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The Shapes and Cavities of Four Cholic Acid Host Systems

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We have continued our efforts to correlate cholic acid host shapes and relative energies by completing the conformational analyses of the m-xylylene diamine cholic acid host (mCAH) (cf report #9 entitled "The Shapes and Cavities of the m-Xylylene Cholic Acid Host"). The strategy previously developed, which utilizes a combination of systematic conformational searching by energy and by distance, was employed for studying the p-xylylene diamine cholic acid host (pCAH) and two derivatives degraded in the side chain, one which contains the m-xylylene diamine spacer (referred to as "chopped" MCAH or cmCAH) and one which contains the p-xylylene diamine spacer (cpCAH).

The report compiles all of the information generated on the shapes and cavities of these four cholic acid host molecules. The detailed methods are included, as well as the conformational analyses results. Consistent with earlier studies, the O_3 (Arm A) -- O_3 (Arm B) distance serves as the qualitative measure for inferring the size of the host cavity. The cavity sizes and shapes are compared to the cavity found in arabinose binding protein (internal width of about 7 Å), and speculations on the ability of monosaccharides to bind in these cavities are made.

In summary, the cmCAH cavity provides the best type of binding site: many conformations of cmCAH generate a "C" shaped geometry of sufficient size for one sugar guest (about 8 Å in width). The pCAH cavity provides the next preferable shape, which is a 8-11 Å disc-like or cup-like cavity, followed by the mCAH, whose "7" shapes could bind a monosaccharide to only one cholic acid arm. The cpCAH does not provide any reasonable geometries for guest binding. Some overall "S" shapes are present; some conformations are quite twisted and distorted. As a result, no type of cavity is generated.

Part 1. Conclusions from the mCAH Study

The distance scan had provided conformations which were within 10 kcal from the absolute minimum energy value and had O_3 to O_3 distances of 12-20 Å. The breakdown in a 13-17 Å window had been:

13.13 - 13.96 Å	17 confs
14.02 - 14.91 Å	19 "
15.01 - 15.99 Å	24 "
16.01 - 16.97 Å	13 "

The total number of conformations generated from this scan by distance was 73, in contrast to a total of 63 generated from a scan by energy using an O_3 to O_3 constraint of 13-17 Å. The reason for the discrepancy between the results from the two methods had not been resolved in the previous technical report (cf #7). We have now accounted for the difference in results by realizing that the energy scan and distance scan did not use the same absolute energy cutoff: the energy scan used -161.86 kcal, whereas the distance scan used -161.00 kcal. The reason for this is that the systematic searching by energy routine uses an internal energy cutoff of 10 kcal relative to the global minimum energy value, whereas the distance scan uses an input absolute value. Since the distance scan used about a 1 kcal larger energy window, more conformations resulted.

The overall shapes of the mCAH low energy conformations generated from the energy scan using distance constraints were assessed. Of the 63 states, 61 were found to be local minima. Every fifth conformation starting from the lowest energy and proceeding to the highest energy was generated graphically and evaluated visually. The shapes generated differed from those previously generated with either a preformed "C" or "S" spacer. These new conformations fell into an "┐" or half "C" shape, wherein one arm remained arched but the other was twisted around itself.

Part 2. The p-Xylylene Diamine Cholic Acid Host (pCAH) Study

The pCAH was built from the cholic acid fragment (Refcode = BUGJES from the Cambridge Crystallographic Database) and the p-xylylene diamine spacer fragment, which had been previously optimized using AM1. The AM1 net charges were used, with minor modification for the O and N of the two amide groups.

Conformational analysis of pCAH (12 bonds, by 60°, Figure 1) evaluated 531,441 (over 1/2 million!) states by energy and resulted in 45 conformations (all of which were local minima), spanning energies from -160.55 to -150.61 kcal (a 10 kcal range). Only 5 conformations existed within a 5 kcal range from the minimum. Interestingly, the global minimum energy structure did not have any neighboring conformations, since the closest conformation was 3.5 kcal higher in energy. A breakdown of the energy components of the global minimum energy structure and of the cutoff structure at 10 kcal (conf #45) revealed that the changing component in the total gas phase energy was the steric contribution. This changed along with the folding pattern observed for the cholic acid host.

	Minimum Energy Conf.	Conf #45
Steric	-126.22	-116.50
Electrostatic	43.12	26.22
HBonding	-76.66	-59.54
Torsional	-0.79	-0.79

A test of the conformational energy analysis results was done by changing the atom type for the H's on the sp^3 oxygens from hydrogen-bonding type hydrogens to aliphatic hydrogens. This would eliminate the possibility for biasing the conformations, and determine if the hydrogen-bonding H's were causing the clam to collapse onto itself. The fact that about the same number of conformations resulted within 10 and 5 kcal windows indicated that the internal folding was not driven by the tendency of the cholic acid arms to hydrogen bond to themselves.

