

**MODELING THE CONFORMATION OF POLYPHENOLS AND
THEIR COMPLEXATION WITH POLYPEPTIDES:
SELF-ASSOCIATION OF CATECHIN AND ITS
COMPLEXATION WITH L-PROLINE GLYCINE OLIGOMERS**

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1. INTRODUCTION

Over the past 10 years, several scientific thrusts have come together in the study of flavanoids that make it possible to move forward into the study of complexation between polyphenols and polypeptides. Enhanced understanding of the conformational properties of flavanoid monomers and polyflavanoids through molecular modeling, combined with the detailed NMR experimental data now in the literature, provide the foundation.¹⁻¹³ Recent work using conformational searching techniques with the GMMX⁶⁻⁸ protocol has shown additional detail about the distribution of pseudo equatorial and pseudo low-energy axial conformers in the ensemble, as shown in figure 1. This leads to information about the relationship



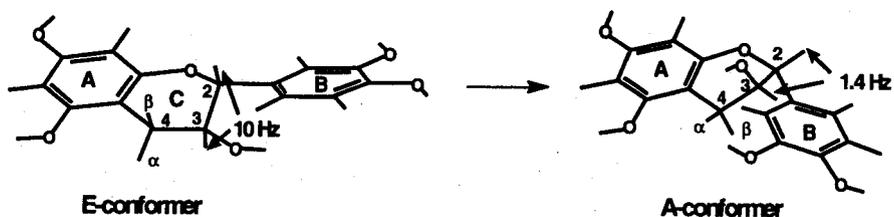


Figure 1. The pseudo equatorial conformer to pseudo axial conformer is illustrated. The structure-sensitive $J_{2,3}$ coupling constant varies between the E-conformer and A-conformer from 10.0 Hz to 1.4 Hz, respectively, as determined by MacroModel with a GB/SA water solvent model.

between the conformer ensemble and the Boltzmann averaged NMR proton coupling constants that one would expect to observe in a solution. Figure 1 also illustrates the pseudo equatorial to axial transformation that takes place in all catechin or (+)-catechin-(4 α →8)-(+)-catechin (B3) dimer complexes during the conformer searches and which would also be expected to occur in solution. Interest continues to further understand the details about this conformer distribution as well as in the prediction of complexation of tannins with metal ions and proteins. Although the GMMX software has given many interesting results, it is limited in handling cases that require systematic conformational searching of molecules combined in a complex. In addition, there are no solvent model options.

Recent NMR studies on procyanidin dimers¹⁴ and NOE results of the complexation of L-proline-glycine compounds with (+)-catechin and polyflavanoid dimers¹⁶ have given data to help guide computational studies. Couple this with the improved molecular computational software available,¹⁶⁻¹⁹ and it becomes possible to explore complexation searching conformational space through Monte Carlo and molecular dynamics protocols using water as a solvent. The importance of this is highlighted by the renewed interest in its pharmacological characteristics such as the antiviral and antitumor behavior of tannins and other polyphenols²⁰ as well as reported interaction of polyphenols with proteins in aqueous solutions.²¹⁻²³ In this chapter, we explore computational models for molecules such as L-proline-glycine and glycl-L-prolyl-glycl-glycine ion (GPGG ion) interacting with (+)-catechin and (+)-catechin-(4 α →8)-(+)-catechin (B3) to form complexes. These results are compared to the close-contact positions obtained from NOE NMR experiments in aqueous solution. The complex structures found using conformational search methods are discussed in terms of the specific hydrophobic and hydrophilic interactions observed.

2. COMPUTATIONAL METHODS

The two protocols applied in this study for searching conformational space are MacroModel¹⁶ version 5.5 and HyperChem 5.1.¹⁷ MacroModel was applied in the Monte Carlo conformational multiple minimum structure searching mode using the Amber and Merck (MMFF 94s) force fields.²⁴ Typically, 5000MC steps were

used within a 16kJ/mol energy window using the GB/SA²⁶ water solvent model. Sometimes a 30kJ/mol energy window was used for the collection of conformer structures over a wider range of energies. The molecules involved in the complex were related to each other during the searches through the MOLS command which selects a molecule in the complex and defines an axis system for independent molecular rotation and translation for complexation. A distance constraint of between 2 to 8 Å was applied between selected atoms. The distance constraint keeps the molecules from flying apart by discarding all structures generated outside of the constraint limits. Since no quantitative NOE distances were available, the FIXD command to keep the molecules at some fixed distance between NOE contacts was not used. All flexible torsion angles for both molecules were rotated and, except for the amide linkage, bond angles were allowed to vary between 0° and ±180°. The catechin pyran torsion angles were varied, but the proline ring was not opened, and the configuration was left *trans*. The number of combinations for bonds rotated at any given MC step was randomly selected between 2 and a maximum of 20 for two catechin molecules. The searching procedure was started with many different initial structure complex combinations; parallel and anti-parallel (+)-catechin molecular forms, for example.

HyperChem was applied using the molecular dynamics protocol with the low-energy complexation structure found from the MacroModel search studies. The MD studies were run for 10ps at 1fs steps. The heating from 0K and cooling to 0K were done in 0.3ps each. The constant temperature bath was examined at 300 and 350K. Structures were examined by sampling the stored structure files accumulated at every 5fs. Ensembles were filtered for computation of distances between selected atoms.

