH, and N the number of sets of titration experiments performed are given in columns 2, 3, and 4, respectively. In all cases the molality of the α-cyclodextrin was 0.0015−0.0035 mol kg^{-1}. The basis of the uncertainties is discussed in the text. [b] Phosphate buffer {(NaH_2PO_4, 0.025 mol kg^{-1}) + (Na_2HPO_4, 0.025 mol kg^{-1}), pH = 6.9}. [c] Acetate buffer {($NaC_2H_3O_2$, 0.1 mol kg^{-1}) + $C_2H_4O_2$, pH = 5.0}. [d] Glycine buffer {($C_2H_5NO_2$, 0.1 mol kg^{-1}) + HCl, pH = 10.0}. [e] Thermodynamic quantities for this reaction could not be determined since a precipitate formed when this buffer was used. [f] K and/or $\Delta_r H°$ for this reaction were too small to measure with the titration calorimeter.

NaH_2PO_4 (m = 0.24 mol kg^{-1}, pH = 5.13) was titrated into aqueous solutions of α- and β-cyclodextrin (m = 0.0032 mol kg^{-1}). The predominant ion at pH = 5.13 is $H_2PO_4^-$. The result of these two control experiments, when corrected for the enthalpy of dilution of this same NaH_2PO_4 solution into pure water, was that there was no measurable interaction of the predominant $H_2PO_4^-$ ion with either of the cyclodextrins. A similar control experiment was also done in which Na_2HPO_4 (m = 0.19 mol kg^{-1}, pH = 8.15) was titrated into solutions of α- and β-cyclodextrin (m = 0.0032 mol kg^{-1}). Again, it was found that there was no measurable interaction of the predominant HPO_4^{2-} ion with either of the cyclodextrins. Upon the basis of these findings, we identify the thermodynamic quantities obtained for the reactions of these cyclodextrins in phosphate buffer as those that would be obtained with a dilute aqueous solution that contains no buffer. However, it is possible (but unlikely) that the standard molar enthalpies of reaction of both $H_2PO_4^-$ and HPO_4^{2-} with α- and β-cyclodextrin are close to zero but that these two ions still bind to these cyclodextrins.

A modified series of control experiments was performed with the acetate buffer. In the first series, a solution containing $NaC_2H_3O_2$ (m = 0.48 mol kg^{-1}) was dissolved in phosphate buffer (m = 0.005 mol kg^{-1}, pH = 6.9). The predominant species at this pH is $C_2H_3O_2^-$. This solution was then titrated into aqueous solutions of α- and β-cyclodextrin (m = 0.0038 mol kg^{-1}) dissolved in the same phosphate buffer used for the $NaC_2H_3O_2$. The result of these two control experiments, when corrected for the enthalpy of dilution of this same $NaC_2H_3O_2$ solution into the phosphate buffer, was that there was no measurable interaction of the (predominant) acetate ion $C_2H_3O_2^-$ with either of the cyclodextrins. In the second series, a solution of $NaC_2H_3O_2$ (m = 0.45 mol kg^{-1}) in glycine buffer (m = 0.10

mol kg^{-1}, pH = 2.2) was used. The predominant species at this pH is $C_2H_4O_2^0$. This solution was titrated into a solution containing α-cyclodextrin (m = 0.0045 mol kg^{-1}) in this same glycine buffer. This experiment, after correction for the enthalpy of dilution of the acetic acid−glycine buffer solution into glycine buffer, yielded the following results for the 1:1 binding of $C_2H_4O_2^0$ with α-cyclodextrin at T = 298.15 K: $K \approx 13$ and $\Delta_r H° \approx -21$ kJ mol^{-1}. Since cyclodextrins hydrolyze slowly in acidic (pH = 2) aqueous solution,[20] the thermal effect due to the interaction of α-cyclodextrin with $C_2H_4O_2^0$ was superimposed on the thermal effect due to the hydrolysis of the α-cyclodextrin and the results are considered to be approximate. However, they do demonstrate that there is a significant interaction of $C_2H_4O_2^0$ with α-cyclodextrin. This interaction with the acetate buffer is understood when one considers that hydrophobic groups generally lead to interactions with α- and β-cyclodextrin and that there is a hydrophobic alkyl group in $C_2H_4O_2^0$. That the neutral acetic acid species $C_2H_4O_2^0$ shows such an interaction while the acetate ion $C_2H_3O_2^-$ does not is consistent with the general trend found in the literature (see Table II in ref 13) that, as a rule, the presence of a charge on a ligand correlates with a smaller equilibrium constant for the reaction of that ligand with either α- or β-cyclodextrin than for the corresponding reaction of the uncharged ligand.

In a similar experiment, $NaC_2H_3O_2$ (m = 0.45 mol kg^{-1}) in glycine buffer (m = 0.10 mol kg^{-1}, pH = 2.2) was titrated into β-cyclodextrin (m = 0.0045 mol kg^{-1}) dissolved in the same glycine buffer. The result was that there was no measurable interaction of $C_2H_4O_2^0$ with β-cyclodextrin. Therefore, K and/or $\Delta_r H°$ for the binding reaction are small. We estimate that $\Delta_r H°$ is approximately −4 kJ mol^{-1} for the 1:1 binding reaction of $C_2H_4O_2^0$ with β-cyclodextrin. This estimate is obtained from the approximate value of −21 kJ mol^{-1} for the standard molar enthalpy for the reaction of $C_2H_4O_2^0$ with α-cyclodextrin and the value of ≈17 kJ mol^{-1} found (see Figure 2 in ref 12) for the difference between the standard molar enthalpies of reactions of the straight-chain alcohols with α- and β-cyclodextrin. Furthermore, in most cases, the differences (see Table 2) between the standard molar enthalpies for the reaction of a given ligand with β-cyclodextrin using these two buffers are small (<2 kJ mol^{-1}). Thus, there is good evidence that the standard molar enthalpy for the 1:1 binding reaction of $C_2H_4O_2^0$ with β-cyclodextrin is small. However, as can be seen from Table 2, there are generally significant differences between the standard molar Gibbs energies for the reaction of a given ligand with β-cyclodextrin using these two buffers. Thus, although the enthalpic interaction between $C_2H_4O_2^0$ and β-cyclodextrin appears to be small, there is excellent evidence for the existence of a binding interaction between $C_2H_4O_2^0$ and both α- and β-cyclodextrin. The origin of this interaction can be attributed to the presence of a hydrophobic alkyl group in $C_2H_4O_2^0$ and its interaction with β-cyclodextrin.

