Structural Effects on the Binding of Amine Drugs with the Diphenylmethyl Functionality to Cyclodextrins. II. A Molecular Modeling Study

Wei-Qin Tong,[1] John L. Lach,[1,2] Ting-Fong Chin,[1] and J. Keith Guillory[1,3]

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Molecular modeling has been used to study the complexation between α, β, or γ-cyclodextrin (CD) and a group of amine compounds having the diphenylmethyl functionality. The computer program SYBYL 5.3 and the Tripos force field (version 5.2) were used for all the calculations. Three-dimensional structures of 13 amine compounds were built individually from their atoms, and CDs were built based on the X-ray crystallographic coordinates. The diphenylmethyl derivative–CD complexes were constructed and optimized. Based on the calculated binding energies accompanying the inclusion process, the preferred method of approach of the compounds to the cavities of the CD molecules, and the structural effects on the binding between amine compounds and three CDs were explored. The calculated binding energies exhibited a good correlation with the stability constants obtained from solution calorimetric titrations. The present study shows that for similar ligand molecules, the molecular modeling technique should enable us to visualize the structure of the inclusion complexes and will also assist us in determining the ability of a potential drug molecule to form a stable complex with CDs.

KEY WORDS: molecular modeling; computer graphics; molecular mechanics; SYBYL; inclusion complex; diphenylmethyl derivatives; cyclodextrins (CDs).

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides composed of six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units (1). A CD ring is externally hydrophilic and relatively apolar internally. Many drug molecules are capable of residing within the central cavity of a CD molecule, thus forming an inclusion complex. The inclusion complexes formed display interesting properties and may increase the stability and solubility of the guest molecules (2,3).

A number of amine compounds used medicinally bear the diphenylmethyl functionality. Complexation with CDs may increase the aqueous solubility and enhance the stability of some of these diphenylmethyl derivatives.

Studies of complexes of these amine compounds with CDs are very limited. In our previous paper, the titration microcalorimetric results for the binding of some amine drugs with the diphenylmethyl functionality to α-, β-, and γ-cyclodextrins in aqueous solution at 25°C were presented (4). Structural effects on the stability constants, thermodynamics, and inclusion complex geometry were explored based on the solution calorimetric results.

In order to support our explanation and to elucidate further the binding mechanism, the determination of the geometries of inclusion complexes is essential. X-ray crystallography requires the synthesis, isolation, and crystallization of the complex (5). Corey–Pauling–Koltun (CPK) models provide a rapid assessment of the relative space-filling properties of ligand and substrate but cannot provide quantitative information about interatomic forces. For the purpose of our analysis, the application of computational methods to problems in molecular structure seems to offer the best combination of speed and accuracy (6–12).

We present herein the results of the molecular modeling studies on the same series of amine drugs and their complexes with α-, β-, and γ-CDs using the Tripos force field (13). Preliminary results have been reported in the preceding paper in this series.

MATERIALS AND METHODS

General Considerations

Molecular mechanical calculations were performed with the program, SYBYL 5.3 (13), using the Tripos force field (version 5.2), and executed on a Silicon Graphics 4D120GTX Graphics workstation. Energy minimizations were carried out using the MAXIMIN 2 energy minimizer with its default values (13). All structures were optimized until the energy change from one iteration to the next was less than 0.05 kcal/mol.

There is one important limitation to the accuracy of our computations. The calculations do not take into account water molecules. Ignoring the solvent could, among other consequences, cause the complexes to appear more flexible than they are in reality. This is because complexation is driven partly by hydrophobic forces that are not incorporated into our calculations. This omission of solvent, unfortunately, is a traditional limitation of computational chemistry. The addition of water molecules to the calculations will increase the computation time dramatically and is not practical for large molecules such as our complexes at the present time.

The principle goal of our research is to compare the energy gain on the formation of the inclusion complexes for a series of compounds with α-CD, β-CD, or γ-CD. Considering the structural similarity between the ligand compounds, it is reasonable to assume that these compounds bind to CD by a similar mechanism. Therefore, the relative magnitudes of energy gain for the series on complex formation should not be critically dependent upon the presence of water molecules. The inclusion of water molecules in the calculation, however, would change the absolute values of total energy.

The potential energy function, E, of the molecule used in the force field includes stretching, bending, torsion, van der Waals, electrostatic interaction, and constraint (including distance, angle, torsion angle and range constraints)

[1] Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242.
[2] Deceased.
[3] To whom correspondence should be addressed.

