Rational Molecular Fragmentation Model As A Method For The Structural Analysis Of Large Drugs: A Case Study Concerning The Conformations Of Carvedilol In Terms Of Quantum Theoretical And Experimental NMR Spectroscopy

Theses of the Ph.D. Dissertation by

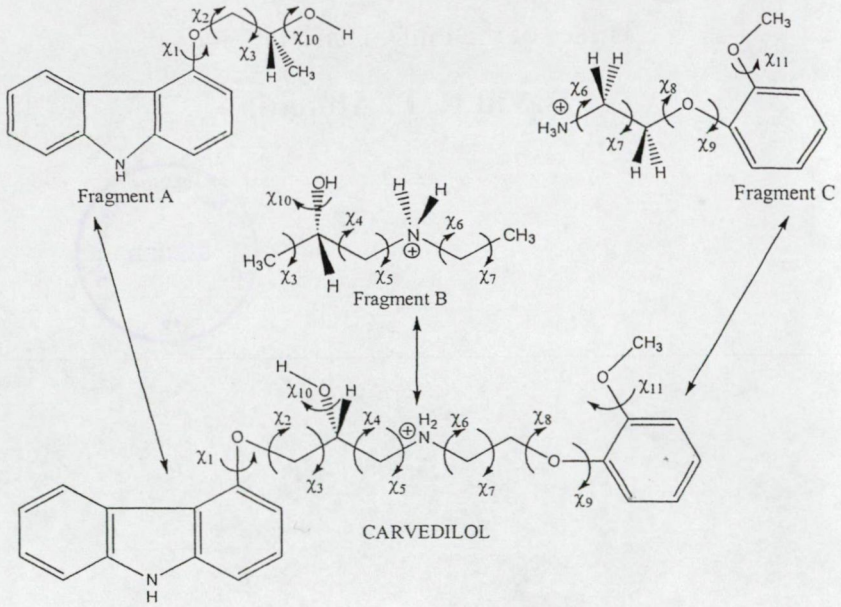
David R. P. Almeida

Institute of Pharmaceutical Chemistry
University of Szeged
Szeged, Hungary

2004

ABSTRACT

The pharmaceutical carvedilol acts as a non-selective beta (β_1/β_2) and selective alpha (α_1) adrenoceptor antagonist, cardioprotector, antioxidant, oxidative phosphorylation uncoupler, and amyloid-beta ($A\beta$) anti-fibrillar agent. Given its diverse pharmacodynamic profile, the resolution of carvedilol's highly populated conformations are a necessary prerequisite to divulging the basis of its molecular interactions. However, given carvedilol's sizeable conformational potential energy hypersurface (PEHS) - 11 torsional angles and 177 147 (3^{11}) conformational possibilities - this task requires a creative approach as traditional brute force multi-dimensional conformational analysis (MDCA) is impractical. Using carvedilol as a case study, a novel theoretical method entitled *rational molecular fragmentation* was developed for the structural and conformational study of complex drug molecules that minimizes computational and experimental resources. Rational molecular fragmentation was based on dividing carvedilol into simpler pharmacophore fragments (carbazole Fragment A, secondary amine Fragment B, and di-substituted benzene Fragment C) with manageable PEHSs but still relevant to the electronic structure of whole carvedilol.