NMR. The complexations of the following ligands by α-, β-, and γ-cyclodextrin were examined with NMR: phenethylamine$^+$, L-α-*O*-benzylglycerol^0, 1-benzylimidazole^0, 4-benzylpiperidine$^+$, 1-butylimidazole^0, (1*S*,2*S*)-(+)-pseudoephedrine$^+$, (1*S*,2*R*)-(+)-ephedrine$^+$, (1*R*,2*S*)-(−)-ephedrine$^+$, hydrocinnamate$^-$, phenyl-β-D-glucopyranoside^0, 3-phenyl-1-propylamine$^+$, and tyramine$^+$. A sequence of typical NMR spectra for the complexation of (1*S*,2*R*)-(+)-ephedrine$^+$ with β-cyclodextrin at varying concentration ratios of (1*S*,2*R*)-(+)-ephedrine to cyclodextrin (i.e., guest to host or [G]/[H] ratio) is shown in Figure 2. It is seen that the addition of ligands leads to variable degrees of changes in the chemical shifts Δδ of both the ligand and the cyclodextrin protons.

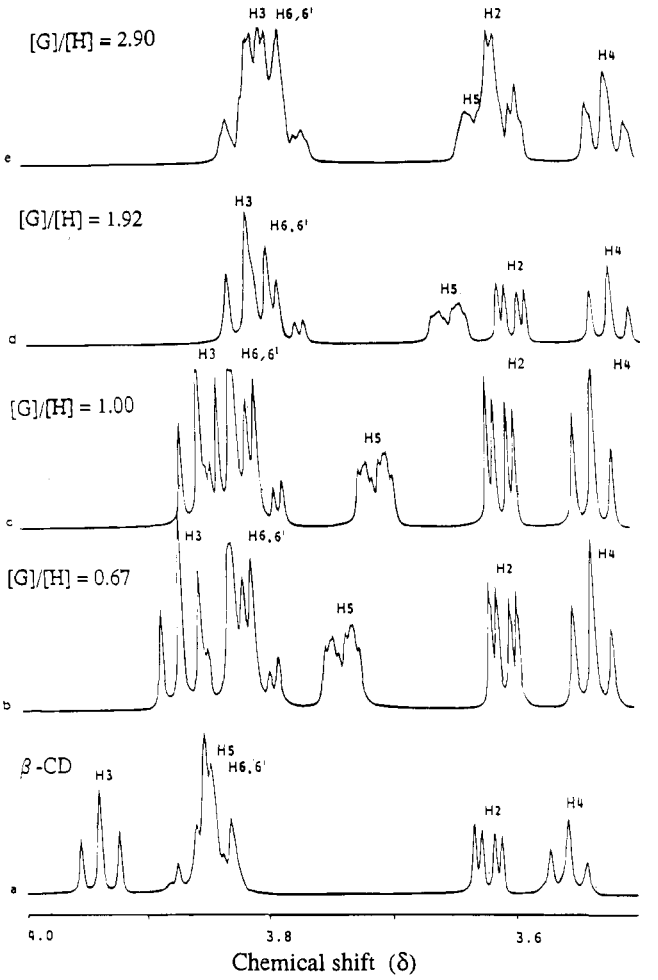

Figure 2. Typical proton NMR spectra at a variety of guest to host ([G]/[H]) ratios for the complexation of (1*S*,2*R*)-(+)-ephedrine$^+$ with β-cyclodextrin in buffered aqueous solutions at pD = 7.0 and T = 298.15 K. Note the large upfield shifts of H3 and H5 in sharp contrast to the small shift changes observed for H2, H4, or H6,6'.

The values of Δδ are critical functions of the position in the molecule and the cavity size of the cyclodextrin as well as the [G]/[H] ratio. In general, the inclusion of an aromatic ligand in the cyclodextrin cavity caused major upfield shifts of the H3 and/or the H5 protons located inside the cyclodextrin cavity. This is attributable to the ring current of the aromatic portion of the molecule that is included in the cavity. The other cyclodextrin protons located outside the cavity showed negligible or only trivial changes. Since the values of Δδ for the cyclodextrin protons obviously increase with increasing ligand concentration and those for the ligand protons decrease with increasing ligand concentration, the variations of Δδ for a variety of cyclodextrin−ligand combinations will be considered at a fixed [G]/[H] ratio near unity; the values of Δδ are given in Table 3. The values of Δδ for all cyclodextrin (H1−H6,6') and ligand protons are shown for each ligand in Figures A2a−A13a and A2b−A13b, which are included in the supporting information deposited with this article.

Discussion

The cyclodextrins are truncated right cylindrical cone shaped molecules 7.9 × 10^{-8} cm high with a hollow tapered central cavity. The top and bottom dimensions of the cavity for α-cyclodextrin (cyclomaltohexaose) are 5.3 × 10^{-8} and 4.7 × 10^{-8} cm, respectively, and those for β-cyclodextrin (cyclomaltoheptaose) are 6.5 × 10^{-8} and 6.0 × 10^{-8} cm, respec-

Table 3. Chemical Shift Changes ($\Delta\delta$) of Cyclodextrin's H3 and H5 Protons upon Complexation with Selected Ligands in Buffered Aqueous Solutions at pD = 7.0 and T = 298.15 K[a]