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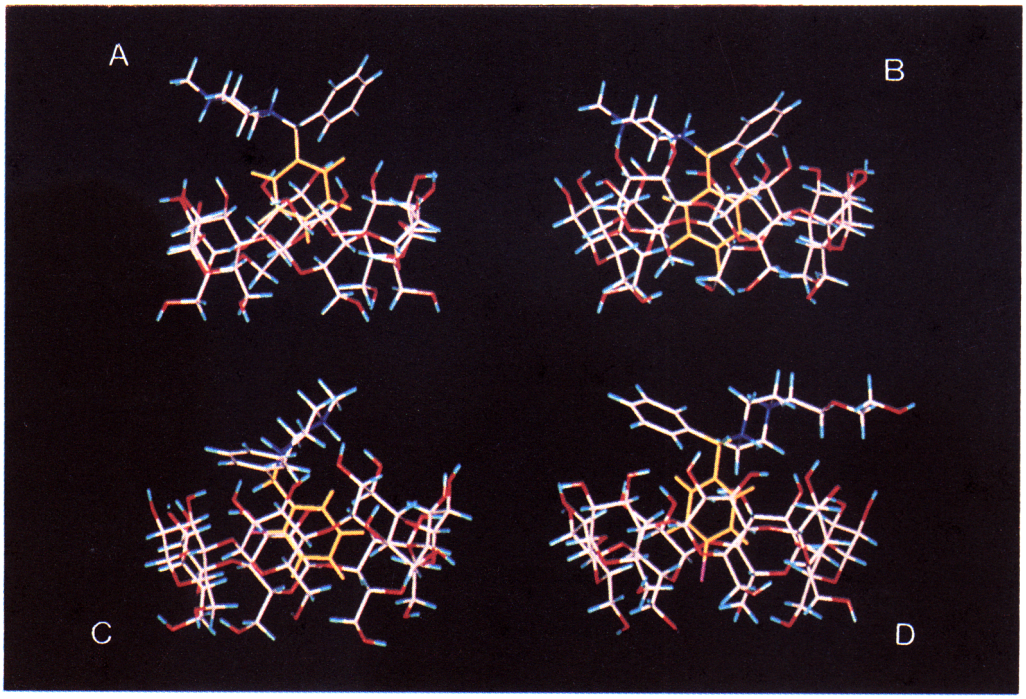

Fig. 1. Computer-generated minimal energy structures. A, cyclizine · 2HCl–α-CD complex; B, cyclizine · 2HCl–β-CD complex; C, chlorcyclizine · 2HCl–β-CD complex; D, hydroxyzine · 2HCl–β-CD complex. Color is coded by atom type: red, O; white, C; cyan, H; blue, N; purple, Cl. Parts of the guest molecules are shown in yellow color for clarity.

terms (14). The charge calculations were performed using the Gast–Hück method, which is a combination of two other charge computation methods: the Gasteiger–Marsili method to calculate the σ component of the atomic charge (15–17) and the Hückel method to calculate the π component of the atomic charge (18,19). *E* is a measure of intramolecular strain relative to a hypothetical situation. By itself *E* has no physical meaning.