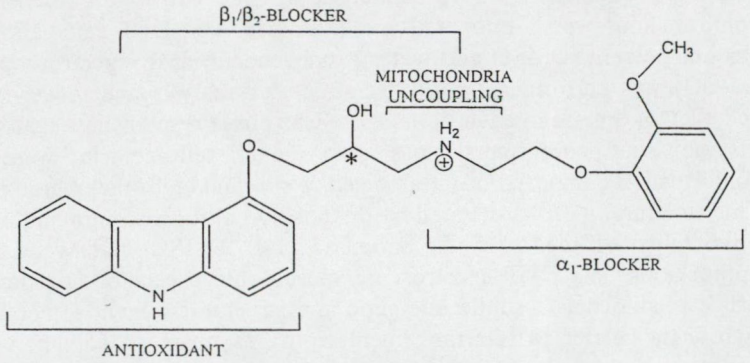


Each fragment was analyzed *via* gas phase Hartree-Fock [RHF/3-21G and RHF/6-31G(d)] and B3LYP/6-31G(d) density functional theory (DFT with the Becke 3LYP hybrid exchange-correlation functional) MDCA to disclose and assess dominant structures, structural motifs, conformational intricacies, stereochemical relationships (i.e., point chirality and axis chirality), multiple proton conformational basicity (particular to primary and secondary amines) parameters, and intramolecular attractive forces (IMAF) relevant to each fragment and to carvedilol. The dominant (low energy) fragment conformations, once optimized and evaluated, were used to hypothesize and predict a total of 240 carvedilol conformers which were initially optimized at the RHF/3-21G level of theory revealing 121 converged structures. An authentic set of nine converged low energy conformations were discovered and examined with DFT calculations in gas and solvent (DMSO and water). Independent NMR spectroscopy (in DMSO) was performed for further structural analysis and to verify the accuracy of this fragmentation method. Gas phase results show that seven of the nine conformers possess a novel tetra-centric spiro-type conformation composed of intramolecular six- and eight-membered rings. This structural motif is dictated by the positive nitrogen centre and by the inflexibility of the carbazole aromatic ring. DMSO and water DFT optimizations and NMR spectroscopy closely mirror each other indicating that carvedilol has a subtle energetic and structural solvent effect and a significant barrier to re-arrangement from gas phase to solvent exists. Given the harmony achieved between theoretical and experimental results, this study suggests the most populated states of carvedilol expected to dominate physical and biological samples and gives credence to the ability of methodically analyzing complex molecular systems by means of theoretical structure-activity fragmentation. Together, this will critically aid the molecular understating of carvedilol's pharmacodynamic mechanisms and structural underpinnings.

1. INTRODUCTION

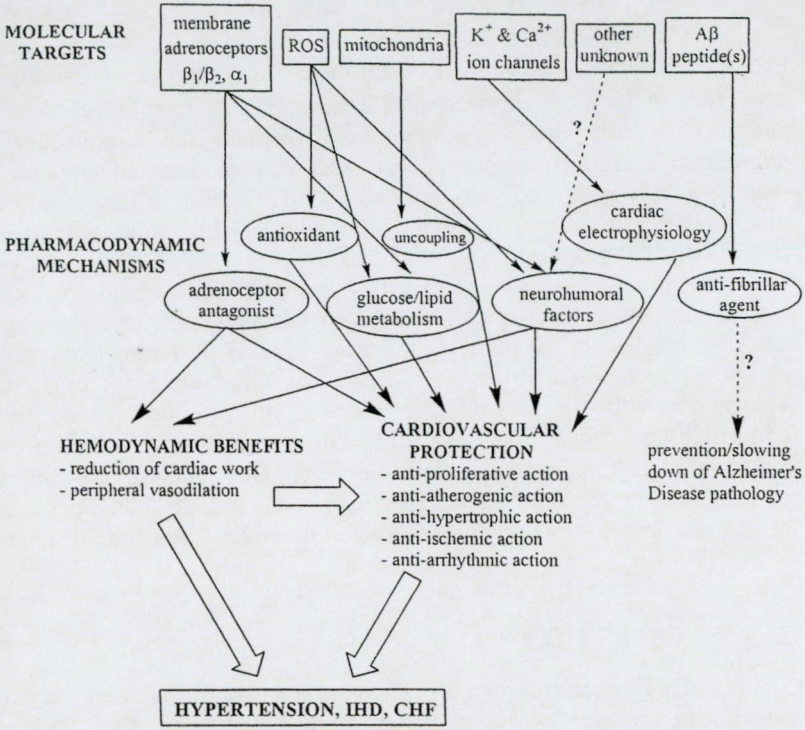
1.1 Biological & Pharmacodynamic Review of Carvedilol

The cardiovascular pharmaceutical carvedilol, 1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-2-propanol ($C_{24}H_{26}N_2O_4$), is a hydrophobic aryloxypropanolamine multiple-action neurohormonal antagonist. Carvedilol is used in the treatment of mild-to-moderate essential hypertension, stable angina pectoris, ischemic heart disease (IHD), and mild-to-moderate chronic congestive heart failure (CHF). (Note that * denotes stereocentre in figure below.)



Carvedilol provides hemodynamic benefits such as peripheral vasodilation and reduction in cardiac work from balanced non-selective β-adrenoceptor blockage (β_1/β_2) and selective α_1-receptor blockage. With regards to cardioprotection, carvedilol exerts anti-proliferative/anti-atherogenic, anti-hypertrophic, anti-ischemic, and anti-arrhythmic actions by means of antioxidant effects, improvement of glucose and lipid metabolism, modulation of neurohumoral factors, and beneficial cardiac electrophysiological properties (K^+ and Ca^{2+} ion channels).