3. CATECHIN/CATECHIN SELF-ASSOCIATION

NOE results showed considerable cross-peak correlation, indicating that self-association was occurring. The structures illustrated below are from among the low-energy conformers in the ensemble found from the molecular searches. Figure 2 shows the lowest energy structure found for the self-association catechin/catechin complex with the MMFF force field. The B-rings are nearly parallel and overlap each other. The hydrogen bonding in the MMFF force field comes from the natural electrostatic and van der Waals interactions. A measure of the complexation binding energy can be obtained from taking the difference in energy between the complex formed and the two molecules separated by a large distance, e.g., 12 Å. This energy difference, ($E_{\text{complex}} - E_{\text{isolated}}$) for catechin/catechin, is -30.0kJ/mol for the complex shown in figure 2. Figure 3 shows the second lowest-energy structure found from a number of Monte Carlo MacroModel searches.

The third lowest-energy structure found for catechin self-association using the MMFF force field and the water solvent was found at an energy of 1.5kJ/mol and is shown in figure 4. This structure is also in parallel form, but translated slightly from that shown in figure 3.

The perspective of the complex formed in figure 2 shows that B-rings for molecules I and II are parallel and aligned, but OH groups are opposed. In that figure,

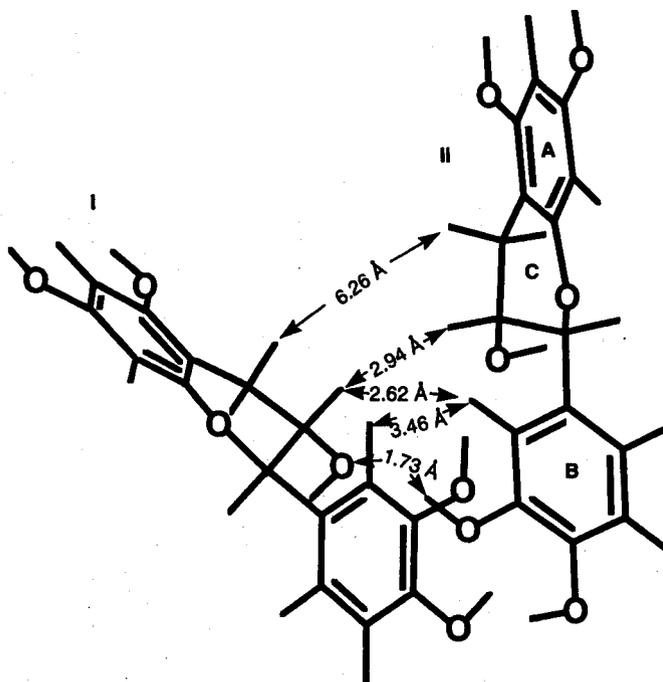


Figure 2. The lowest energy catechin/catechin complex found in a typical MacroModel MMFF force field search, having an energy of 122.9 kJ/mol (0.0 kJ/mol relative energy). The B-rings are nearly parallel and overlap, with the hydroxyl groups opposed. The distances listed show the closest hydrogen atom contact points.

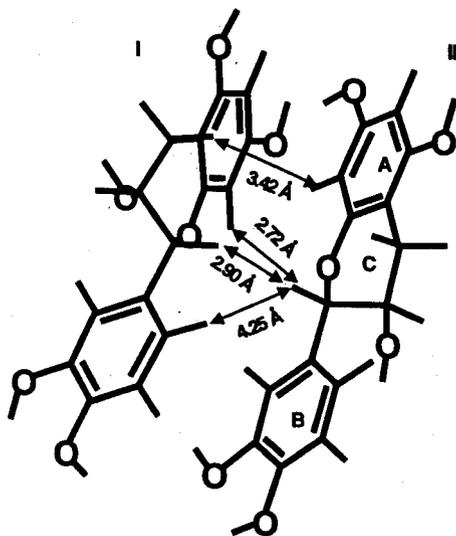


Figure 3. The second energy of the lowest three catechin/catechin complexes found in a typical MacroModel MMFF force field search has a relative energy of 0.3 kJ/mol. This structure and that in figure 2 are nearly parallel, but molecule II is rotated approximately 180° around the long axis relative to molecule I. The distances listed show the closest hydrogen atom contact points.

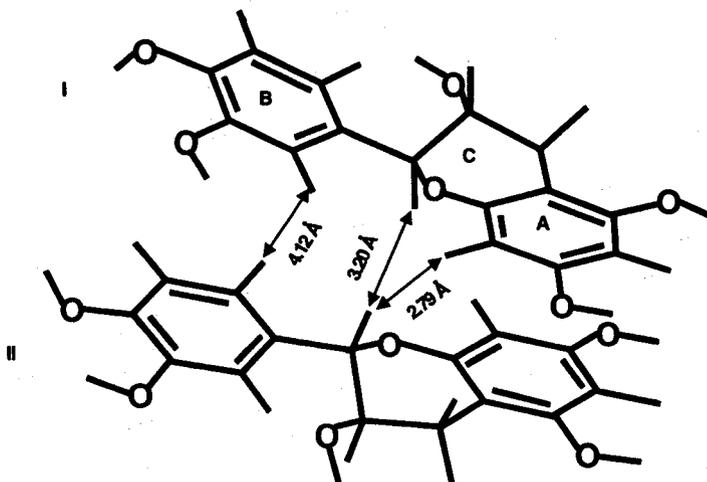


Figure 4. The third lowest-energy complex found from the MacroModel MC MMFF force field search for catechin/catechin complexes. The conformer relative energy is 1.5 kJ/mol. The molecules are rotated nearly 180° relative to each other.