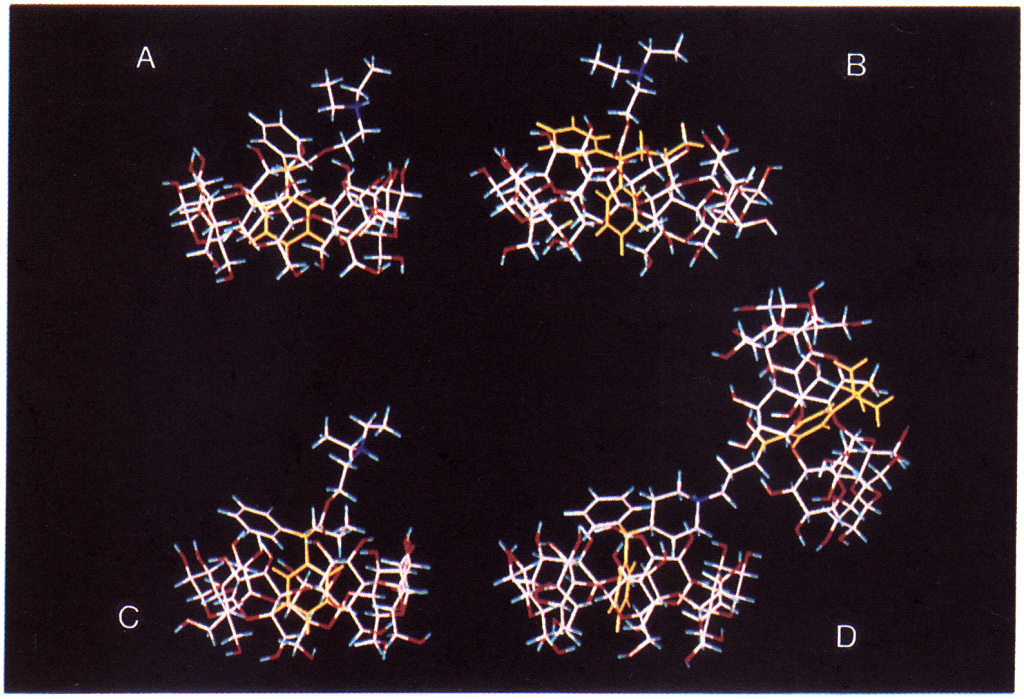

Fig. 2. Computer-generated minimal energy structures. A, adiphenine · HCl–β-CD complex; B, proadifen · HCl–γ-CD complex; C, proadifen · HCl–β-CD complex; D, terfenadine · HCl–β-CD complex (1:2 stoichiometry). Color is coded by atom type: red, O; white, C; cyan, H; blue, N. Parts of the guest molecules are shown in yellow color for clarity.

Building of Individual Amine Compounds and CD Molecules

Three-dimensional structures of all the amine compounds were built individually using the SKETCH function in the small molecule building package of SYBYL. The amine functional groups were protonated prior to calculation. After energy minimization, the SEARCH routine of SYBYL, a conformational search for sterically allowed, low-energy conformations was performed on all molecules. The molecule with the lowest energy conformation was then minimized again using MAXIMIN 2. The resulting structure was taken as the optimized one and was used subsequently for the construction of the inclusion complex. The X-ray structure of adiphenine · HCl was available (20) and was retrieved from the Cambridge data base (21) for comparison purposes. Fairly good agreement was found between the calculated adiphenine · HCl structure and the crystallographic structure.

Detailed X-ray diffraction data have been reported for α-, β-, and γ-CDs (22–26). The structures retrieved through the Cambridge data base were used as the starting point for the optimization procedure. After deleting other molecules in the coordinate system, including the solvent molecules, the initial CD structures were then optimized by MAXIMIN 2.

Construction and Optimization of Inclusion Complexes

The construction of the diphenylmethyl derivative–CD complexes was accomplished by using individual amine compounds and CDs, separately optimized, as building blocks. The ligand and substrate molecules were first put into separate molecule areas but on the same screen, after which they were arranged to the proper position to facilitate docking. Then both molecules were frozen and merged together. The merged structure was considered as the starting geometric arrangement for the designed complex. The complex was then optimized either in batch mode or in interactive mode.

Initially, a rough "distance vs energy" curve for the approach of compounds into the cyclodextrin cavity was estimated. Thus, the energy decreases as the compound enters the cavity until one "pushes too far" and the energy increases dramatically. Calculations were obtained repeatedly near the apparent energy minimum area while randomly rotating the compound relative to the CD. At least four different initial geometries were considered for a given complex. The complex having the lowest relative energy was then located. Finally, a conformation search was performed and the complex with minimum energy was chosen as the preferred complex structure.

In SYBYL there are two ways to reach a global energy minimum: by dynamic run and conformation search (14). In our experience, the dynamic run up to 500 K was not successful because the merged molecules tended to fall apart as the temperature increased.

Therefore, the conformation search was performed in order to locate the energy minimum. There are too many bonds in our complexes to make a rigorous conformational search, however. Therefore, only the bonds linked to the carbon of the methyl group of the diphenylmethyl functionality were set to be rotatable, because the groups directly linked by these bonds were the main structural parts which interacted with the CD cavity. The increment for the rotation angles was set at 5°.

RESULTS AND DISCUSSION

Molecular mechanical calculations were performed on all thirteen compounds and three CDs, as well as compound–CD complexes. Gas-phase binding energies, ΔE's, were defined as $E_{components} - E_{complex}$. The structure which gives the highest binding energy was considered as the final optimized structure for the complex. Apparently, no X-ray structures for complexes between diphenylmethyl derivatives and CDs have been reported.