	α-cyclodextrin ($\Delta\delta$)				β-cyclodextrin ($\Delta\delta$)				γ-cyclodextrin ($\Delta\delta$)			
ligand	[G]/[H]	H3	H5	H5/H3	[G]/[H]	H3	H5	H5/H3	[G]/[H]	H3	H5	H5/H3
phenethylamine$^+$	1.04	0.04	0.00	0.0	0.94	0.03	0.06	2.0				
L-α-*O*-benzylglycerol^0	0.94	0.05	0.00	0.0	0.59	0.03	0.09	3.0	0.62	0.01	0.03	3.0
1-benzylimidazole^0	0.85	0.08	0.03	0.4	0.77	0.09	0.18	2.0	0.78	0.02	0.04	2.0
4-benzylpiperidine$^+$	0.71	0.07	0.00	0.0	0.94	0.14	0.22	1.6	1.03	0.03	0.05	1.7
1-butylimidazole^0	0.76	0.08	0.04	0.5	0.95	0.05	0.09	1.8				
(1*S*,2*S*)-(+)-pseudoephedrine$^+$	1.00	0.06	0.00	0.0	0.98	0.07	0.12	1.7				
(1*S*,2*R*)-(+)-ephedrine$^+$	0.79	0.04	0.00	0.0	1.00	0.07	0.12	1.7				
(1*R*,2*S*)-(−)-ephedrine$^+$	0.83	0.04	0.00	0.0	0.97	0.06	0.10	1.7				
hydrocinnamate$^-$	1.03	0.05	−0.03	−0.5	0.92	0.04	0.10	2.5				
phenyl-β-D-glucopyranoside^0	0.91	0.04	0.00	0.0	0.81	0.02	0.05	2.5				
3-phenyl-1-propylamine$^+$	0.58	0.04	0.00	0.0	1.03	0.10	0.12	1.2	0.90	0.01	0.03	0.3
tyramine$^+$					0.80	0.05	0.07	1.4				

[a] The quantity [G]/[H] is the ratio of the concentration of the ligand (the guest) to the concentration of the cyclodextrin (the host).

Table 4. Thermodynamic Quantities K, $\Delta_rH°$, $\Delta_rG°$, and $\Delta_rS°$ from the Literature for the Reactions of α-Cyclodextrin and β-Cyclodextrin with Organic Ligands at T = 298.15 K[a]

ligand	K	$\Delta_rH°$ (kJ mol^{-1})	$\Delta_rG°$ (kJ mol^{-1})	$\Delta_rS°$ (J K^{-1} mol^{-1})	ref
	α-Cyclodextrin(sln) + Ligand(sln) = α-Cyclodextrin·Ligand(sln)				
phenethylamine	26.4 ± 0.4				Wong et al. (1983)
hydrocinnamic acid	1260 ± 300	−31.4 ± 0.4	−17.7 ± 0.7	−46 ± 3	Lewis and Hansen (1973)
	β-Cyclodextrin(sln) + Ligand(sln) = β-Cyclodextrin·Ligand(sln)				
hydrocinnamic acid	200				Pauli and Lach (1965)

[a] All reactions were carried out in aqueous solutions. The pHs at which the reactions occurred were not specified.

tively.[36] The most likely mode of binding consists of insertion of the less polar portion of the ligand into the cavity with the charged group of the ligand remaining solvent exposed at the wide top end of the cavity. The binding reaction involves the rearrangement of the solvent (water) in both the cyclodextrin and ligand and interactions (e.g., van der Waals, hydrogen bonding) between the two species. The resultant of all these processes is described by the thermodynamic quantities.

Thermodynamic Results. Inspection of Tables 1 and 2 shows that the values of $\Delta_rS°$ for all of the reactions involving α-cyclodextrin are negative and that the values of $\Delta_rS°$ for the reactions involving β-cyclodextrin are either negative or small. Also, the values of $\Delta_rH°$ are all negative with the exception of the approximate value for the reaction of *n*-hexyl-β-D-glucopyranoside with β-cyclodextrin, which is very close to zero. Therefore, these reactions are "enthalpy driven" at T = 298.15 K. We have also constructed plots of the standard molar enthalpy of reaction as a function of the standard molar entropy of reaction. From the plots of $\Delta_rH°$ vs $\Delta_rS°$ for the reactions involving α- and β-cyclodextrin, we obtain respective slopes of 239 ± 40 K and 254 ± 32 K. The respective intercepts are −9.3 ± 1.6 and −10.2 ± 0.7 kJ mol^{-1}. Following Bertrand et al.[11] and our earlier practice,[8,12] we have also compared the differences in thermodynamic quantities for the exchange reactions:

$$\text{α-cyclodextrin·ligand(sln)} + \beta\text{−cyclodextrin(sln)} = \text{α-cyclodextrin(sln)} + \beta\text{−cyclodextrin·ligand(sln)} \quad (3)$$

These quantities are obtained by combination of the appropriate thermodynamic quantities for the reactions of the various ligands with α- and β-cyclodextrin. From a plot of $\Delta_rH°$ vs $\Delta_rS°$ for these exchange reactions, we obtain a slope of 251 ± 30 K and an intercept of −1.9 ± 1.2 kJ mol^{-1}. All of these slopes are smaller than the slopes of 274−360 K obtained in earlier studies.[8,11,12]

Comparisons with Earlier Literature. The thermodynamic results in the literature on the reactions of these ligands with α- and β-cyclodextrin are summarized in Table 4. Although these various workers did not report the pHs at which the respective reactions occurred, it appears likely that, under the experimental conditions used, the predominant species of phenethylamine has a charge number of +1 and that hydrocinnamic acid has a charge number of −1. On this basis, the result of K = 26.4 ± 0.4 of Wong et al.[15] for the reaction of phenethylamine with α-cyclodextrin, obtained with a potentiometric method, is twice as large as the result obtained in this study. The literature contains two results for the reaction of hydrocinnamic acid with α- and β-cyclodextrin. Lewis and Hansen[37] reported K = 1260 ± 300 for the reaction of hydrocinnamic acid with α-cyclodextrin and Pauli and Lach[38] gave K = 200 for the reaction of hydrocinnamic acid with β-cyclodextrin. This latter result is comparable with the result of K = 141 ± 3 obtained herein. However, the result of Lewis and Hansen for the reaction of hydrocinnamic acid with α-cyclodextrin differs greatly from the result of K = 31.3 ± 0.6 obtained in this study. Lewis and Hansen also reported $\Delta_rH°$ = −31.4 ± 0.4 kJ mol^{-1} for this reaction, a result that is substantially different from our value of −15.5 ± 0.2 kJ mol^{-1}. It does not seem possible to arrive at a definitive explanation for the differences between our results and those in Table 4. However, we believe that the results obtained[12] on test reactions such as the hydrolysis of sucrose and the reactions of cyclohexanol and 1-hexanol with α-cyclodextrin as well as the careful characterization of the substances used in this study lend confidence to the accuracy of the results given herein.

