

I. Introduction

Generally, the structural elucidation of molecules should be performed in the following hierarchy: chemical composition (1-dimensional, 1D), constitution (2-dimensional, 2D), configuration and conformation (3-dimensional, 3D, *Figure 1*). It is worth to note that while the 1D and 2D structures are static properties, the 3D ones are dynamic molecular properties. Due to dynamic conformational flexibility, a molecule in a given conformation may possess optical activity. This is the reason why we can talk about conformational chirality and the concepts of configuration and conformation have the same rank with respect to structural determination.

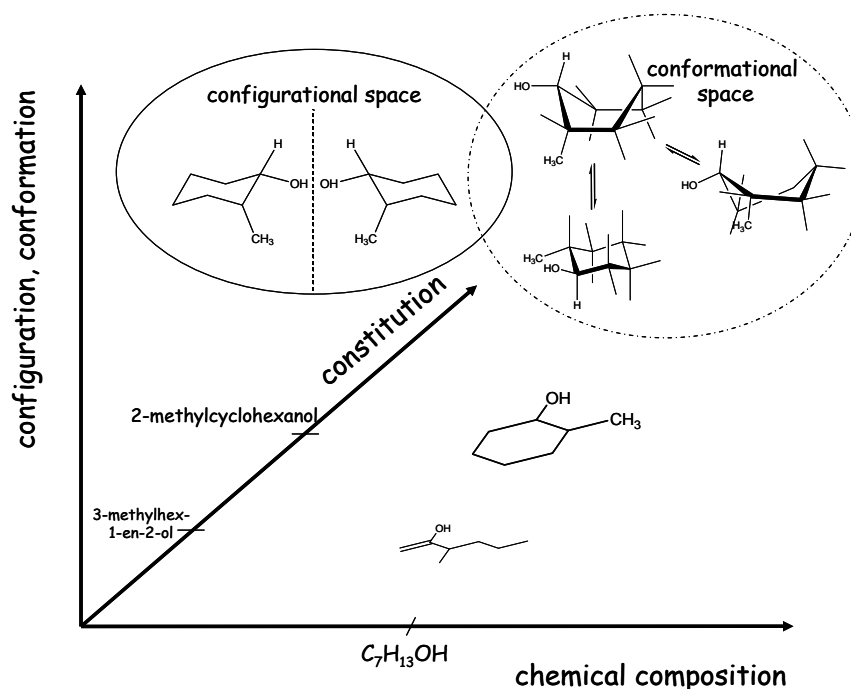


Figure 1. Hierarchy of the Elucidation of Molecular Structures.

In order to differentiate 2D structural isomers of a molecule, we should define chemical composition and distinct constitutions. Furthermore, the complete characterization in 3D needs the description of the molecular configurational and conformational space.

Nowadays the determination of the chemical composition and constitution of a molecule is a routine task. In some cases, the elucidation of the possible molecular conformations is not so difficult either: e.g., NMR and/or XRD studies of biopolymers. However, experimental determination of the geometries of all the

conformers of a molecule is a great challenge and rather hopeless task. The source of the problem is the difference between the cardinalities of the configurational and conformational spaces. Generally, the number of elements in the conformational space is substantially greater than the number of configurational states. In case of molecules possessing chirality the number of chiral isomers is not greater than 2^n , where n is the number of geometrical elements causing chirality. Whereas, in case of conformational isomers the upper limit is d^n , where n is the number of torsional angles and d is the maximum of the local minimums occurring at the individual free torsional angles (usually $d \gg 2$). Identification of the configuration of a typical protein (300-400 monomers) would be really difficult if the nature had not preferred one of the amino acid configurations. Of course, there are some favored conformations, so called conformers and/or active conformers; however, observing and identifying such structures are rather difficult. The theoretical quantum chemical methods open the door to treat molecules as individual objects and to investigate the conformational hypersurfaces of them.