the B-ring for molecule II is oriented in the plane of the page. The A-rings are oriented out of the page plane. There is a strong hydrogen bond formed between I (OH)-3_C and II (OH)-3_B. This structure suggests that strong interaction should appear between II H-3_C and I C-ring protons along with some weak interaction between B-rings, for example, H-2_B and H-2_B. Examination of the figure 3 complex shows that the two catechin molecules are parallel to each other, but have the OH-3_C groups rotated around the long axis by nearly 180° to each other. The contact points are clearly close enough to cause NOE behavior, especially the 2.90 Å between protons II H-2_C and I H-2_C, and the 2.72 Å between protons II H-2_C and I H-8_A. There is also contact between the I H-(4 α)_C proton on catechin I and the H-8_A proton on catechin II. These structures are crossed over at the pyran ring.

In the figure 4 structure, notice that there is NOE contact between molecules I and II through the H-2_C protons at 3.20 Å, and across from the H-2_C proton to the H-8_A proton at 2.79 Å. In the conformational searches, it was common to observe that the C-ring protons are involved as contact points in these catechin self-associated complexes.

Figure 5 illustrates the HyperChem molecular dynamics low-energy structure as determined from a self-association simulation in a water-box containing about 1665 water molecules. Simulations were run at a constant temperature of 300 K. The structure is in the anti-parallel form with the A-ring and B-rings paired. The complex structure actually has a cross-shape. There is contact between B- and A-ring protons H-2_B to H-8_A of 3.64 Å, as predicted by NOE results. However, again it would appear that the H-2_C protons should show strong NOE behavior with a

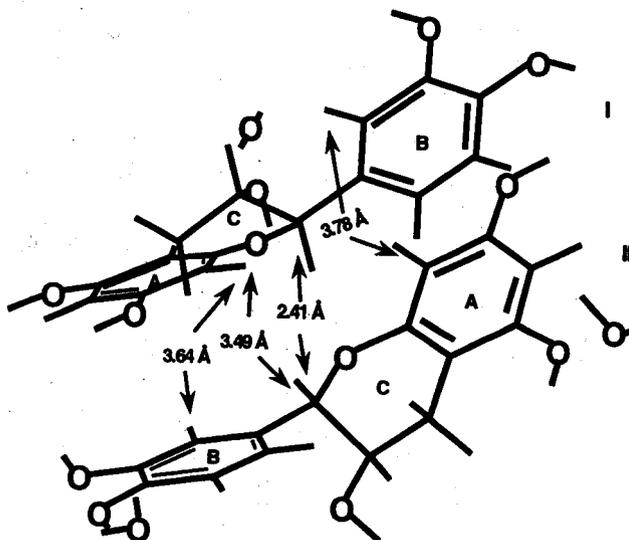


Figure 5. A low-energy complex form stabilized during the MD HyperChem run in a water solvent box. The structure shows the anti-parallel form. The closest contact distance is 2.41 Å between the H-2_C protons between molecules I and II. For clarity, all but a few water molecules have been removed.

separation of 2.41 Å. This has not been observed by NMR experiments.¹⁶ In figure 5, the water molecules have been cut away with only several neighbors retained. There are problems in trying to achieve the minimization of energy for a structure in a water box, since it is mainly the individual water molecules that are moved. If the water molecules are removed, minimization can substantially change the interaction, allowing the individual molecules of the complex in some cases to drift apart. One of the benefits of the GB/SA water model when used in molecular dynamics simulations is that explicit water molecules are not present. Molecular dynamics experiments were run by MacroModel to check some of the structures.

One example of the interaction between catechin molecules when one is in the axial configuration is shown in figure 6. This particular MacroModel complex conformer has a relative energy of 8.6 kJ/mol above the lowest one found (fig. 2) and so would contribute only slightly to the Boltzmann distribution. However, this axial/equatorial anti-parallel form does give a picture of one type of structure that may be needed to explain coupling constant values. A lower relative energy value is needed, however, for there to be a significant contribution to the Boltzmann properties.

Figure 7 shows the minimum energy complex structure found in a run with a modification of the electrostatic hydrogen bonding function in the MMFF force field with a Lennard-Jones 6-12 potential. This might be the lowest energy [119 kJ/mol] catechin/catechin complex. The next higher one in this series is only 1.0 kJ/mol

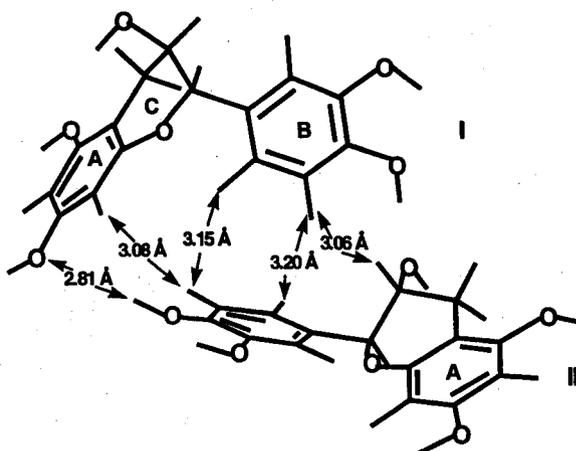


Figure 6. The complexation of an axial catechin conformer I with the equatorial conformer II in an anti-parallel form showing the interaction of the B-ring protons with the A-ring protons. This higher energy complex is 8.6 kJ/mol above the global minimum found so far in MacroModel MC conformational searching.

higher than the one shown in figure 2. It is also a cross-like structure with good interaction between the A- and B-rings, as shown in figure 8.