Correlation with Structural Features. The ligands used in this study were selected so as to allow us to examine how several structural features might affect the thermodynamics of complexation reactions of cyclodextrins. The features examined are (1) the number of CH_2 groups separating the charge at the amino group and the aromatic ring on the ligand, (2) steric effects, (3) the presence of an additional functional group (amino, hydroxy, methoxy, and methyl) attached to position 4

(36) Li, S.; Purdy, W. C. *Chem. Rev.* **1992**, *92*, 1457−1470.

(37) Lewis, E. A.; Hansen, L. D. *J. Chem. Soc., Perkin Trans. 2* **1973**, 2081−2085.

(38) Pauli, W. A.; Lach, J. L. *J. Pharm. Sci.* **1965**, *54*, 1745−1750.

in the aromatic ring, (4) the presence and location of hydroxy group(s) on the ligand, (5) changes in the chirality of the ligand, and (6) the flexibility of the organic molecules attached to the aromatic ring. In the discussion to follow, some detailed comparisons are made between the thermodynamic quantities for these reactions and the structural features of the various ligands. These comparisons neglect contributions due to the solvation of the reactants and product. Nevertheless, a reasonably consistent picture of the trends in the results is obtained. All comparisons of thermodynamic quantities that follow refer to $T = 298.15$ K and to the reactions occurring in the noninteracting phosphate buffer.

Separation of Charge from the Aromatic Ring. First, we shall examine how the thermodynamics of the complexation reactions correlate with the number of alkyl groups separating the charge at the amino group and the aromatic ring on the ligand. The CH_2 increment in the values of $\Delta_r G°$ for the reactions of phenethylamine$^+$ (two CH_2 groups) and 3-phenyl-1-propylamine$^+$ (three CH_2 groups) with β-cyclodextrin is -3.7 kJ mol^{-1}. The substance 4-benzylpiperidine$^+$, in which six CH_2 groups (five in a cyclic structure) are located between the charge at the amino group and the aromatic ring, has a still more negative value of $\Delta_r G°$ for its reaction with β-cyclodextrin than do phenethylamine$^+$ and 3-phenyl-1-propylamine$^+$. The value of -3.7 kJ mol^{-1} for the CH_2 increment is in the range of values of $\Delta_r G°$ for the transfer of CH_2 groups from liquid organic phases to water (-3 to -4 kJ mol^{-1}) which is considered to be typical for hydrophobic interactions.[9,39] Similar CH_2 increments have been reported[7-9,40] for reactions of straight-chain alcohols and diols with both α- and β-cyclodextrin. However, the CH_2 increment is reduced to -1.0 to -1.5 kJ mol^{-1} for the reactions of phenethylamine$^+$, 3-phenyl-1-propylamine$^+$, and 4-benzylpiperidine$^+$ with α-cyclodextrin. A possible explanation for this reduction in the value of the CH_2 increment is that the α-cyclodextrin cavity is smaller than the β-cyclodextrin cavity. Thus, the steric effects due to the presence of the aromatic ring and, for 4-benzylpiperidine$^+$, a cyclohexane ring, in the α-cyclodextrin cavity may not allow for the complete hydrophobic interaction found for the corresponding reactions of these ligands with β-cyclodextrin and of less bulky ligands with α-cyclodextrin.[7-9,40]

We now compare the thermodynamic quantities for the reactions of *n*-hexyl-β-D-glucopyranoside and of 1-hexanol with α-cyclodextrin. For the reaction of *n*-hexyl-β-D-glucopyranoside with α-cyclodextrin, $K = 839 \pm 35$ and $\Delta_r H° = -18.5 \pm 0.3$ kJ mol^{-1}. For the reaction of 1-hexanol with α-cyclodextrin, $K = 871 \pm 46$ and $\Delta_r H° = -18.5 \pm 0.4$ kJ mol^{-1};[12] the values of the thermodynamic quantities are the same within their experimental errors. These results are consistent with the view that only the *n*-hexyl part of these substances is included in the α-cyclodextrin cavity and that the glucopyranose part of the *n*-hexyl-β-D-glucopyranoside is excluded from the α-cyclodextrin cavity due to steric effects. Pursuing this idea further, it is seen that the equilibrium constants for the reactions of L-α-*O*-benzylglycerol with α- and β-cyclodextrin are larger than the corresponding equilibrium constants for the reactions involving phenyl-β-D-glucopyranoside, particularly in the case of the reaction with β-cyclodextrin. These results are also consistent with the idea that the glucopyranose part of the phenyl-β-D-glucopyranoside is also excluded from the α-cyclodextrin cavity. Thus, the cyclodextrin complexes formed with L-α-*O*-benzylglycerol appear to be stabilized by the presence of hydrophobic CH_2 groups and by OH groups; the latter groups can hydrogen bond to the cyclodextrin cavity. However, this type of stabilization is not possible with the cyclodextrin complexes formed with phenyl-β-D-glucopyranoside.

Effect of Para-Substituted Functional Groups. Tables 1 and 2 contain thermodynamic quantities for the reactions of several substances that differ from phenethylamine$^+$ only in the presence of an additional functional group attached to position 4 in the aromatic ring. These functional groups are amino, hydroxy, methoxy, and methyl. The respective substances that contain these groups are 2-(4-aminophenyl)ethylamine$^+$, tyramine$^+$, 4-methoxyphenethylamine$^+$, and 4-methylphenethylamine$^+$. The order of the values of the equilibrium constants for the formation of these complexes of these substances with α-cyclodextrin is $CH_3 > OCH_3 >$ phenethylamine$^+ >$ OH $> NH_2$; for β-cyclodextrin, the order is $OCH_3 > CH_3 >$ OH $> NH_2 >$ phenethylamine$^+$. The order of the values for the standard molar enthalpies of reaction with α-cyclodextrin is OH $< OCH_3 < CH_3<$ phenethylamine$^+$; for β-cyclodextrin, the order is OH $< NH_2 < OCH_3 < CH_3 \sim$ phenethylamine$^+$. The order of the values for the standard molar entropies of reaction with α-cyclodextrin is OH $< OCH_3<$ phenethylamine$^+ < CH_3$; for β-cyclodextrin, the order is OH $< NH_2<$ phenethylamine$^+$ $< OCH_3 < CH_3$. The amino group is not included in the series for the standard molar enthalpy and standard molar entropy of reaction with α-cyclodextrin because neither the equilibrium constant nor the standard molar enthalpy of reaction could be measured for the reaction of 2-(4-aminophenyl)ethylamine$^+$ with α-cyclodextrin. The OH group, and to a lesser extent the NH_2 group, provides a significant enthalpy stabilization for the ligand–cyclodextrin complex. However, this stabilization is offset by unfavorable entropy changes. Also, the values of $\Delta_r H°$ for the reaction of all of these substances with α-cyclodextrin are less than the values of $\Delta_r H°$ for the corresponding reactions with β-cyclodextrin. However, the values of $\Delta_r S°$ for the reaction of all of these substances are also less than the values of $\Delta_r S°$ for the corresponding reactions with β-cyclodextrin. These differences in the values of $\Delta_r S°$ more than compensate for the differences in the values of $\Delta_r H°$. Therefore, the equilibrium constants for all of these reactions with α-cyclodextrin are less than the corresponding equilibrium constants for the reactions with β-cyclodextrin.