Carvedilol protects mitochondria from oxidative stress by uncoupling oxidative phosphorylation *via* a weak protonophoretic (proton transfer) mechanism involving the amino group (pK_a = 7.9) of its side-chain. This phenomenon known as "mild uncoupling" occurs when a small decrease in mitochondrial electric potential induces a significant reduction in the ROS produced by the mitochondrial respiratory chain. It has been shown that carvedilol and its active hydroxylated analogues act as novel anti-fibrillar agents able to inhibit amyloid-beta (Aβ) fibril formation and may have uses in the prevention or slowing down of Alzheimer's disease (AD) pathology. It is currently not known if carvedilol binds to Aβ monomers, dimers, or other oligomers or what type of interaction occurs between carvedilol and the Aβ peptide(s).



Given the multi-faceted nature of carvedilol, it is necessary to reveal its complete molecular identity and conformational profile as a means to fully divulge the structural properties of its adrenoceptor binding conformation and further clarify its function in hemodynamic and cardioprotective mechanisms. Likewise, to expound carvedilol's role in antioxidant pathways, the uncoupling of oxidative phosphorylation in mitochondria, and with Aβ peptide(s) in AD, its conformational identity is requisite as conformation is a fundamental part of all of these processes. However, given carvedilol's 11 associated torsional angles and 177 147 (3^{11}) conformational possibilities [total is arrived at by multi dimensional conformational analysis (MDCA) where each torsional angle can assume *gauche plus*, *anti*, or *gauche minus* orientations], this task is exceptionally extensive. To remedy this quandary, we have developed a method entitled *rational molecular fragmentation* to study large complex molecular systems in great detail. The cardiovascular pharmaceutical carvedilol will be used as a case study illustrating how this method is developed, drawn on, and delegated to reveal the structural and conformational intricacies of a particular molecular system. Given the lack of novel methods available, it is hoped that this model can be extrapolated for further investigations.

1.2 Stereogenic Units of Carvedilol

Carvedilol exists as a racemic mixture of both enantiomers *R*(+) and *S*(-). However, carvedilol's enantiomers show marked stereoselective properties in that both stereoisomers have equal α_1-adrenoceptor blocking activity but only the *S*(-) enantiomer possesses the nonselective β-adrenergic antagonist activity. This represents an unusual situation in which enantiomers of an optically active drug differ not only quantitatively in terms of potency but also qualitatively in that they possess distinct pharmacologic profiles.

Optical isomerism provides evidence about the chiral disposition of electron density in space around the nuclei. Essentially, the chirality of the electron density exists irrespective of whether it is associated with a carbon carrying four different substituents (point chirality) or if it is associated with the asymmetric electron distribution caused by a conformational twist (axis chirality). Since chirality is central to the structure of any molecular system, the stereogenic units of carvedilol are investigated to highlight how point and axis chirality can be used to predict electronic structure and energetics of corresponding chiral minima.

2. AIM OF STUDY

This dissertation is carried out with a fourfold aim: (1) devise a novel structure-activity fragmentation method with substantial predictive power and minimal computational cost to assess and analyze the conformations of selected carvedilol molecular fragments and to predict the conformations of carvedilol; (2) evaluate the stereochemical relationships, emphasizing point chirality and axis chirality, of carvedilol since its pharmacologic profiles are unique in their stereochemical reliance; (3) examine the dependence of basicity (proton affinity) on conformation as is relevant to carvedilol's role as a physiological base in mitochondria uncoupling; (4) discover, determine, and depict the electronic structure of carvedilol's most populated (lowest energy) conformations expected to dominate physical and biological samples.

The comprehensive solution of carvedilol's conformational character and related structural parameters will greatly aid the molecular discernment of its pharmacodynamic mechanisms. Furthermore, satisfactory success of the application of this developed rational molecular fragmentation method for the structural analysis of carvedilol may indicate its viability for the study of other complex molecular systems with regards to broad prediction and analysis of electronic structure, stereochemical, and conformational parameters.

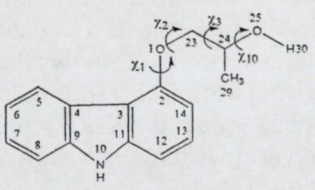
3. METHODS

Gas and solvent (Onsager solvent reaction field method) phase theoretical *ab initio* [restricted Hartree-Fock, RHF/3-21G and RHF/6-31G(d)] and density functional theory [DFT, B3LYP/6-31G(d)] quantum chemical molecular orbital (MO) computations were employed for the development of the rational molecular fragmentation method and for all carvedilol and fragment structural, stereochemical, and proton affinity investigations. Additionally, MO computations were used to gauge the accuracy and ability of the devised method to predict the preferred conformations of carvedilol. All computations were carried out using GAUSSIAN98 software program. NMR spectroscopy was utilized for the structural analysis of carvedilol, to compare experimentally- and theoretically-determined structures, and to further assess the success of the molecular fragmentation approach.