According to the work by Hatano and Hemingway,¹⁵ the cross-peak interactions in the catechin solutions in water were observed between the molecule I H-2_B proton and the molecule II H-8_A proton. In the lowest energy conformers found so far by MC conformational searching, there is considerable B-ring/B-ring

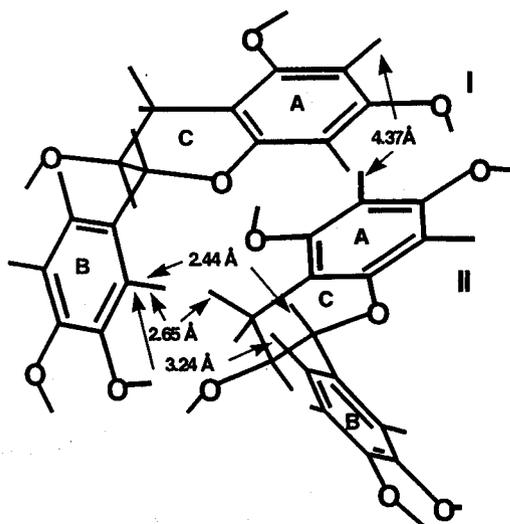


Figure 7. This is the lowest energy structure found in a modified Monte Carlo MacroModel run with the hydrogen bonding function changed to a Lennard-Jones 6-12 potential. The energy of 119 kJ/mol is lower than that shown in figure 1, but this may be due to H-bonding function changes.

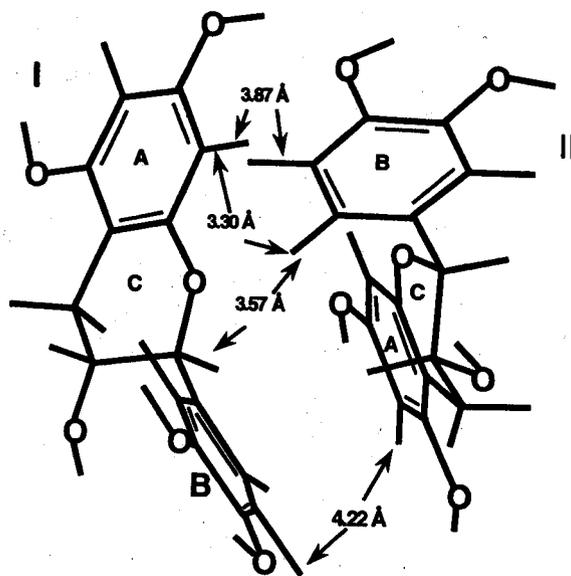


Figure 8. The second highest-energy structure from this run with the hydrogen bonding set to a different function. The hydrogen bonding parameter in the MacroModel program was set to run a Lennard-Jones 6-12 potential. The structures cross over at the pyran rings. The ensemble shows a number of good NOE contacts (hydrophobic interactions) between the A- and B-rings.

association with definite interaction between the H-4_C proton and the H-3_B proton; see figures 7 and 8. These results suggest that further work should examine the NMR spectra for indication of C-ring proton involvement in the complexes through hydrophilic and hydrophobic interaction. It is possible that it might not appear because of relaxation properties.

In the MacroModel study, several of the above structures were found by using complexes started in the anti-parallel structural form. However, there did not seem to be a propensity for the complexes to end with the B-ring of one molecule interacting directly (that is, overlaid) with the A-ring of another moiety. Specifically, the protons of the B-ring need to be oriented directly into the A-ring as would be required by Hatano's NMR results.¹⁶ A number of higher energy conformers found in MacroModel ensembles appear to have properties that fit with the NOE NMR results.

To better examine the hydrophobic interactions of the ensemble, filtered data were studied. The distance data given below were filtered from several MacroModel runs. The interatomic distance filter was set between 2.0 and 4.0 Å with contact numbers collected on structures within this distance window. Although a Boltzmann average was not done, a couple of examples of percentages are given here showing interactions between molecules I and II. Run A, with 788 complexes in the ensemble (50 kJ/mol energy window), had 6.35 percent H-2_B to H-6_B, and

4.57 percent H-2_B to H-2_B. Run B, 388 complexes in an energy window of 30 kJ/mol, had 13.4 percent H-8_A to H-6_B, 12.3 percent H-6_A to H-5_B, and 7.73 percent H-2_B to H-2_B. Run C, 325 complexes, 30 kJ/mol energy window, had 12.9 percent H-2_C to H-2_C, 9.5 percent H-2_B to H-6_B, and 8.9 percent H-8_A to H-2_B.