Theoretical study of the conformational flexibility of biologically active molecules is of utmost importance. Knowledge of each possible conformer is essential, since it is far from certain that the biologically active conformer corresponds to the global minimum of the molecular potential energy surface. The simplest class of molecules with high conformational flexibility is that of the unbranched alkanes. In spite of numerous studies and even books on this topic, it is still not clear how many conformers exist for the individual members of this homologous series. Even for an n -alkane molecule due to its flexible carbon backbone the question of the number of possible conformers arises. Further important questions are: (i) what are the relative stabilities of the conformers and (ii) how can they transform to each other? After all, the macroscopic physical and chemical properties of n -alkanes are statistical averages over the ensembles of the conformers. The conformational enumeration problem of n -alkanes cannot be solved by means of abstract mathematics only and the current advanced

experimental techniques cannot give a decisive argument in this case either. At present, the best solution seems to be the combined application of theoretical chemical (physical) and abstract mathematical tools. By conventional quantum chemical treatment, the various conformers of a given molecule are related to the local minima of its conformational potential energy surface (PES) deduced from the approximate solution of the non-relativistic, time-independent Schrödinger equation within the Born–Oppenheimer approximation. As far as the saddle points of the conformational PES are concerned, the most important first-order ones (transition states) connect the conformers to each other. Accordingly, the topology of the conformational PES determines the conformational properties of a molecule. Figure 2 shows the well known conformational energy diagram of butane.

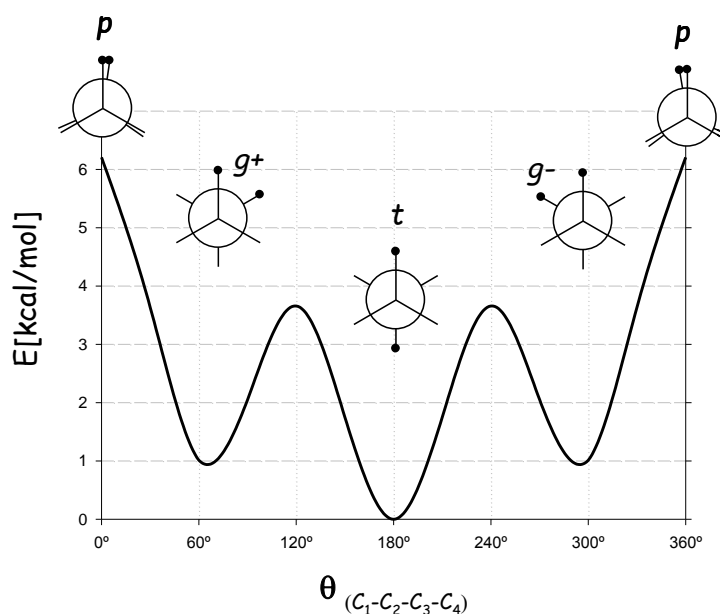


Figure 2. The Conformational Diagram of Butane.

gauche-type conformations ($g^+ \sim +60^\circ$, $g^- \sim -60^\circ$), antiperiplanar ($t \sim 180^\circ$), synperiplanar ($p \sim 0^\circ$)

The learned conformational situation occurring at butane use to extend to higher elements of homolog series and assume that, the all-trans conformer belongs to the global minima of the potential energy surface. In combinatoric studies, it is generally assumed that any isomer of n -alkanes can be embedded into the crystal

lattice of diamond. It follows from the foregoing that, the number of possible conformers is not larger than 3^n , where n is the number of the rotatable C-C bonds.