THESES

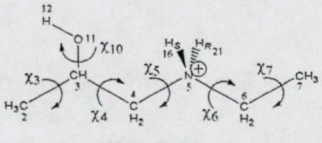
Thesis #1

Using the pharmacophore, I have divided carvedilol into three molecular fragments as the basis of the model of rational molecular fragmentation: *R*- and *S*-4-(2-hydroxypropoxy)carbazol (Fragment A), 2(*R* and *S*)-1-(ethylamonium)propane-2-ol (Fragment B), and aminoethoxy-2-methoxy-benzene (Fragment C).



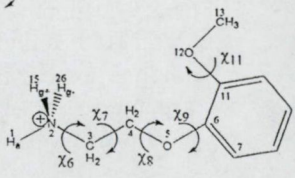
χ_1 = C23-O1-C2-C3 χ_3 = C29-C24-C23-O1
χ_2 = C24-C23-O1-C2 χ_{10} = H30-O25-C24-C23

Fragment A



χ_4 = N5-C4-C3-C2
χ_5 = C6-N5-C4-C3
χ_6 = C7-C6-N5-C4
χ_{10} = H12-O11-C3-C2

Fragment B

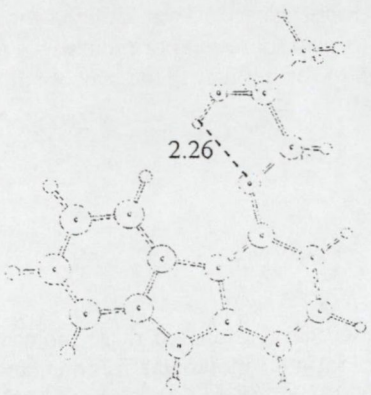


χ_7 = O5-C4-C3-N2 χ_9 = C7-C6-O5-C4
χ_8 = C6-O5-C4-C3 χ_{11} = C13-O12-C11-C6

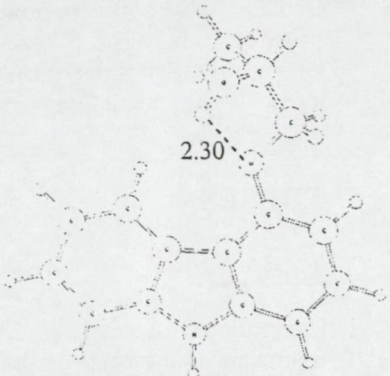
Fragment C

Thesis #2

I have performed gas phase MDCA on *S*- and *R*-Fragment A at the RHF/3-21G, RHF/6-31G(d), and B3LYP/6-31G(d) levels of theory and discovered 19 DFT optimized minima out of a possible 81 conformational states (23%). I have presented the dominant intramolecular attractive forces (IMAF) of *R*-Fragment A to be H-bonding between O1 and H30 leading to the formation of an intramolecular five-membered ring which confers both electrostatic (*via* the H-bond) and steric (*via* the five-membered ring) relaxation to the side-chain of Fragment A. (DFT relative energies of the structures below is indicated in parentheses in Kcal•mol^{-1}.)



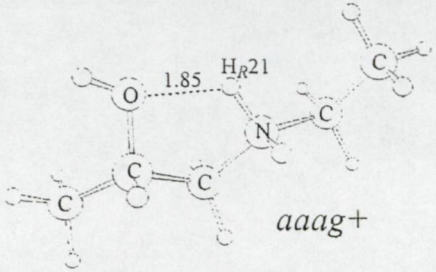
aaag+ (0.00)



aag-g- (0.87)

Thesis #3

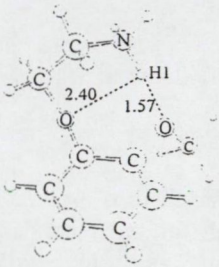
I have optimized *S*-Fragment B with MDCA at the RHF/3-21G level of theory to uncover a total of 18 converged minima out of a possible 81 (22.2%). I found the dominant structural characteristic to be that all minima possess torsional angle χ_{10} in the *g*+ position and all low energy structures possess an H-bond between O11 and the H_S/H_R protons resulting in a five-membered ring similar to the global minima *aaag*+.