Although the lowest energy conformer found might not show all of the experimental NOEs, it is important to examine the higher energy structures. Intermolecular distances were filtered from the 111 conformer complexes found over a 16 kJ/mol window for structures from the search shown in figures 7 and 8. The following percentage contact was noted for an interatomic distance window between 2.0 Å and 4.0 Å: I H-2_B to II H-8_A found a 28.2 percent population; and II H-2_B to I H-8_A, 16.2 percent. This shows a large number of potential NOE contacts that agree with the noted experimental NMR results.

Searches made with NOE distance constraints on those conformers allowed in the ensemble were not performed. Besides not having the quantitative data needed, it is also possible that the relaxation time for some nuclei in the complexes are very fast and may or may not contribute to the NOEs in expected ways.²⁶ Therefore, some close interactions may not lead to observed NOEs.

4. CATECHIN/L-PROLINE-GLYCINE OLIGOMER COMPLEXES

Although many of the complexes between catechin and L-proline and proline-glycine complexes have been examined, just three complexes will be discussed here. These are catechin/L-prolyl-glycine, the catechin/glycyl-L-prolyl-glycyl-glycine tetramer zwitterion, and the dimer B3/glycyl-L-prolyl-glycyl-glycine tetramer ion complex. Searches were started by placing the L-proline derivative near the catechin or the B3 dimer [(+)-catechin-(4 α →8)-(+)-catechin] in a position guided by the NMR NOE data. However, a number of different starting structures were examined to allow many possible complexes over the thousands of MC steps in the search routine. Since the *trans* form of proline was predominant in the mixtures studied,¹⁵ except for glycyl-L-proline, only the *trans* form of the proline was considered in this study.

The NMR NOE results for catechin/L-prolyl-glycine show strong cross-peaks from both the H-6_A and the H-8_A to the glycine protons. In addition, the *trans* form showed very weak cross-peaks with the B-ring protons. The catechin/L-prolyl-glycine structure complex given in figure 9 shows that the proline ring settles over the C-ring in a planar fashion. The glycine unit shows hydrogen bonding between the NH₂ and carboxylate group. This compound was modeled in the neutral state. Examination of the figure shows interaction between the methylene protons and H-2_B of 4.1 Å, and the proline (C₈H₂) and the catechin H-(4 β)_C proton of 2.6 Å.

In this low-energy catechin/L-proline-glycine complex, and in nearly all modeled cases, there has been a lot of interaction between the proline ring protons (γ and δ) to H-4_C as well as to the H-2_C and H-3_C protons from catechin. In these studies, there has been indication that the C-ring protons from catechin should be involved in NOEs for the complexes. It is not clear yet why these have not been observed.

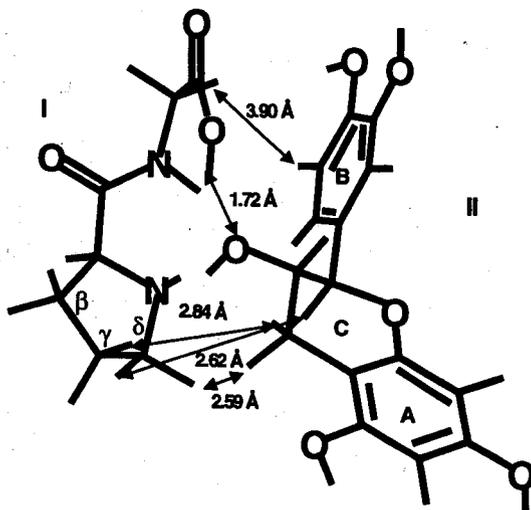


Figure 9. The lowest-energy catechin/L-proline-glycine complex. The proline-glycine is *trans*, and the amide structure is locked in the planar position. In this case, the glycine C-OH was allowed to rotate, and it shows a strong H-bond (1.72 Å) to the catechin. Considerable interaction is noted from H-2_B and H-3_B to the γ and δ L-proline ring protons, as well as from the glycine methylene proton to the H-5_B proton. The structure is shown here in the neutral form.

First, the structural characteristics of the GPGG ion tetramer were examined. This was accomplished by carrying out MacroModel searches on the GPGG ion using the water solvent model, GB/SA. After starting the structure in many different conformers, the lowest energy forms found always showed a β -turn structure. An example of the lowest energy structure is given in figure 10. The β -turn structure is confirmed by a number of studies, including NMR and Raman spectroscopy as well as with modeling.^{27,28}

There appears to be some controversy about the nature of this tetramer structure. Perly et al. predicted a II β -turn structure.²⁷ Although we came up with a β -turn structure, not all of the torsion angles agreed with Perly's NMR paper. In our study, the torsion angle ϕ_4 agreed exactly with the NMR study done in DMSO, but for ϕ_2 , ψ_2 Perly found -60° , 120° . Our structure is more like a I β -turn structure (fig. 10); ϕ_2 , ψ_2 , ϕ_3 , and ψ_3 values are -60° , -30° , -90° , and 0° , respectively.^{28,29} The NH...O=C hydrogen bonding across the glycine arms is predicted satisfactorily. This structure became the starting point for forming complex structures with the catechin molecule and with the B3 dimer. In each search case, all non-ring torsion angles were allowed to vary. Figure 11 shows one of the lower-energy catechin/GPGG ion complexes found with a MacroModel search and the MMFF force field. Here we see the arms of the GPGG ion structure interacting through hydrogen bonding with the (OH)-7_A. The methylene of the N-terminal glycine interacts hydrophobically with the H-6_A (4.62 Å) proton of catechin and the C-terminal (CH₂) _{α} interacts with H-8_A proton (3.46 Å). The proline γ and δ CH₂