Hydroxyl Groups. We now consider more fully the role of the OH groups and hydrogen bonding in the formation of these complexes. The most direct comparison is made between the standard molar enthalpies for the reactions of tyramine$^+$, which has an OH group in position 4, and phenethylamine$^+$, which does not contain an OH group, with both α- and β-cyclodextrin. The differences are -8.2 ± 1.6 and -7.4 ± 0.4 kJ mol^{-1}, respectively. We take an approximate difference of -8 kJ mol^{-1} as the effect due to the addition of an OH group to the aromatic ring in these substances. Some comparisons are now made between the standard molar enthalpies of reaction for the reactions with β-cyclodextrin of various pairs of substances that differ only in the presence or absence of an OH group. Such pairs of substances are 4-*O*-methyldopamine$^+$ + 4-methoxyphenethylamine$^+$, 3-hydroxytyramine$^+$ + tyramine$^+$, and 3-*O*-methyldopamine$^+$ + 3-methoxyphenethylamine$^+$. The respective differences in the standard molar enthalpies of reaction for these pairs are -5.3 ± 0.2, -2.7 ± 0.3, and $+6.0 \pm 1.8$ kJ mol^{-1}. Thus, simple additivity comes close to working only for the first pair of substances. The difference obtained for the pair 3-*O*-methyldopamine$^+$ + 3-methoxyphenethylamine$^+$ is strikingly different from the others and is indicative of some structural difference(s) vis-á-vis the other pairs of substances.

(39) Tanford, C. *The Hydrophobic Effect*; Wiley-Interscience: New York, 1980.

(40) Rymdén, R.; Carlfors, J.; Stilbs, P. *J. Inclusion Phenom.* **1983**, *1*, 159–167.

A similar approach taken with the standard molar entropies of reaction yields differences ranging from -22 to $+4$ J K^{-1} mol^{-1} for the various pairs of substances considered above. However, the first two of these pairs and the pair tyramine$^+$ + phenethylamine$^+$ have values very close to -18 J K^{-1} mol^{-1}. This value could be considered as a representative difference for the standard molar entropy of reaction due to the addition of an OH group to the aromatic ring in these substances. The outlier in this comparison is the value of $+4$ J K^{-1} mol^{-1} for the pair 3-*O*-methyldopamine$^+$ + 3-methoxyphenethylamine$^+$, which was also the outlier in the comparison made for the standard molar enthalpies of reaction. Again this is indicative of some structural difference(s) vis-á-vis the other pairs of substances. A possibility is that the hydroxyl group in 3-methoxyphenethylamine$^+$ is not located in the β-cyclodextrin cavity.

There is a substantial increase in the equilibrium constants for the formation of the complexes of the ephedrines with β-cyclodextrin in comparison to the equilibrium constant for the complex phenethylamine forms with β-cyclodextrin. Since the standard molar entropies of reaction are comparable, this stabilization is enthalpy driven and ranges from -2 to -6 kJ mol^{-1}. These values are at the low end of the range of values found for the effect of the addition of an OH group to the aromatic ring in such substances as phenethylamine$^+$ and the methoxyphenethylamines$^+$. However, this additional stabilization is not seen in the reactions of the ephedrines with α-cyclodextrin. A possible explanation for this is that these hydrogen bonds cannot be established in the smaller α-cyclodextrin cavity because of steric effects.

Methoxy Groups. Some comparisons can be made of the results involving those substances related to phenethylamine by the addition of one or more methoxy groups. Here, comparison of the results for phenethylamine$^+$, 3-methoxyphenethylamine$^+$, 4-methoxyphenethylamine$^+$, and 2,5-dimethoxyphenethylamine$^+$ shows that the stabilization of the complex formed with α- and β-cyclodextrin due to the presence of a methoxy group is primarily enthalpic, particularly for the reactions of these substances with α-cyclodextrin. The stabilization is most pronounced for the reactions involving 4-methoxyphenethylamine$^+$. There is also some entropic stabilization for the reaction of this substance with β-cyclodextrin. The complex formed between 2,5-dimethoxyphenethylamine$^+$ and β-cyclodextrin is less stable than the respective complexes formed between 3-methoxyphenethylamine$^+$ and 4-methoxyphenethylamine$^+$ with β-cyclodextrin. It is likely that this destabilization arises from a steric effect.

Chirality. A result of this study is that there are significant differences in the thermodynamic quantities for the reactions of the chiral isomers of ephedrine and pseudoephedrine in their reactions with β-cyclodextrin. These differences may also exist for the reactions of these substances with α-cyclodextrin. An enthalpy−entropy compensation effect is also present (see Figure 3). The slopes of the straight lines in this enthalpy−entropy plot are 318 ± 42 and 364 ± 70 K for the reactions of the ephedrines and pseudoephedrines with α- and β-cyclodextrin, respectively. These slopes are in agreement with slopes obtained in earlier studies.[8,11,12] The respective intercepts ($\Delta_r S° = 0$) are -6.6 ± 1.4 and -10.9 ± 0.3 kJ mol^{-1}. It is interesting that the relative order of the ephedrines and pseudoephedrines in the enthalpy−entropy plot is the same for the reactions of these substances with both α- and β-cyclodextrin. Thus, while the results of this study provide some limited answers to the questions concerning the interactions of chiral substances with cyclodextrins, there is a need for additional studies on the reactions of other chiral substances with both cyclodextrins and with other host substances.