aaag+

Thesis #4

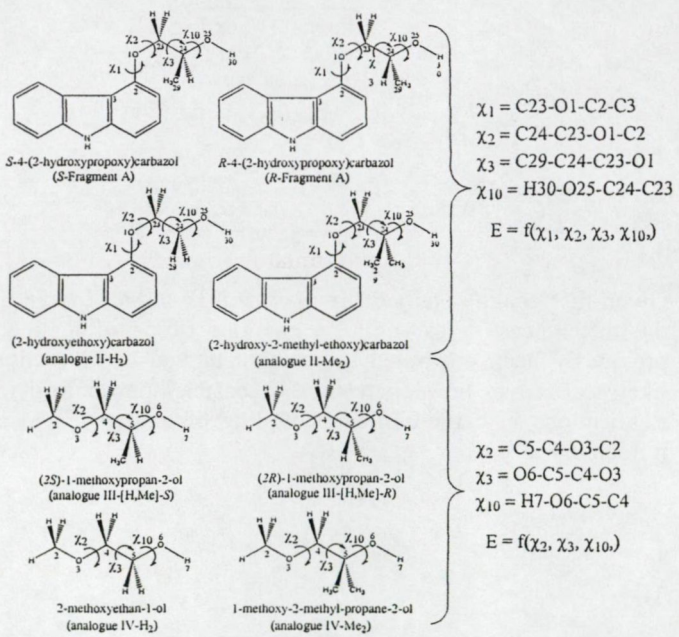
I have performed MDCA on Fragment C at the RHF/3-21G level of theory and have revealed that, instead of the expected 81 conformers, a total of 24 converged minima were found (30% convergence). I have discovered that the Fragment C PEHS global minima is conformer *g+g+g+g-* which possess an eight-membered ring formed by a short H-bond between H1 and O12 and a longer H-bond between H1 and O5 forming a five-membered ring.



g+g+g+g-

Thesis #5

I have analyzed and presented the structural and energetic basis of point chirality and axis chirality by MDCA of *S*- and *R*-Fragment A along with designed pro-chiral and chiral analogues II-H_2, II-Me_2, III-[H,Me]-*S*, III-[H,Me]-*R*, IV-H_2, and IV-Me_2. All structures were optimized at the RHF/3-21G, RHF/6-31G(d), and B3LYP/6-31G(d) levels of theory to illustrate the stereochemical relationships of carvedilol and its fragments.



Thesis #6

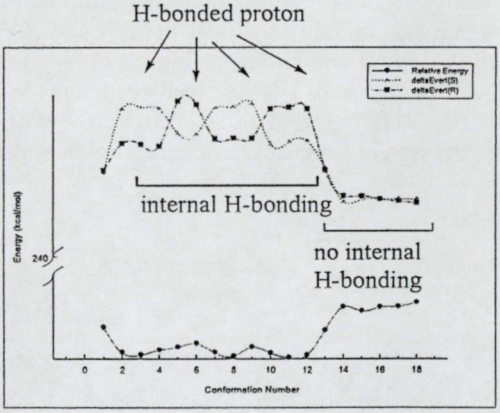
I have determined that the energetic and structural *R*-carvedilol parameters presented can be promptly extrapolated to *S*-carvedilol as all enantiomeric minima will be paired up according to the equation below. Optimized parameters for a converged minima of *R*-carvedilol require the switching of point chirality from the *R*- to *S*-stereoisomer followed by the switching of optimized torsional angle geometries (i.e., axis chirality) from clockwise to counterclockwise rotation.

$$E_R = E_S$$

$$f_R(\text{index of } \chi_1 \text{ to } \chi_{11}) = f_S(\text{index of } -\chi_1 \text{ to } -\chi_{11})$$

Thesis #7

I have shown that there is a direct dependence of multiple proton basicity on conformation for the secondary amine Fragment B and the primary amine Fragment C as protons will be biased depending on conformation and IMAF adopted. For both fragments, conformers devoid of IMAF display lower energies of deprotonation. (The graph below displays the basicity character of Fragment B.)