Circadian rhythms are fundamental to the behavior and physiology of all higher organisms expressed at the level of organism, tissue, and cell. In mammals, the suprachiasmatic nuclei contain the principal circadian clock governing daily rhythms of physiology and behavior. The rhythm is entrained to the 24-hour period by the daily light-dark cycle, with hormone levels. Melatonin is a neurohormone, which is secreted by the pineal gland and the compound itself is a product of the tryptophan catabolism. The pineal gland reacts sensitively, when the intensity of light changing. If light get into the retina then the pineal gland suspends melatonin synthesis. As a consequence, the endocrine melatonin level is high during darkness and so melatonin has become known as the chemical expression of darkness or the hormone of darkness. Studies related to melatonin are fairly ramifying (Alzheimer disease, oxidative stress), nevertheless the most intensively investigated area connected to daily i.e. circadian rhythm (sleep disturbances, sleeplessness, jet-lag, shift-work, ageing). Melatonin provides information not only concerning the time of the day but the period of the year (seasonal - winter depression, annual - breeding). G-protein receptors mediate the chronobiological effects of melatonin. In mammals two high affinity melatonin receptors have been cloned until now. The role of different receptors has not clean yet, but despite of high level homology it seems that active sites are dissimilar. Abnormalities and disorders related to circadian rhythm can be treated by melatonin; however the use of melatonin as a drug is limited by its short half-life time (20-30 min) as well as its poor selectivity of action. From this point of view, design of receptor selective analogs has great significance.

II. Aims

According to the scientific literature, we think that the following studies are worthwhile:

❖ Alkanes

Our aim was to investigate the conformational potential energy surface of normal alkanes using *ab initio* and an approximate quantum chemical method parameterized on small hydrocarbon molecules and to enumerate the possible conformers.

❖ Melatonin

The active conformation of melatonin has not been identified yet and there are some contradictory publications concerning the geometry of the global minimum. Calculations at high level of theory have not been performed on the conformational potential energy surface of melatonin. Our aim was to accomplish a high level quantum chemical conformational search involved all those torsional angles, which affect the topology of the potential energy surface.

III. Methods

On account of the size of problems and limited available resources, the following generally accepted quantum chemical approach was used to explore the conformational space: the non-relativistic, time-independent Schrödinger equation was numerically solved within the Born–Oppenheimer approximation using the Hartree-Fock and LCAO-MO method.

III.a. Used software packages

Ab initio calculations were completed with the Gaussian 94 as well as the Gaussian 98 program packages. In order to generate initial geometries and evaluate

the results, computer programs were written.

III.b. Conformational Analysis of Alkanes

The SEOEM semiempirical model was used to find the conformers of alkanes until undecane. In case of butane, pentane, hexane, heptane and octane molecules HF/6-31G* and MP2/6-311G** calculations were also accomplished. The *ab initio* calculations were performed with the Gaussian 94 package on CRAY C90 and IBM SP2 computers. The initial geometries were prepared and the results were evaluated on a PC with the PcMol package. The SEOEM calculations were performed with the SEHMO program on CRAY C90, IBM SP2 and personal computers. For the enumeration of different conformers of unbranched aliphatic alkanes, a FORTRAN program was written. With the help of structural similarity calculations, the unique (nonisomorphic) conformers of *n*-alkanes were determined.

III.c. Conformational Analysis of Melatonin

In order to characterize the conformational space of the molecule, five torsional angles were selected (*Figure 3.*).

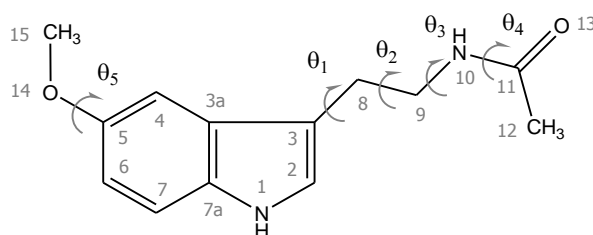


Figure 3. The Torsional Angles of Melatonin

First, 1500 trial geometries were generated by Monte Carlo method, which were then fully optimized without symmetry constraints at Hartree-Fock level of theory with STO-3G basis set (HF/STO-3G) to determine the local minima, i.e. conformers, on the conformational potential energy surface. Second, the

equilibrium geometries of the conformers obtained at HF/STO-3G level were fully optimized with 6-31G* basis set (HF/6-31G*). Through calculation of the eigenvalues of the Hessian, all the stationary points found were checked, i.e. the conformations kept were true local minima on the PES. Finally, on the basis of symmetry considerations further trial conformations were generated and then optimized. In order to generate these geometries and to classify conformers into equivalence classes Kylix and FORTRAN programs were written.