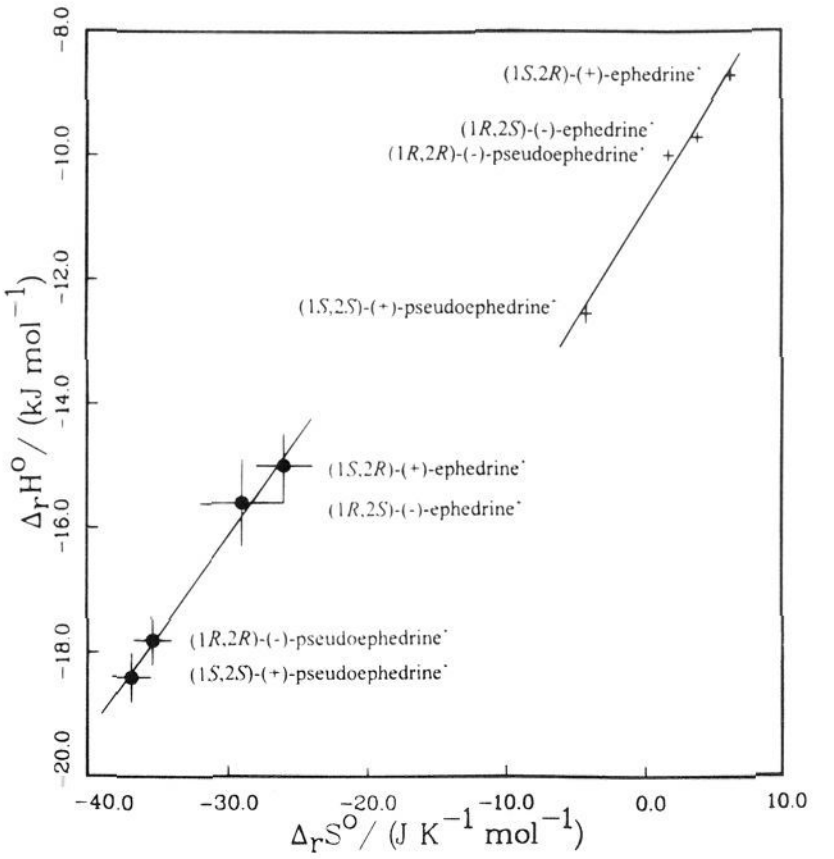

Figure 3. Standard molar enthalpies $\Delta_r H°$ for the reactions of (1*S*,2*R*)-(+)-ephedrine$^+$, (1*R*,2*S*)-(−)-ephedrine$^+$, (1*R*,2*R*)-(−)-pseudoephedrine$^+$, and (1*S*,2*S*)-(+)-pseudoephedrine$^+$ with α-cyclodextrin (●) and with β-cyclodextrin (+) as respective functions of the standard molar entropies $\Delta_r S°$ for these reactions. The straight lines are the least-squares fits to the results. Note that the relative order of the ephedrines and pseudoephedrines in the enthalpy−entropy plot is the same for the reactions of these substances with both α- and β-cyclodextrin.

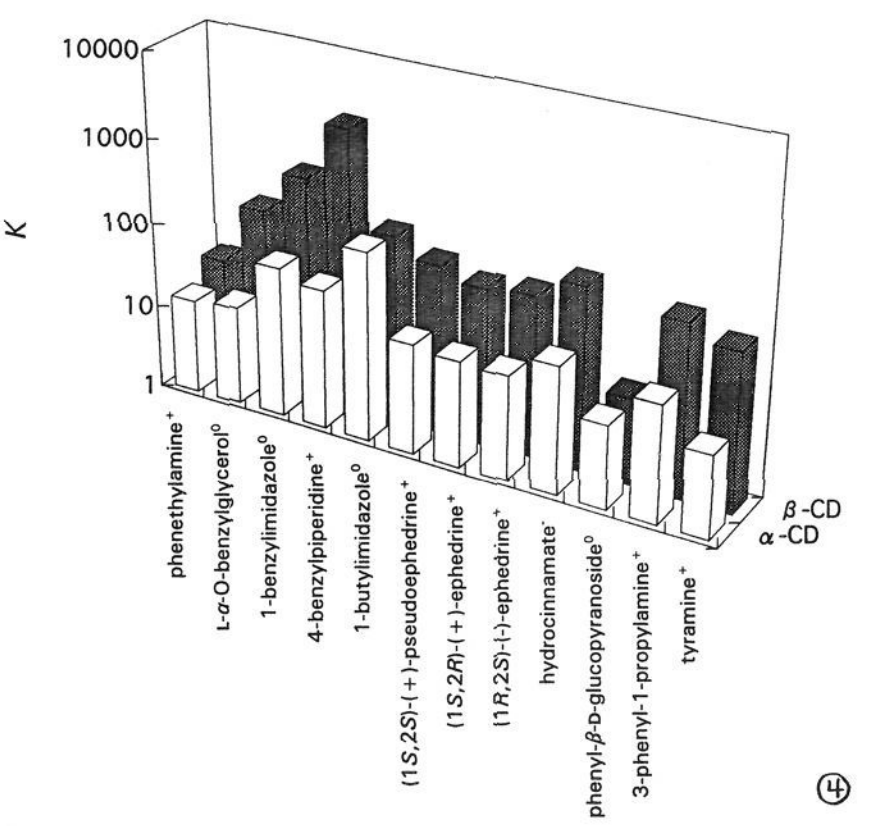

Figure 4. Profiles of equilibrium constants K for the reactions of representative ligands with α- and β-cyclodextrin in buffered aqueous solutions at pH = 6.9 and $T = 298.15$ K. Compare this profile with that of the NMR chemical shift changes ($\Delta\delta$) shown in Figure 5.