Conformational analysis <u>by distance</u>, using an O_3 to O_3 interatomic distance constraint of 8-12 Å and an absolute energy limit of -149.5 kcal (about a 10 kcal window), resulted in 9 conformations, having distances ranging from 8.04 to 11.73 Å. Using a window of 12-20 Å for the distance constraint, 28 conformations resulted.

All of the 9 conformations above were generated graphically and analyzed visually. While none of these adopted a "C" shape specifically, a "cup-shaped" cavity was seen in many of the shape types, which were twisted "C"'s or "S"'s, depending on the shape of the p-xylylene spacer, either  or  . When a good cup shape was observed, all of the hydroxyl groups were facing the same direction, so that the overall cavity was like a disc with the hydroxyls pointing upward. This is similar to the observed cavity of 7 Å width found in arabinose binding protein. Therefore, the pCAH conformations provide several possibilities for one monosaccharide molecule to complex with the two arms of the pCAH.

All of the 28 conformations above were also analyzed. Most of the conformations were definitely "S" shaped, with the size of the cavities being very large, 12.53 to 19.89 Å. The tendency to maximize van der Waals contacts resulted in very compact structures, so that an O_3 to O_3 line did cut diagonally through the whole molecule. This tendency toward the "S" shape might provide an opportunity for each arm to bind one sugar molecule.

Part 3. The cmCAH Study

The cmCAH was built from mCAH by deleting two methylene groups in each side chain of the cholic acid and editing the length of the new $C(CH_3)$--$C(=O)$ bond to 1.51 Å. The same AM1 net charges as were on the atoms in the full mCAH were used, with modification of the C, O and N atomic charges in the two amide groups. As with the full cholic acid hosts, a Cl replaced the CH_3 group in each side chain.

Conformational analysis of cmCAH (10 bonds, by 60°, Figure 2) evaluated 6,561 states by energy and resulted in 7 conformations within 10 kcal from the global minimum energy value of -151.68 kcal. Only the global minimum energy structure was identified as a local minimum, meaning that the 7 conformations were all related to each other and belonged in the same conformational family. With the reduction in number of rotatable bonds, a finer grid resolution could be used for the rotation of the bond from the alpha-methylene group to the aromatic ring. Subsequent conformational analysis (8 bonds by 60°, 2 bonds by 30°) resulted in evaluation of 104,976 states. A total of 89 conformations fell within 10 kcal from the minimum, with 7 being local minima. The global energy minimum structure remained at -151.68 kcal.

A test of the effect of hydrogen-bonding hydrogens was done by changing the 6 hydrogens on the sp^3 oxygens to aliphatic hydrogens (no hydrogen-bonding possible) and rerunning the energy scans two different ways as described above. The resulting number of conformations and minima were equivalent to the above energy scans (7/1 and 89/8 conformations/minima versus 7/1 and 89/7, respectively). In the later scan, 9 minima existed within 20 kcal (194/9). We concluded, therefore, that the tendency toward folding did not arise from a bias for internal hydrogen bonding.

Conformational analysis by distance, using an internal interatomic distance constraint, was not necessary due to the few number of conformations arising from the scans by energy. The "chopped" clam system is very restricted in flexibility, so that the number of energetically reasonable conformations is severely limited.

All of the above 9 minima were generated graphically and examined visually. Three conformations provided good "C" shapes, with an internal width of 8 Å or slightly larger. Four shapes provided a more disc-shaped surface with the hydroxyl groups oriented in the same direction. Only two shapes would not be amenable for binding a monosaccharide guest.