IV. New results

IV.a. Alkanes

With the help of *ab initio* HF/6-31G* and MP2/6-311G**, as well as semiempirical SEOEM calculations the conformational potential energy surface of pentane, hexane, heptane, octane, nonane and decane molecules was determined (Figure 4. a). According to the quantum chemical studies, one has to take into consideration two further favorable conformations; these conformers contain extended torsional angles $x^+ \sim +95^\circ$, $x^- \sim -95^\circ$ (Figure 4. b).

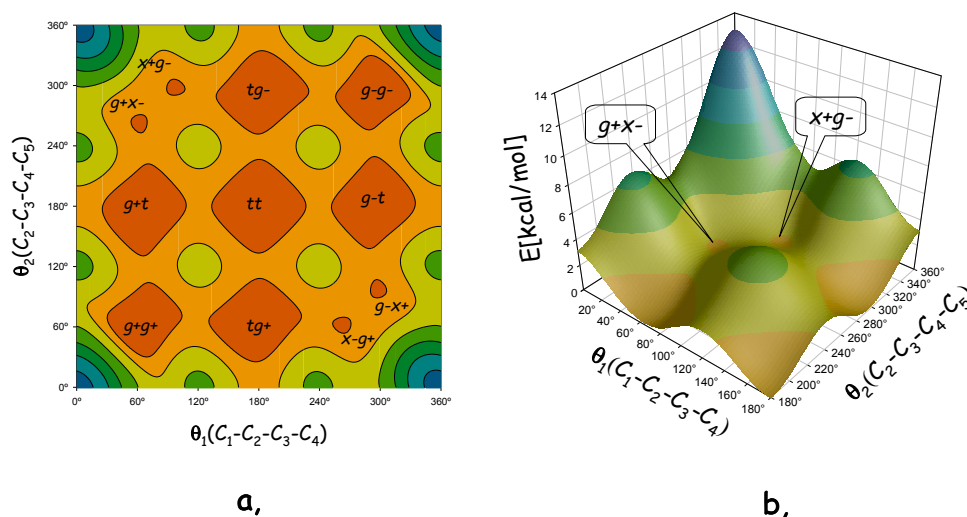


Figure 4. a, The Conformational Contour Plot of Pentane
 b, The Conformational Potential Energy Surface of Pentane Close to $g+x^-$ and $x+g^-$ conformations

The gas-phase standard heats of formation of unbranched alkanes were

determined from the quantum chemical calculation results. The calculated values for the C₅-C₁₀ molecules closely matched the experimental values. The results obtained for pentane, hexane and heptane were utilized to derive four rules with which the number and sequences of the existing conformers up to decane could be reproduced (Table 1.). The validity of the rules was confirmed at Hartree-Fock and second-order Moeller-Plesset levels too. The rules demonstrate that the most important factors governing the conformational behavior of unbranched alkanes are the non-bonded repulsive-attractive (van der Waals) interactions between the hydrogen atoms attached to the carbon atoms at positions 1-4, 1-5, 1-6 and 1-7.

Table 1. Number of Possible Conformers According to the Four Rules and Founded by SEOEM Method as well as Experimental and Calculated Gas-Phase Standard Heats of Formation of Unbranched Alkanes

<i>n</i> -alkane	n	3 ⁿ	(I)-(IV) rules	Found conformers	Experimental value [kJ/mol]	Calculated value [kJ/mol]
butane	1	3	3	3		
pentane	2	9	11	11	-146.9	-146.8
hexane	3	27	35	35	-167.1	-167.0
heptane	4	81	107	107	-187.7	-187.4
octane	5	243	339	339	-208.6	-207.8
nonane	6	729	1073	1073	-228.2	-228.2
decane	7	2187	3375	3375	-249.5	-248.7
undecane	8	6561	10633	10633	-270.9	-269.8

We solved the whole conformational enumeration problem of *n*-alkanes. Some recurrence relations were derived to enumerate conformers. Most probably the rules determining the sequences of the conformers slightly depend on the force field applied. Nevertheless, the graph theory method can be applied in every case.