Effects of Substituted Imidazoles. Some comparisons of thermodynamic quantities for the reactions of α- and β-cyclodextrin with substances containing the imidazole ring are now made. The value of the equilibrium constant for the reaction of 1-benzylimidazole with β-cyclodextrin is much larger than the value of the equilibrium constant for the reaction of 1-phenylimidazole with β-cyclodextrin. This is primarily an entropic effect. A possible reason for this could be that the

increased flexibility of the 1-benzylimidazole molecule over the 1-phenylimidazole molecule allows for several possible configurations of the former compound in the cyclodextrin cavity. This would lead to a higher entropy for the complex than if the ligand were constrained to have only one configuration. Also, a comparison of the thermodynamic quantities for the reactions of 1-butanol (data for the reactions of this substance were obtained from ref 8) and 1-butylimidazole with both α- and β-cyclodextrin shows that the thermodynamic quantities are quite different from each other. This behavior would be consistent with the view that the imidazole ring participates in the formation of the cyclodextrin complexes. A similar comparison of the results for the reactions of 1-imidazole (data for the reactions of this substance were taken from ref 41) and of 1-phenylimidazole and 1-benzylimidazole with both α- and β-cyclodextrin also shows dissimilar values. Again, this is indirect evidence for the participation of more than the imidazole ring in both 1-phenylimidazole and 1-benzylimidazole in the formation of the cyclodextrin complexes. This matter was dealt with in a more definitive way with ROESY NMR measurements (see below).

NMR. Only the H3 proton, of the seven different α-cyclodextrin protons, shows a significant upfield shift upon complexation. The other cyclodextrin protons show either much smaller or negligible deviations, i.e. $|\Delta\delta| < 0.02$. Benzylimidazole^0 and butylimidazole^0 were the only ligands to cause small but definite upfield shifts of H5 in addition to those of H3. This anomalous upfield shift for α-cyclodextrin is consistent with the inclusion of the imidazole portion of the molecule (which is smaller than the phenyl portion) into the cavity. This is demonstrated by the large values of $\Delta\delta$ of the imidazole protons in benzylimidazole^0 and in butylimidazole^0.

It is also interesting to note that the complexation of hydrocinnamate$^-$ leads to a downfield shift of the H5 proton and an unusual upfield shift of the H2 proton. Usually, the latter proton never deviates from its original position upon inclusion of the other ligands, i.e. $|\Delta\delta| < 0.01$. Therefore, it is inferred that the carboxyl group in hydrocinnamate$^-$ is hydrogen-bonded to the cyclodextrin's peripheral hydroxyl group probably at C2, thus affecting the chemical shift of the H2 proton. Unfortunately, the direct observation of a hydrogen bond is not possible in a D_2O solution with NMR spectroscopy.

On the other hand, complexation of the same series of ligands with β-cyclodextrin produced much larger upfield shifts of both H5 (dominant) and H3 (subordinate) protons as shown in Table 3. The trends in the values of $\Delta\delta$ for γ-cyclodextrin are quite similar to those for β-cyclodextrin but much smaller in intensity. The H3 and H5 protons are positioned inside the cyclodextrin cavity in this sequence from the wider opening of the cyclodextrin cavity, and the observed upfield shifts of these protons are evidently caused by the ring current of the aromatic portion of the ligands. Hence, the magnitude of the upfield shifts, $\Delta\delta$(H3) and $\Delta\delta$(H5), and their relative ratio, $\Delta\delta$(H5)/$\Delta\delta$(H3), may be used respectively as quantitative measures of the complex stability and the depth of inclusion of the ligand into the cavity.

As seen in Table 3, the $\Delta\delta$(H5)/$\Delta\delta$(H3) ratios are typically 0 for α-cyclodextrin and 2 for β- and γ-cyclodextrins. However, the value of $\Delta\delta$(H5) varies, ranging from −0.03 to 0.04, from 0.05 to 0.22, and from 0.03 to 0.05 for α-, β-, and γ-cyclodextrin, respectively. Since the depth and strength of complexation depend critically upon the match of the ligand's size and shape, the small values of $\Delta\delta$(H5)/$\Delta\delta$(H3) and $\Delta\delta$(H5) for

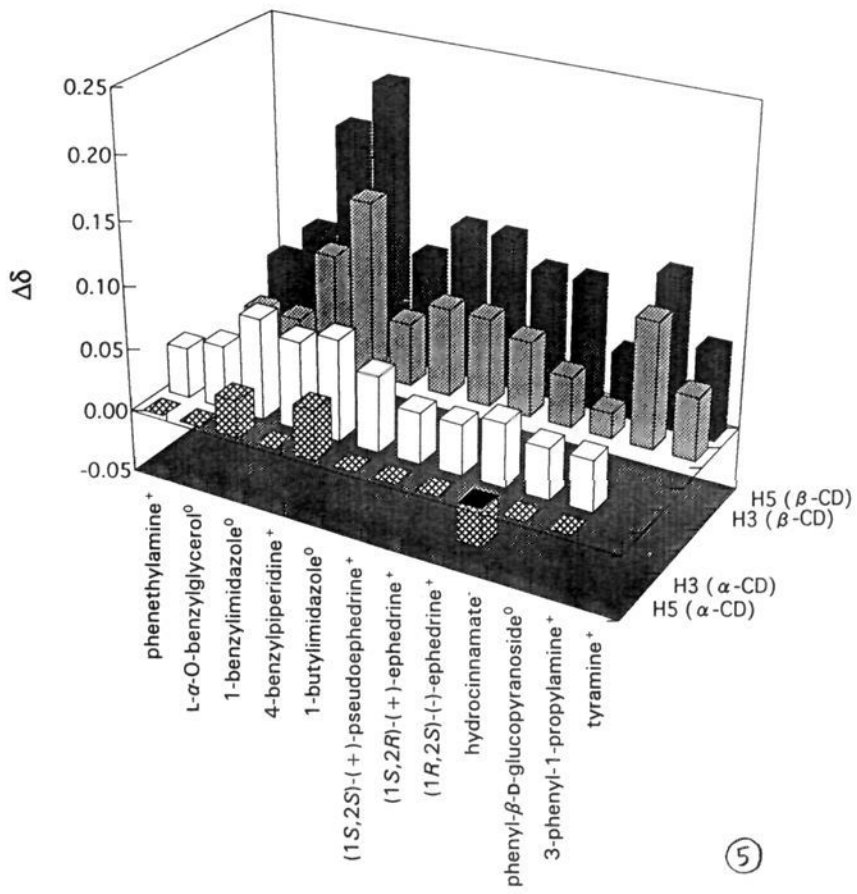

Figure 5. Profiles of the NMR chemical shift changes ($\Delta\delta$) of H3 and H5 protons in α- and β-cyclodextrin upon complexation with representative ligands. Compare this profile with that of the equilibrium constants shown in Figure 4.

α-cyclodextrin clearly indicate that the ligands employed are too large to be fully accommodated in the relatively small cavity of the α-cyclodextrin. Therefore, it appears that these ligands are situated on the edge of the cavity and affect only the nearest H3 protons.