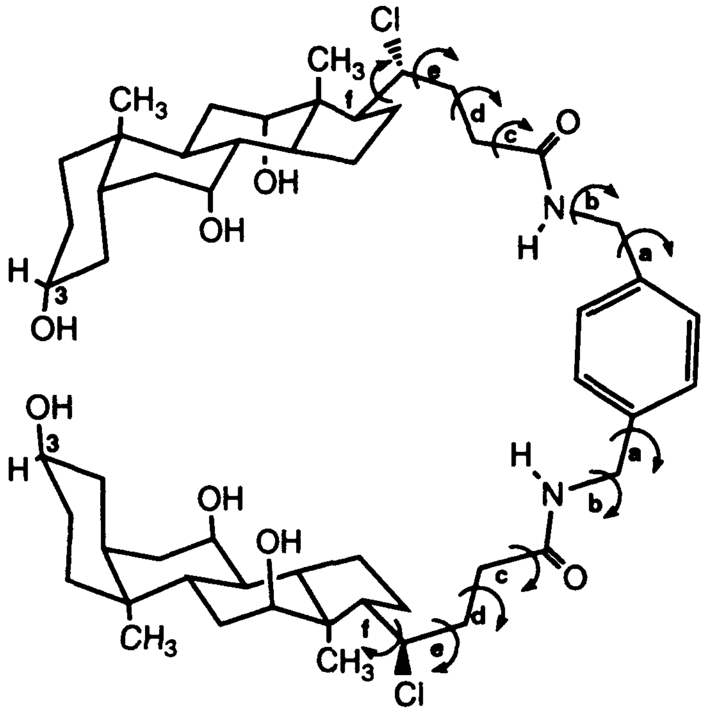
Part 4. The cpCAH Study

The cpCAH was built from the reference state of the pCAH by deleting two methylene groups in each side chain of the cholic acid host, editing the length of the new $C(CH_3)$--C(=O) bond to 1.51 Å, and adjusting the AM1 net charges on the C, N and O atoms of the two amide groups.

The conformational analyses of cpCAH by energy were done as for cmCAH (described in Part 3.). Scan 1 (10 bonds, by 60°, Figure 3) evaluated 6,561 conformations by energy and resulted in 40 conformations within 10 kcal from the global minimum, with the one local minimum (the global minimum energy structure itself). Scan 2 (8 bonds by 60°, 2 bonds by 30°) evaluated 104,976 states by energy and resulted in 675 conformations within 10 kcal, with 6 conformations identified as local minima. Scans 3 and 4 were done with aliphatic hydroxyl hydrogens (no hydrogen bonding possible) and resulted in 40/1 and 676/6 conformations/minima within 10 kcal, respectively. Since the results with and without hydrogen-bonding type hydrogens were essentially the same, again we concluded that the scan results were not biased toward compact structures by the atom types used.

All of the 6 minima above were generated graphically and examined visually. Two conformations were extended "S" shapes with OH's pointing in opposite directions; two were "V" shapes with the OH's pointing in opposite directions; two were globular shapes with nestled cholic acid arms. Therefore, in the cpCAH, no reasonable shapes for a host which could bind a monosaccharide were found.

Figure 1. Conformational Analysis Details for pCAH



12 rotatable bonds

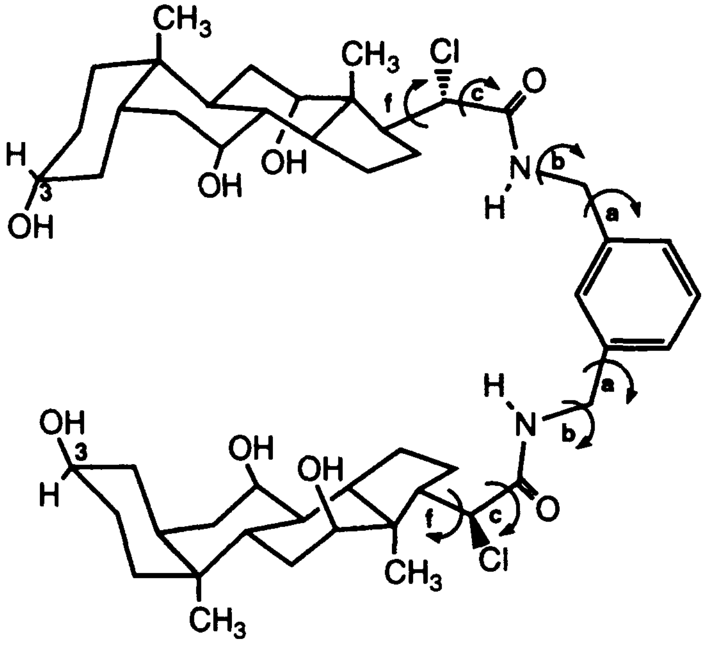
Contributions to energy:

Steric -- Hopfinger 6-12 Potential Set

Electrostatics -- AM1 Net Charges

Torsional Fns. -- Bond **a** free rotation
Bond **b** methylformamide
Bond **c** acetaldehyde
Bonds **d,e,f** ethane