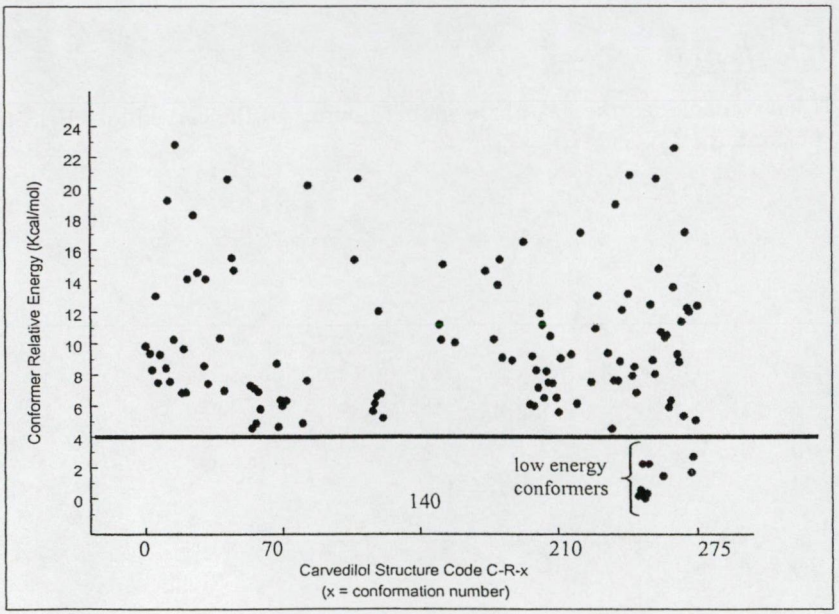
Given the results obtained for Fragment B and C, I have postulated that the differences in carvedilol's energies of deprotonation, which have previously been attributed to uncharacterized conformation factors, are likely related to the dependence of basicity on conformation. This has implications in carvedilol's uncoupling of oxidative phosphorylation in mitochondria.

Thesis #8

I have utilized converged low energy (defined as possessing a conformer relative energy ≤2.00 Kcal•mol^{-1}) Fragment A, B, and C conformations to predict a comprehensive list of 240 non-redundant *R*-carvedilol conformers hypothesized to be low energy states. I have optimized the 240 predicted structures at the RHF/3-21G level of theory and discovered a total of 121 unique carvedilol conformations spanning a rage of 275 conformational assignments (121/275 = 44%) and possessing a range in relative conformer energy of ~23 Kcal•mol^{-1}.

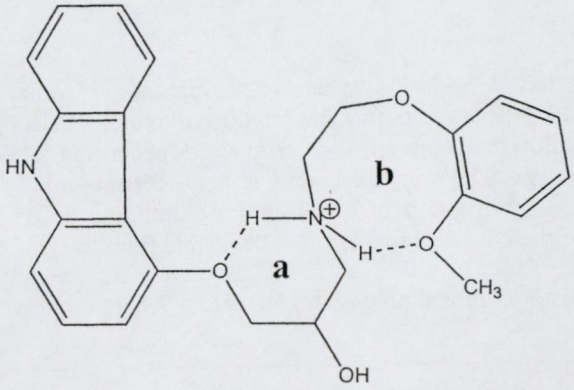
Thesis #9

I have selected a definitive and authentic set of nine Hartree-Fock optimized low energy conformers for the carvedilol PEHS by analyzing a graphical plot of all converged conformations according to their respective relative energy. The nine low energy structures are bound by a conformer relative energy of less than four Kcal•mol^{-1} and are clearly divided from the rest of the converged structures. I selected the nine conformations, C-*R*-246 to C-*R*-251, C-*R*-258, C-*R*-272, C-*R*-273, for further gas and solvent phase optimization with high level DFT calculations.