The structures of diphenylmethyl derivatives basically consist of three portions: two aromatic (or substituted aromatic) substituents and the part containing the amine functional group. From our molecular modeling study, it is clear that only a portion of the ligand molecule can be included within the CD cavity. Furthermore, the entire ligand molecule cannot pass through the cavity of α- or β-CD owing to the steric hindrance of the other two parts. Theoretically all three parts of the molecule could be the potential binding sites.

In the case of complexes formed with α- or β-CD, only one of the aromatic groups from the ligand molecule can fit into the CD cavity at one time. The optimized structures of cyclizine · 2HCl complexes with α- and β-CDs are shown in Figs. 1A and B as examples. The phenyl ring does not fit into

Table I. Results from Molecular Mechanical Calculation and Binding Constants (K) from Titration Calorimetry for the Interactions Between Diphenylmethyl Derivatives and α-Cyclodextrin

Compound	$E_{component}$	$E_{complex}$	ΔE (kcal/mol)	K (M^{-1})	ln K
Diphenidol · HCl	21.75	105.32	24.67	43.8	3.780
Diphenhydramine · HCl	18.01	105.95	20.31	44.3	3.791
Orphenadrine · HCl	18.00	103.13	23.12	35.4	3.567
Bromodiphenhydramine · HCl	19.80	86.42	41.63	1355.7	7.212
Diphenylpyraline · HCl	20.29	104.78	23.75	31.5	3.450
Cyclizine · 2 HCl	57.85	139.89	26.20	48.4	3.879
Chlorcyclizine · 2 HCl	58.54	127.39	39.39	1116.6	7.018
Meclizine · 2 HCl	51.59	118.41	41.42	865.0	6.763
Hydroxyzine · 2 HCl	58.39	129.90	36.73	1175.4	7.069
Proadifen · HCl	21.93	103.95	26.23	184.2	5.216
Adiphenine · HCl	19.08	104.61	22.71	11.5	2.442
α-Cyclodextrin	108.24	—	—	—	—

Table II. Results from Molecular Mechanical Calculation and Binding Constants from Titration Calorimetry for the Interactions Between Diphenylmethyl Derivatives and β-Cyclodextrin

Compound	$E_{component}$	$E_{complex}$	ΔE (kcal/mol)	K (M^{-1})	ln K
Diphenidol · HCl	21.75	111.45	27.79	936.9	6.843
Diphenhydramine · HCl	18.01	103.63	31.87	1148.5	7.046
Orphenadrine · HCl	18.00	99.65	35.84	1282.0	7.156
Bromodiphenhydramine · HCl	19.80	95.48	41.81	2156.1	7.676
Diphenylpyraline · HCl	20.29	93.27	44.51	2252.0	7.720
Cyclizine · 2 HCl	57.85	142.39	32.95	1215.2	7.103
Chlorcyclizine · 2 HCl	58.54	131.20	44.83	2461.9	7.809
Meclizine · 2 HCl	51.59	129.17	39.91	2404.5	7.713
Hydroxyzine · 2 HCl	58.39	136.28	39.60	2316.7	7.748
Proadifen · HCl	21.93	110.82	28.61	914.4	6.818
Adiphenine · HCl	19.08	97.46	39.11	2750.4	7.920
β-Cyclodextrin	117.49	—	—	—	—

the α-CD cavity as deeply as into the β-CD cavity. Apparently, the phenyl ring cannot fit into the narrowest ring formed by six H (C-5) in the interior of α-CD. In other words, there is an energy barrier which prevents the aromatic ring from further penetrating into the α-CD cavity. On the other hand, the phenyl group can fit into a β-CD cavity very easily. The aromatic ring is located approximately in the center of the CD cavity. The ring appears to be tilted over to a certain extent, as can be seen from the complex structure. This tilting is necessary in order to reduce the repulsion between another aromatic ring in the diphenylmethyl functional group and the CD ring.

In the case of complexes formed with γ-CD, it is possible that both aromatic rings of the ligand molecules could fit into the CD cavity. However, the structure with one phenyl ring tilted into the CD cavity and the other ring partially in the cavity also gave similar binding energy. Therefore, some uncertainty exists in determining the final optimized structures of complexes with γ-CD. An example of an optimized complex is given in Fig. 2B.