Figure 2. Conformational Analysis Details for cmCAH



8 rotatable bonds

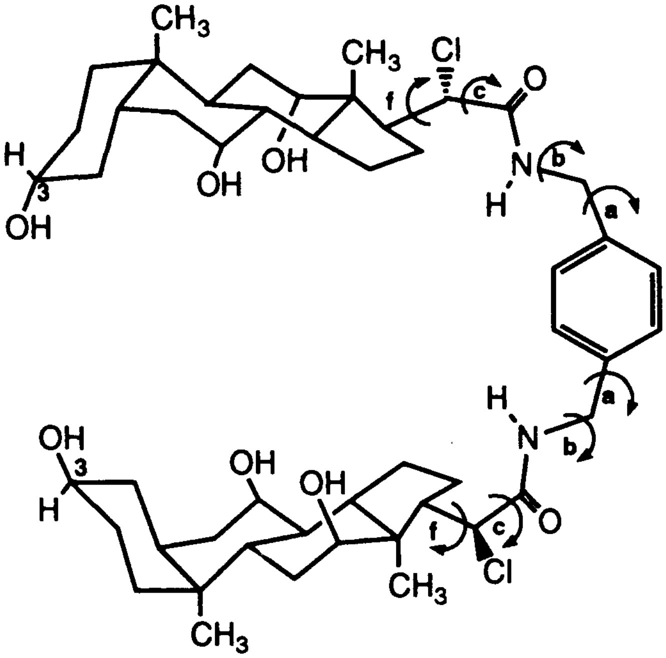
Contributions to energy:

Steric -- Hopfinger 6-12 Potential Set

Electrostatics -- AM1 Net Charges

Torsional Fns. -- Bond **a** free rotation
Bond **b** methylformamide
Bond **c** acetaldehyde
Bond **f** ethane

Figure 3. Conformational Analysis Details for cpCAH



8 rotatable bonds

Contributions to energy:

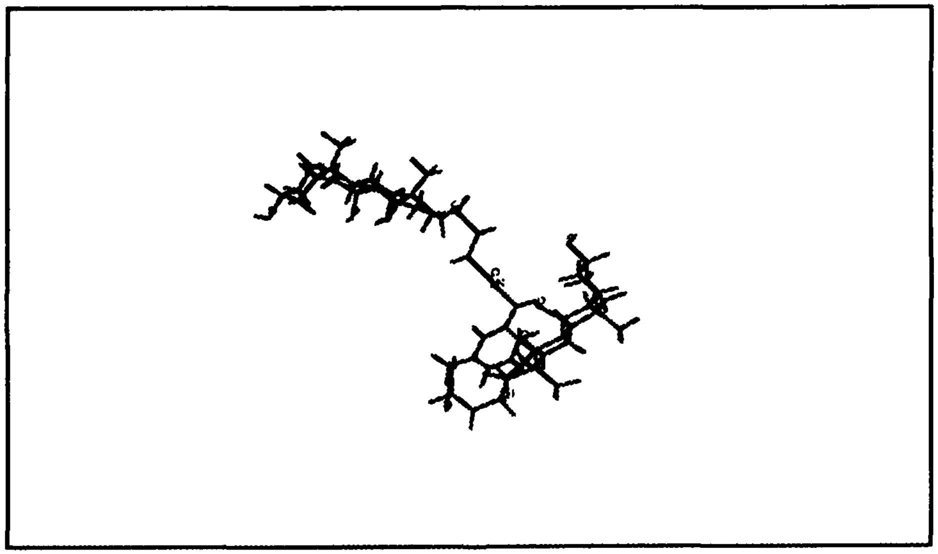
Steric -- Hopfinger 6-12 Potential Set

Electrostatics -- AM1 Net Charges

Torsional Fns. -- Bond **a** free rotation
Bond **b** methylformamide
Bond **c** acetaldehyde
Bond **f** ethane