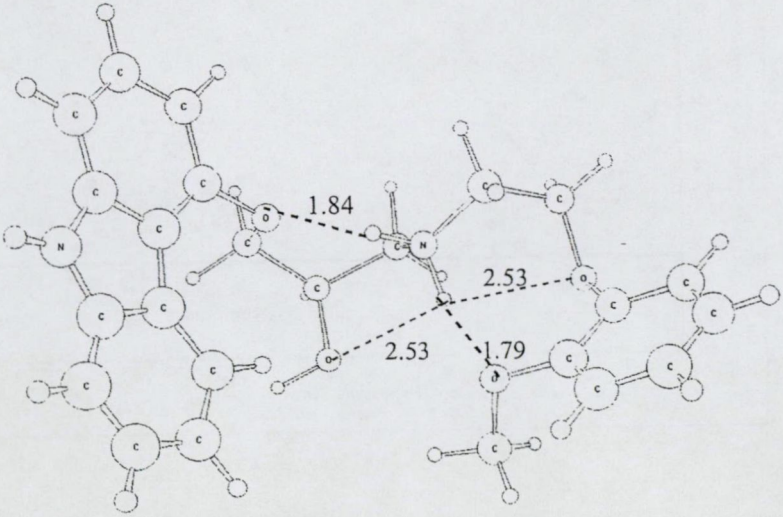
Thesis #10

I have optimized the nine low energy carvedilol structures in the gas phase at the B3LYP/6-31G(d) level of theory (all nine conformations exhibit a gas phase relative energy of less than two Kcal•mol^{-1}) and determined that seven (C-*R*-246 to C-*R*-250, C-*R*-258, and C-*R*-272) of the nine conformations possess a novel "tetra-centric" structural motif. The tetra-centric conformation consists of the 13-membered aromatic carbazole ring (centre 1), an O···H-N H-bonded six-membered ring (ring **a**; centre 2), an O···H-N H-bonded eight-membered ring (ring **b**; centre 3), and the di-substituted benzene ring (centre 4).



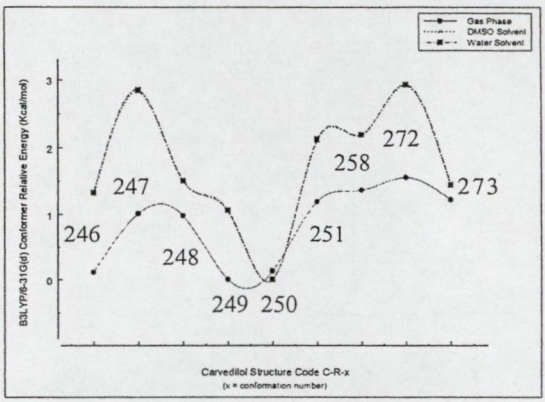
Thesis #11

I have concluded the gas phase global minima of the carvedilol PEHS to be conformer C-*R*-249.



Thesis #12

I have characterized the solvent effect of carvedilol with DFT DMSO and water solvation optimizations and have displayed that carvedilol has a subtle solvent effect with regards to energetics and electronic structure. On the whole, DMSO and water produced the same solvent effect and most structures preferred the tetra-centric motif seen in the gas phase suggesting there are significant conformational transition state energy barriers that affect the interconversion rates between the different conformational states. (Note that the DMSO and water relative energies extensively overlap each other in the figure below.)



Thesis #13

I have theoretically resolved carvedilol's conformational surface along with all torsional angle orientations necessary for carvedilol to assume its low energy states (summarized in the equation below).

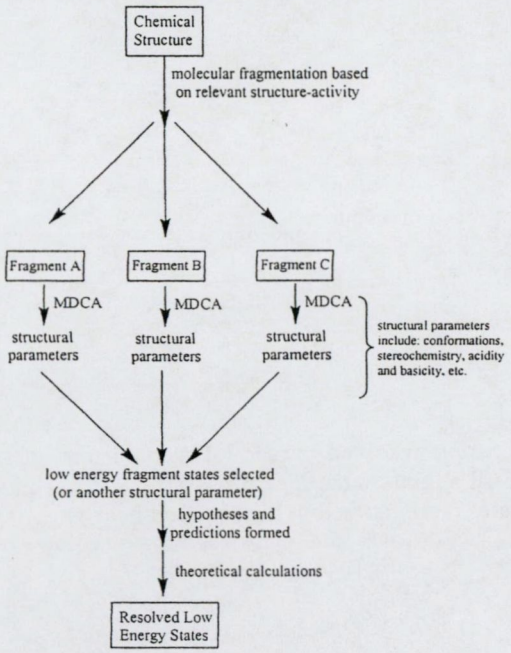
$\chi_1 = g+/a$ $\chi_2 = a$ $\chi_3 = g-$
$\chi_4 = g+$ $\chi_5 = g+/a$ $\chi_6 = g+/a/g-$
$\chi_7 = g+/g-$ $\chi_8 = g+/a/g-$ $\chi_9 = g+/g-$
$\chi_{10} = g+$ $\chi_{11} = g+/a/g-$

Thesis #14

I have determined from ROESY spectra (in DMSO) that there is rigid motion about torsional angles χ_1, χ_2, χ_3, χ_4, and χ_5 as determined from the fact that protons H39/H40 and H44/H45 are not equivalent, and as a result, rotation about these torsional angles is likely hindered by strong and persistent intramolecular H-bond networks. Thus, I have concluded that the independent NMR ROESY spectra substantiate the DFT optimized structures.