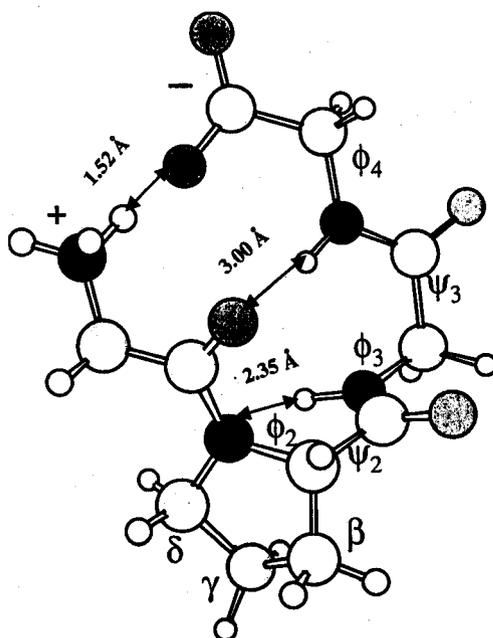


Figure 10. The low-energy conformer for GPGG zwitterion from the Macro-Model search shows a β -turn structure. The ϕ_2 , ψ_2 , ϕ_3 , ψ_3 , and ϕ_4 values are -80° , -29° , -98° , 20° , and 169° , respectively. Some similar form of β -turn structure is found with all of the low-energy catechin complexes.

hydrogen atoms also interact with the catechin H-8_A hydrogen atom. According to work done by Hatano and Hemingway,¹⁵ there is strong association only between the C-terminal glycine methylene group and the H-8_A proton and the other N-terminal methylene group and the H-5_B proton. Their work might suggest a preferred extended structure for the polypeptide oligomer, but the observations could arise by averaging NOEs from several low energy complexes, for example; see figure 12. It should be noted here that the energy difference ($E_{\text{complex}} - E_{\text{isolated}}$) between the complexed molecules in figure 11 and the isolated molecules minimized by MMFF and in water was -32.0 kJ/mol. This again supports the favorable energy lowering by molecular association.

In figure 12, the next higher-energy conformer in this series (1.7 kJ/mol) shows the glycine arms interacting primarily with the pyran ring oxygen atom. There is close contact between the H-8_A proton of catechin and the NH₃⁺-CH₂ group (3.08 Å) and proline (CH₂)_δ (3.41 Å). The proline (CH₂)_γ hydrogen atoms interact directly with H-2_C, and H-2_B, and the H-5_B and H-2_B catechin hydrogen atoms have close contact with C-terminal (CH₂)_α protons (3.68 Å, 2.92 Å). This again shows the hydrophilic interaction helping to tie the molecules together coupled with hydrophobic interactions as well. This study indicates that proline methylene hydrogen atoms are involved in many hydrophobic interactions. Conformer complexes arise with the catechin situated in the axial form, too. Figure 13 shows that the (OH)-3_C is axial, and that it is in good position to interact through hydrogen bonding with the C-terminal and N-terminal glycine arms. The energies of these complexes are typically in the order of 8 kJ/mol higher and consequently not low

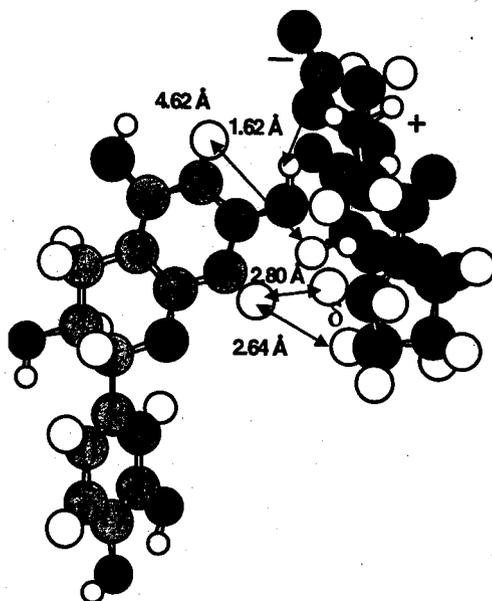


Figure 11. NMR experiments show NOE between H-8_A of catechin and the methylene of the C-terminal glycine as well as between the H-6_B proton of catechin and the C_α methylene of the C-terminal glycine unit. Molecular search results show preference for both hydrophobic and hydrophilic interactions. The numbers of favorable interactions are large, and relative binding energies are low. The arms of the β-turn tetramer interact through hydrogen bonding with the (OH)-7_A group. Methylene protons from NH₃⁺-CH₂ and the C-terminal (CH₂)_α methylene group interact with H-6_A and H-8_A, respectively. The γ and δ hydrogen atoms on the proline ring interact hydrophobically with the H-8_A proton at 2.64 Å and 2.80 Å, respectively.

enough to play a significant role in Boltzmann summations, e.g., in NMR coupling constants and the weighting of NOE data. This relative energy dropped substantially with the Amber force field.