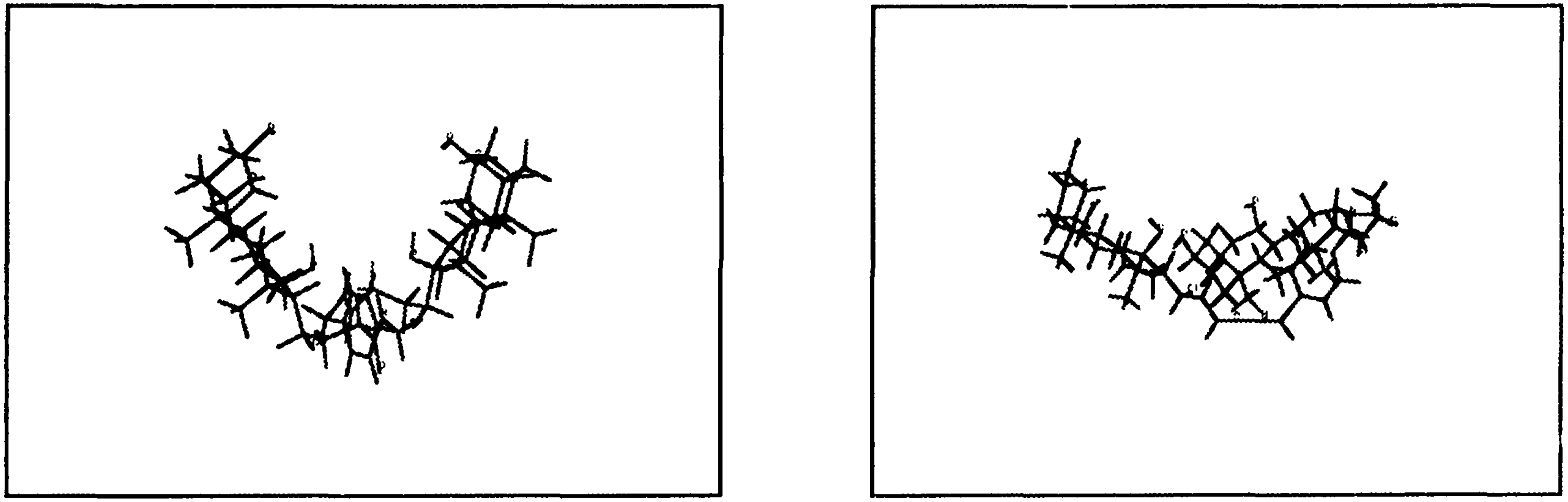
Representative Three-dimensional Shape for mCAH

One Cholic Acid Arm Available for Binding Sugar Guest



Representative Three-dimensional Shapes for pCAH

Cavity Widths: a) 8.04 Å, b) 11.25 Å

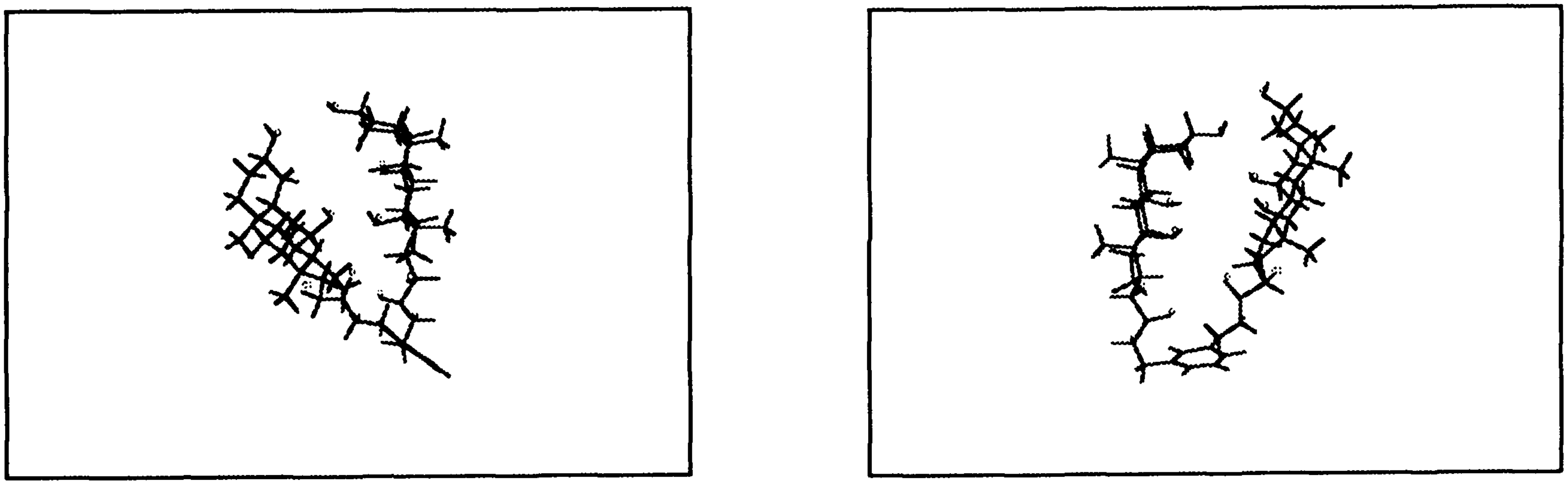


a Cup Shape

b Disc Shape

Representative Three-dimensional Shapes for cmCAH

Diagonal Cavity Widths ~8 Å



"C" Shapes

Representative Three-dimensional Shapes for cpCAH

No Cavities Formed