Accordingly, the much larger $\Delta\delta$(H5)/$\Delta\delta$(H3) ratios, which are as high as 1.2−3.0 for β- and γ-cyclodextrin, indicate a much deeper inclusion by these hosts. As judged from the smaller values of $\Delta\delta$(H5) for γ-cyclodextrin, the complexation appears to be tight-fitting with β-cyclodextrin but seems to be loose with γ-cyclodextrin. Therefore, it may be interesting to compare the absolute value of $\Delta\delta$(H5) obtained here with the complex stability constant K or the standard molar Gibbs energies of complexation $\Delta_r G°$ obtained from the titration calorimetry measurements. The values of $\Delta\delta$(H3) and/or $\Delta\delta$(H5) shown in Figure 5 are qualitatively similar to the values of the equilibrium constants shown in Figure 4.

In sharp contrast to most ligand protons that have small values ($|\Delta\delta| < 0.1$) and somewhat puzzling upfield/downfield shifts upon complexation, the imidazole protons of benzylimidazole^0 and butylimidazole^0 show large upfield shifts. This indicates a substantial change in the environment around the imidazole portion of the molecule. Thus, it is suggested that the imidazole portions of benzylimidazole^0 and butylimidazole^0, though fairly hydrophilic, are also included in the cavity, probably in addition to the phenyl group of benzylimidazole^0. Therefore, the inclusion behavior of the phenyl and the imidazole moieties of benzylimidazole^0 with β-cyclodextrin were examined in detail by ROESY measurements. As seen in Figure 6, both the phenyl and the imidazole protons of benzylimidazole^0 display moderate NOE signals upon irradiation at the H3 and the H5 protons of β-cyclodextrin, but the relative NOE intensity of both protons is inverted, depending upon the position of irradiation. Thus, the irradiation at H5 gives a larger NOE for the phenyl portion, whereas that at H3 affords a larger NOE for the imidazole portion. These results indicate that the phenyl portion is accommodated in the cyclodextrin cavity in the proximity of H5 and that simultaneously the imidazole portion is also

(41) Rekharsky, M. V.; Nemykina, E. V.; Eliseev, A. V.; Yatsimirsky, A. K. *Thermochim. Acta* **1992**, *202*, 25−33.

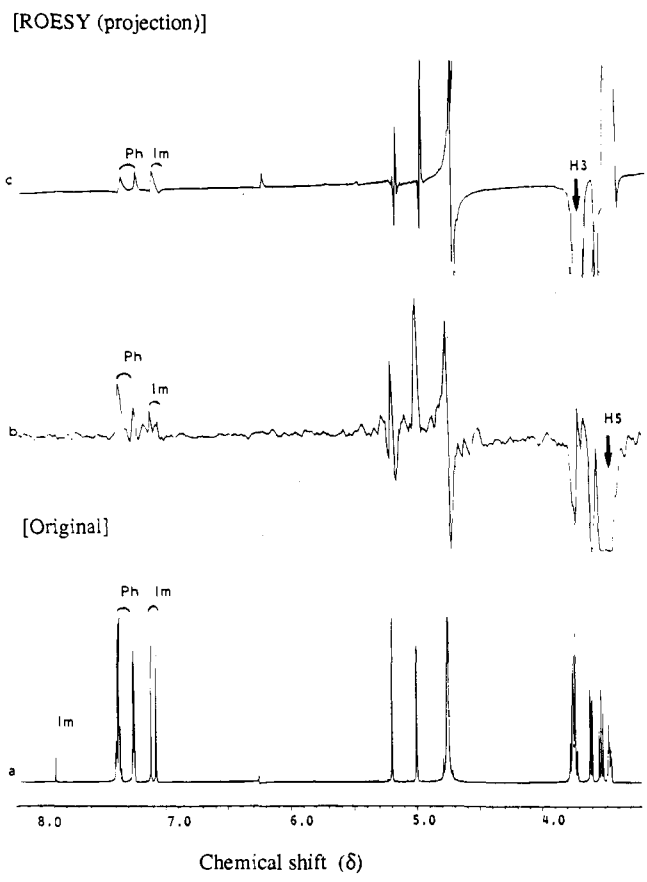

Figure 6. Selective enhancements of the phenyl and imidazole proton signals, respectively, upon irradiation of the H5 and H3 protons of β-cyclodextrin through the intermolecular NOE effect observed for the complexation of 1-benzylimidazole with β-cyclodextrin. The ratio [G]/[H] is 5.5.

perching at the cyclodextrin periphery, stabilizing the phenyl portion's deep insertion into the cavity.

Conclusions

The interactions of phenethylamine, ephedrines, and the related substances studied herein with the cyclodextrins can be qualitatively understood in terms of steric effects, hydrophobic interactions, and hydrogen bonding. The difference in the values of the thermodynamic quantities for the 1:1 binding reactions of these substances with α- and β-cyclodextrin in phosphate and acetate buffers is attributable to the presence of a hydrophobic CH_3 group in the neutral acetic acid molecule and its interaction with the cyclodextrins. Significant differences in the thermodynamic quantities for the reactions of the chiral isomers of ephedrine and pseudoephedrine in their reactions with β-cyclodextrin have been measured. A plot of the standard molar enthalpy vs the standard molar entropy for the reactions of these chiral isomers with α- and β-cyclodextrin is linear; the relative order of the ephedrines and pseudoephedrines in the enthalpy−entropy plot is the same for the reactions of these substances with both α- and β-cyclodextrin. NMR studies demonstrated that the magnitude of the upfield shifts of the cyclodextrin's H3 and H5 protons, $\Delta\delta$(H3) and $\Delta\delta$(H5), and their relative ratio, $\Delta\delta(H5)/\Delta\delta(H3)$, can be used, respectively, as a measure of the complex stability and the depth of inclusion of the ligand into the cavity. The equilibrium constants determined by titration calorimetry correlate well with $\Delta\delta$ determined by NMR.

Acknowledgment. We thank Drs. Robert Ashton and Bruce Coxon for their careful reading of this paper. Y.I. and Y.Y. are grateful to the technical support of the Instrumental Analysis Center, Faculty of Engineering, Osaka University, in measuring the NMR spectra.

Supporting Information Available: Thirteen figures related to the NMR results (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA951456X