Some of the diphenylmethyl derivatives in our series have halogen or methyl substituents on one of the aromatic rings. The complex structures in which the substituted aromatic ring resides in the CD cavity gave higher binding energies compared to the structures having the unsubstituted benzene ring in the CD cavity. These results are consistent with our findings in calorimetric titrations that substituents on the aromatic ring enhanced the binding. The calculated structures of chlocyclizine · 2HCl and hydroxyzine · 2HCl complexes with β-CD are shown in Figs. 1C and D as examples of these cases.

Apparently, all compounds approach the CD cavity preferentially from the more open side of CD. Insertion of one of the aromatic rings into the CD cavity from the smaller aperture of the CD results in more contact between the other parts of the ligand molecules and the primary hydroxyl groups. The latter groups are more hydropilic in comparison with the apolar interior the CD cavity. Also, our calculations show that it is energetically unfavorable to fit a protonated amine group into the CD cavity.

In the case of the terfenadine · HCl–β-CD complex, the value of ΔE increases from 31.89 to 68.42 kJ/mol upon the addition of a second CD molecule. For cinnarizine · 2HCl, this ΔE increase is from 31.91 to 60.02 kJ/mol. It is clear, as shown in Fig. 2D, that no van der Waals repulsion exists between the two β-CD molecules in the terfenadine · HCl–β-CD complex. The additional favorable interaction between the second β-CD and the aromatic ring at the other end of the terfenadine · HCl or cinnarizine · HCl molecule results in enhanced stability. These results strongly support the findings that these two compounds form 1:2 (compound:β-CD) complexes with β-CD.

Table III. Results from Molecular Mechanical Calculations and Binding Constants (K) from Titration Calorimetry for the Interactions Between Diphenylmethyl Derivatives and γ-Cyclodextrin

Compound	$E_{component}$	$E_{complex}$	ΔE (kcal/mol)	K (M^{-1})	ln K
Diphenidol · HCl	21.75	121.38	25.09	373.5	5.923
Diphenhydramine · HCl	18.01	118.36	30.38	—	—
Orphenadrine · HCl	18.00	118.57	30.15	964.3	6.871
Bromodiphenhydramine · HCl	19.80	108.78	41.74	2430.3	7.796
Diphenylpyraline · HCl	20.29	125.74	25.27	777.7	6.656
Cyclizine · 2 HCl	57.85	158.00	30.57	731.9	6.596
Chlorcyclizine · 2 HCl	58.54	157.26	32.01	1108.5	7.011
Meclizine · 2 HCl	51.59	149.36	32.95	843.0	6.737
Hydroxyzine · 2 HCl	58.39	157.29	31.82	1307.1	7.176
Proadifen · HCl	21.93	124.02	28.64	1125.2	7.026
Adiphenine · HCl	19.08	126.24	23.56	404.9	6.004
γ-Cyclodextrin	130.72	—	—	—	—

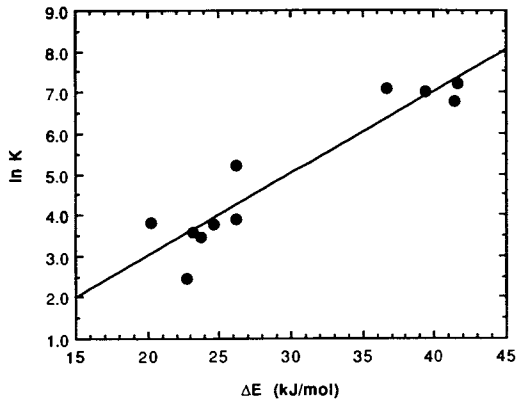

Fig. 3. Plot of ΔE vs ln$K$ for the interaction between α-CD and diphenylmethyl derivatives. The R^2 is 0.881.

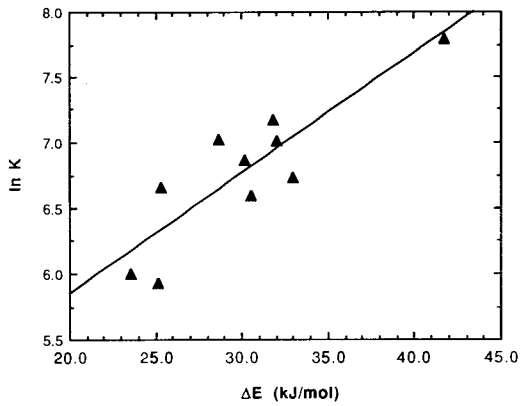

Fig. 5. Plot of ΔE vs ln$K$ for the interaction between γ-CD and diphenylmethyl derivatives. The R^2 is 0.760.