Searches were also carried out with the Amber force field. One of the low-energy conformers is shown in figure 14. Primary contacts are through the β-turns interacting with both the (OH)-3_B and (OH)-4_B hydroxyl groups, and with the proline methylene hydrogen atoms interacting directly with the C-ring hydrogen atoms. The Amber force field favored A-conformer catechin low energy conformers.

5. CATECHIN-(4α→8)-CATECHIN/GPGG ION COMPLEX

Only a few searches were done on this complex structure, and figure 15 illustrates one of the low-energy structures found using the MacroModel protocol. Several low-energy structures were found that showed the glycine arms expanded between the upper and lower units of catechin. The lowest-energy axial conformer

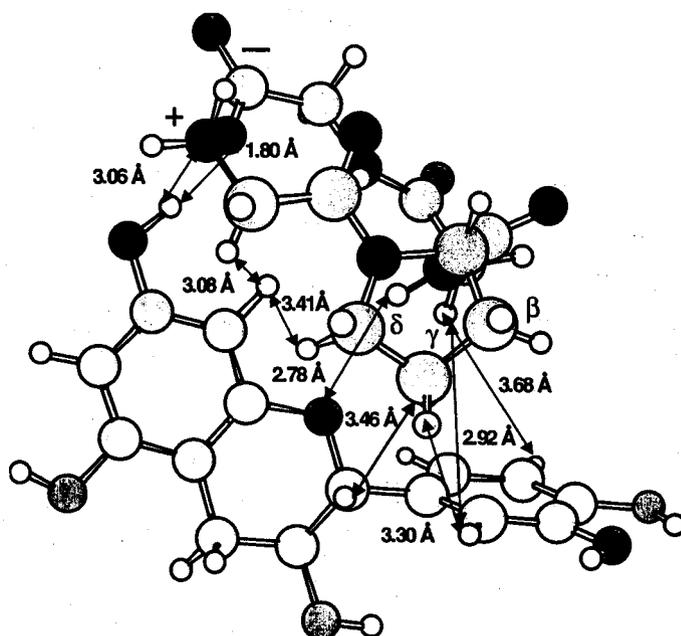


Figure 12. This figure illustrates the diversity of interactions with the combination of hydrophilic and hydrophobic contacts. Here, the β -turn arms are interacting directly with the pyran oxygen atom. The C-terminal α -methylene group shows interaction to the H-8_A proton (3.46 Å) whereas the proline methylene hydrogen atoms (γ and δ) interact through hydrophobic contact with the H-2_C, H-2_B and H-5_B protons.

complex found is higher in energy by nearly 8 kJ/mol from the equatorial counterpart. One structure, illustrated in figure 15, shows the β -turn arms interacting with the upper A-ring and lower E-ring. There is also sufficient hydrophobic interaction between the proline hydrogen atoms and the A-ring. Specifically, the $\text{NH}_3^+\text{-CH}_2$ is 2.70 Å from H-6_A and 5.38 Å from H-8_A, with the $\text{C}_{\text{terminal}}\text{-(CH}_2\text{)}_1$, 2.59 Å from H-8_A. The proline ($\text{C}_\gamma\text{H}_2$) is 4.17 Å from H-8_A and the ($\text{C}_\delta\text{H}_2$) is 2.86 Å from H-6_A. Several close hydrogen-bonding contacts are present; for example, NH to (OH)-7_A, 2.36 Å. The terminal CO^- to (OH)-4_E shows an unrealistically short hydrogen bonding distance of 1.3 Å. This is probably what causes the β -turn to rotate into a more open configuration for the outer glycine torsion angles. This result comes from two 3000-step MC MacroModel searches, so other potentially low-energy conformer complexes are possible. However, this shows the propensity for interaction between the upper and lower units as was observed in the NOE experiments.¹⁵

6. CONCLUSIONS

Molecular modeling has been used to search conformational space for the self-association of catechin and the complexation of catechin with L-proline

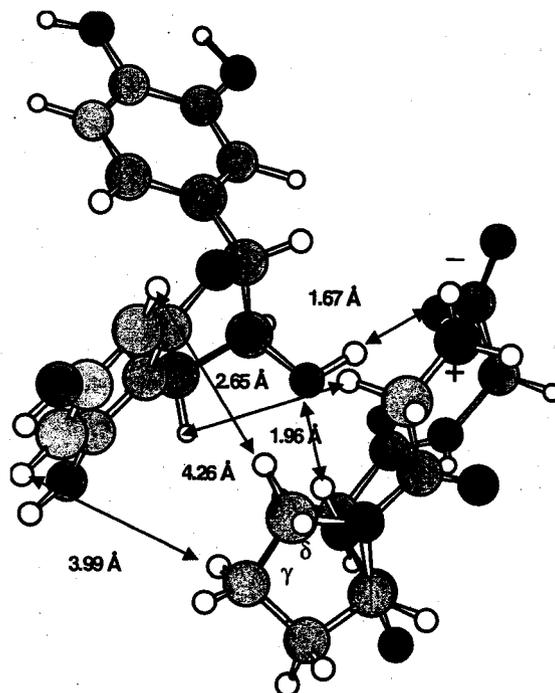


Figure 13. A low-energy search conformation found with interaction between H-4_C of catechin and glycine in GPGG ion. Catechin in an A-conformation is always seen in the ensemble of low-energy complexes. The relative energy here is 3.0 kJ/mol. The ¹H NMR spectrum shows $J_{2,3} = 8$ Hz, indicating some axial-conformation. There is commonly H-H contact between the H-4_C, H-8_A, and methylenes of glycine. Close contact is seen between H-8_A and proline C_δ hydrogens (2.80 Å) in this conformer.