Thesis #15

I have evaluated that, according to conformation distribution, eight (χ_2, χ_5, χ_6, χ_7, χ_8, χ_9, χ_{10}, and χ_{11}) of the 11 torsional angles were accurately predicted (72.7%) using the rational molecular fragmentation model. Although carvedilol possesses an exorbitant amount of conformational possibilities, by utilizing a fragmentation approach based on the basic thermodynamics precept that only low energy states are significantly occupied, the highly populated states of carvedilol have been resolved. The rational molecular fragmentation method applied to carvedilol relies not on profligate computing force, but rather on the study of rationally-constructed fragments to generate PEHS points with some amount of energy minimization. This approach simplifies PEHS sampling because it is focused on hypothesized highly populated states.



ACKNOWLEDGEMENTS

The author wishes to acknowledge the encouragement and moral support of Professor Ferenc Fülöp. The author is also grateful to Mr. Jose Almeida, Ms. M. Fernanda P. Almeida, Ms. Donna M. Gasparro, Mr. Luca F. Pisterzi, Dr. Tamàs A. Martinek, and Professor Imre G. Csizmadia for their inspiration and guidance during this research as well as during the preparation of this dissertation.

The Dissertation is based on the following Published or Accepted or Submitted Papers

1. **David R. P. Almeida**, Luca F. Pisterzi, Gregory A. Chass, Ladislaus L. Torday, Andras Varro, Julius Gy. Papp, and Imre G. Csizmadia. A density functional molecular study on the full conformational space of the *S*-4-(2-hydroxy propoxy)carbazol fragment of carvedilol (1-(9H-Carbazol-4-yloxy)-3-[2-(2-methoxy-phenoxy)ethylamino]-2-propanol) in vacuum and in different solvent media, J. Phys. Chem. A, 106 (**2002**), 10423-10436.

   IF: **2.765**

2. **David R. P. Almeida**, Donna M. Gasparro, Luca F. Pisterzi, Ladislaus L. Torday, Andras Varro, Julius Gy. Papp, and Botond Penke. Gas phase conformational basicity of carvedilol Fragment B, 2(S)-1-(ethylamonium)propane-2-ol: An ab initio study on a protonophoretic of oxidative phosphorylation uncoupling, J. Mol. Str. (THEOCHEM), 631 (**2003**), 251-270.

   IF: **1.014**[§]

3. **David R. P. Almeida**, Donna M. Gasparro, Luca F. Pisterzi, Ladislaus L. Torday, Andras Varro, Julius Gy. Papp, Botond Penke, and Imre G. Csizmadia. Molecular study on the enantiomeric relationships of carvedilol Fragment A, 4-(2-hydroxypropoxy)carbazol, along with selected analogues, J. Phys. Chem. A, 107 (**2003**), 5594-5610.

   IF: **2.765**[†]

4. **David R. P. Almeida**, Donna M. Gasparro, Luca F. Pisterzi, Jason R. Juhasz, Ferenc Fülöp, and Imre G. Csizmadia. Conformational-dependent basicity of carvedilol Fragment C: An ab initio study on the primary amine, aminoethoxy-2-methoxy-benzene, J. Mol. Str. (THEOCHEM), 666-667 (**2003**), 557-580.

   IF: **1.014**[§]

5. **David R. P. Almeida**, Donna M. Gasparro, Luca F. Pisterzi, Jason R. Juhasz, Ferenc Fülöp, and Imre G. Csizmadia. Predicting the conformations of carvedilol based on its pharmacophore fragments: A gas phase and solvation ab initio and density functional study, J. Mol. Str. (THEOCHEM), 666-667 (**2003**), 537-545.

   IF: **1.014**[§]

6. **David R. P. Almeida**, Donna M. Gasparro, Ferenc Fülöp, and Imre G. Csizmadia. Pharmacophore fragment-based prediction and gas phase ab initio optimization of carvedilol conformations, J. Phys. Chem. A, (**2004**), in press.

   IF: **2.765**[†]

7. **David R. P. Almeida**, Donna M. Gasparro, Tamas A. Martinek, Ferenc Fülöp, and Imre G. Csizmadia. Resolution of carvedilol's conformational surface via gas and solvent phase density functional theory optimizations and NMR spectroscopy, J. Phys. Chem. A, submitted for publication (**2004**).

   IF: **2.765**[†]

PUBLISHED OR ACCEPTED PAPERS: **6**

Cumulative IF: **11.337**

SUBMITTED PAPERS: **1**

First Author in: **7**

[§] J. Mol. Str. (THEOCHEM) 2002 impact factor used because 2003 impact factor value not available at the time of printing this dissertation.

[†] J. Phys. Chem. A 2002 impact factor used because 2003 and 2004 impact factor values not available at the time of printing this dissertation.