The values for $E_{components}$, $E_{complex}$, and ΔE are summarized in Tables I, II, and III. The binding constants for each compound with α-, β-, or γ-CD from solution calorimetric studies are also listed for comparison purposes. The plots of ΔE vs ln$K$ are shown in Fig. 3, Fig. 4, and Fig. 5. The correlation between K and ΔE in each case is obvious. K values increase as ΔE increases. The best correlation was found in the case of complexes formed with β-CD. This suggests that the van der Waals interaction between the compounds studied and CDs is a very important contributor to the stability of the inclusion complexes. Note that the parameters from single linear curve fitting are different for complexes formed with different CDs. This is not surprising because, as we found in the solution calorimetric studies, the ligand molecules do not bind to different CDs by the same mechanism.

The case of the adiphenine · HCl–β-CD complex is somewhat unusual. In the molecular modeling result we did not see the expected hydrogen bond between the carbonyl group and the hydroxyl group. Therefore, the ΔE is lower than anticipated. We suspect that this hydrogen bond may be bridged through a water molecule in the complex. If this is the case, the absence of a water molecule in our calculation will preclude the formation of a hydrogen bond in the modeled structure. Addition of a water molecule to the proper position on the complex system did promote the formation of hydrogen bonds and lower the binding energy. However, addition of the water molecule to other complexes also resulted in decreased binding energy due to the van der Waals interaction between the water molecule and the complexes. Therefore, no quantitative information can be obtained by this operation. Figures 2A and C show the optimized structures of complexes formed between β-CD and adiphenine · HCl or proadifen · HCl. The proadifen does not penetrate into the CD cavity as deeply as adiphenine · HCl and the aromatic ring does not tilt as much inside the CD cavity because of steric hindrance from the propyl group in the proadifen · HCl molecule.

In conclusion, this computational study demonstrates the application of molecular modeling to provide meaningful structural information on the inclusion complexes between diphenylmethyl derivatives and CDs. Results from the calculations allow us to explain satisfactorily the differences in the binding abilities of a series of amine compounds with CDs. These results are in agreement with our solution calorimetric studies. Thus, for similar ligand molecules, the molecular modeling technique not only should enable us to picture the structure of the inclusion complexes, but also will assist us in determining the ability of a potential drug molecule to form a stable complex with CDs.

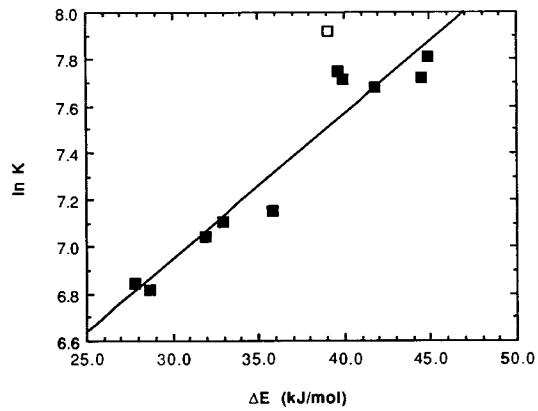

Fig. 4. Plot of ΔE vs ln$K$ for the interaction between β-CD and diphenylmethyl derivatives. R^2 is 0.924. The data point for adiphenine HCl (□) is not included in the regression.

REFERENCES

1. J. Szejtli. *Inclusion Compounds and Their Complexes,* Akademiai Kiadó, Budapest, 1982.
2. D. Duchêne, C. Vaution, and F. Glomot. Cyclodextrins, their value in pharmaceutical technology. *Drug Dev. Ind. Pharm.* 12:2193–2216 (1986).
3. K. Uekama and M. Otagiri. Cyclodextrins in drug carrier systems. *Crit. Rev. Ther. Drug Carrier Syst.* 3:1–40 (1987).
4. W. Q. Tong, J. L. Lach, T. F. Chin, and J. K. Guillory. Structural effects on the binding of amine drugs with the diphenylmethyl functionality to cyclodextrins. I. A microcalorimetry study. *Pharm. Res.* 8:951–957 (1991).
5. T. Sterner, W. Hinrichs, and W. Saenger. A side chain of diastereomeric iloprost protrudes from the cage in the complex with cyclomaltoheptaose (β-cyclodextrin): Crystal structure of (β-cyclodextrin)$_2$ iloprost · 20.5 H_2O. *Carbohydr. Res.* 192:43–49 (1989).
6. U. Burkert and N. L. Allinger. *Molecular Mechanics,* American Chemical Society, Washington DC, 1982.