glycine oligomeric peptides, including the glycy-L-prolyl-glycyl-glycine zwitterion tetramer (GPGG). Monte Carlo MacroModel conformational searching was applied using the Merck force field (MMFF) with the GB/SA water solvent model. Interatomic contact distances between the two moieties in the complexes were compared with results from NOE NMR experiments. Searching for polyphenol complexes looks very promising for giving insight into ways in which catechin might self-associate, and how polyphenols interact with proline-based molecules. Binding energies calculated from the difference in energy between the complex and the isolated molecules show significant association. The MMFF force field in its native state, however, seems to overweight hydrogen bonding even when using a water solvent model. Catechin/catechin self-association has been established by NMR NOE experiments and verified by MacroModel molecular complex conformational search methods. A series of loosely-bound low-energy catechin/catechin conformer complexes that show both hydrophilic and hydrophobic interactions

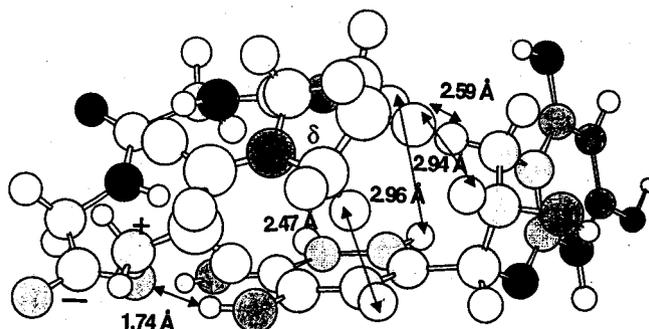


Figure 14. This $-1,067$ kJ/mol GPGG ion/catechin conformer (Amber force field), with a relative energy of 1.7 kJ/mol, shows the interaction of the β -turn arms with the B-ring hydroxyl groups. There is considerable hydrophobic interaction between the proline H-atoms and H-2_C and the glycine methylene groups and H-2_B and H-5_B. The catechin is favored in A-conformer form.

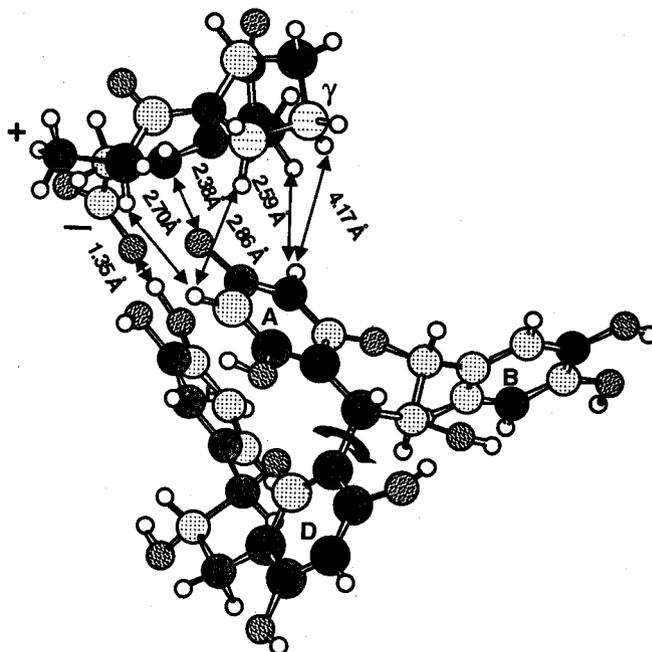


Figure 15. This shows the lowest energy structure found so far in the search in the interaction of the GPGG ion with the B3 dimer [(+)-catechin-(4 α \rightarrow 8)-(+)-catechin]. There is typically interaction found between the glycine arms and the upper and lower units of B3. For example, NH₃⁺-CH₂ to H-6_A is 2.70 Å.

were found. The low-energy structures were B-ring aligned (parallel) and crossed at the pyran rings. These show NOE contact properties like those found in the NMR NOE experiments.

Catechin/GPGG ion complexes have been shown to exist by molecular conformational analysis using MacroModel and the MMFF and Amber force fields. It has been shown that the GPGG zwitterion occurs in a β -turn structure, and that the glycl arms interact with the various OH groups of catechin through hydrogen bonding. The methylene groups and proline hydrogen atoms show hydrophobic contact with the catechin A- and B-rings. Further work is needed with some of the other hydrogen-bonding functions that are available. It appears that the strong hydrogen bonding for the ions may be over-weighted. Finally, this work shows that the combination of the interplay between molecular modeling experiments and NMR experiments is important to guide the direction of modeling structural studies. Complexes formed with the B3 dimer show interactions between the GPGG ion and the upper A-ring and the lower E-ring. Other low-energy conformers for the dimer structure are possible.

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