7. S. P. Jones, G. D. Parr, and D. Jackson. Computer graphics as an aid for predicting drug molecule stability in a cyclodextrin inclusion complex. *J. Pharm. Pharmacol.* 38(Suppl.):94P (1986).
8. H. Höltje and M. Hense. A molecular modeling study on binding of drugs to calmodulin. *J. Comput. Aided Mol. Des.* 3:101–109 (1989).
9. F. M. Menger and M. J. Sherrod. Docking calculations on ferrocene complexation with cyclodextrins. *J. Am. Chem. Soc.* **110**:8606–8611 (1988).
10. M. J. Sherrod. Exploration of cyclomalto-oligosaccharide (cyclodextrin) chemistry with molecular mechanics: Docking calculations on the complexation of ferrocenes with cyclodextrins. *Carbohydr. Res.* **192**:17–32 (1989).
11. M. Ohashi, K. Kasatani, H. Shinohara, and H. Sato. Molecular mechanics studies on inclusion compounds of cyanine dye monomers and dimers in cyclodextrin cavities. *J. Am. Chem. Soc.* **112**:5824–6830 (1990).
12. T.-X. Lü, D.-B. Zhang, and S.-J. Dong. Molecular-mechanical study of cyclodextrin and its inclusion complexes. *J. Chem. Soc. Faraday Trans. 2* **85**:1439–1445 (1989).
13. TRIPOS Assoc. Inc., St. Louis, MO.
14. *SYBYL Theory Manual.* TRIPOS Assoc. Inc., St. Louis, MO.
15. J. Gasteiger and M. Marsili. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. *Tetrahedron* **36**:3219–3228 (1980).
16. M. Marsili and J. Gasteiger. π Charge distribution from molecular topology and π orbital electronegativity. *Croat. Chem. Acta* 53:601–614 (1980).
17. J. Gasteiger and M. Marsili. Prediction of proton magnetic resonance shifts: The dependence on hydrogen charges obtained by iterative partial equalization of orbital electronegativity. *Organ. Magn. Reson.* **15**:353–360 (1981).
18. A. Streiwieser. *Molecular Orbital Theory for Organic Chemists,* Wiley, New York, 1961.
19. W. P. Purcell and J. A. Singer. A brief review and table of semiempirical parameters used in the Hückel molecular orbital method. *J. Chem. Eng. Data* **12**:235–246 (1967).
20. J. J. Guy and T. A. Hamor. Crystal and molecular structure of adiphenine hydrochloride, a muscarinic antagonist of acetylcholine. *J.C.S. Perkin II* 942–947 (1973).
21. *The Cambridge Crystallographic Database,* University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.
22. K. Harata. The structure of the cyclodextrin complex. V. Crystal structures of α-cyclodextrin complexes with *p*-nitrophenol and *p*-hydroxybenzoic acid. *Bull. Chem. Soc. Jpn.* **50**:1416–1424 (1977).
23. K. Lindner and W. Saenger. β-Cyclodextrin dodecahydrate: Crowding of water molecules within a hydrophobic cavity. *Angew. Chem. Int. Ed. Engl.* 17:694–695 (1978).
24. K. Lindner and W. Saenger. Topography of cyclodextrin inclusion complexes. XVI. Cyclic system of hydrogen bonds: structure of α-cyclodextrin hexahydrate, form (II): comparison with form (I). *Acta Cryst.* B38:203–210 (1982).
25. K. Harata. Crystal structure of γ-cyclodextrin at room temperature. *Chem. Lett.* 641–644 (1984).
26. B. Klar, B. Hingerty, and W. Saenger. Topography of cyclodextrin inclusion complexes. XII. Hydrogen bonding in the crystal structure of α-cyclodextrin hexahydrate: The use of a multicounter detector in neutron diffraction. *Acta Cryst.* B36:1154–1165 (1980).