IV.b. Melatonin

The conformational analysis conducted by us is one of the most extended and the highest level investigations concerning the conformational space of

melatonin up to now. All those torsional angles were involved, which could significantly affect the topology of the conformational PES of the molecule. 1500 geometries generated by Monte Carlo method were optimized at Hartree-Fock *ab initio* level using STO-3G and 6-31G* basis sets. With the help of equivalence relations, the conformational hyper surface was divided into equivalence classes. According to the ρ equivalence relation, the 128 conformers can be divided into 17 equivalence classes and all the classes can be divided into two subclasses applying σ except two (*Figure 5*). In case of ρ $128=15\cdot 8+2\cdot 4$, considering σ $128=30\cdot 4+2\cdot 4$. It can be concluded that the side chains of melatonin are enantiotopic groups and conformers are correlated with each other as enantiomer, epimer, or diastereomer pairs. Since the active conformation of melatonin is probably a folded structure, we can say that melatonin has conformational planar docking chirality. Based on these considerations, it is possible to explain the outcomes of restricted chiral melatonin analog experiments. It is suggested that conformers belonging to different equivalence classes should react with different receptor sites and elements of equivalent classes of *3re* and *5si* face side of the indole ring prefer the MT₁, while *3si* face side ones favor the MT₂ melatonin receptor site. We think that the knowledge of stereochemistry of the hormone of darkness can be the key to understand the interaction between the central chiral melatonin analogs and their receptors.

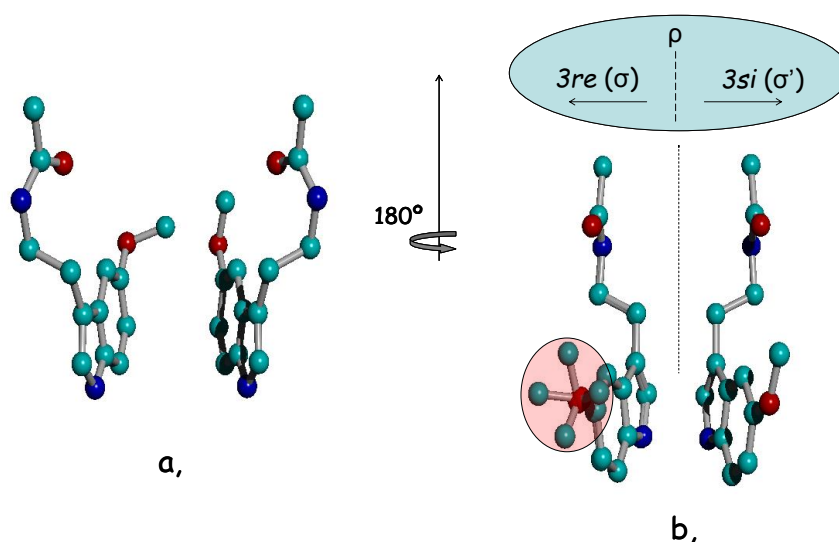


Figure 5. The Equivalence Relations ρ and σ .

a, two elements from a ρ class ($x^+ t x^+ t x^+$ and $x^- t x^- t p$)

b, connection between ρ and σ classes (left side: superposition of 4 conformers belong to σ , right side: an element of class σ' , it has a mirror image pair in σ)

There have been published several papers including different implicit requirements considering the active conformation of melatonin. We think that one of the most important result of our investigations is the relationship between implicit models established by Sicsic, Marot, Sastre and the HF/6-31G* conformational space of melatonin obtained by us. As the conformers $x^+ g^- x^- p x^+$ and $x^- g^+ x^+ p x^-$ meet all the requirements of the three quite different sets of conditions, one should pay distinctive attention to them (Figure 6).

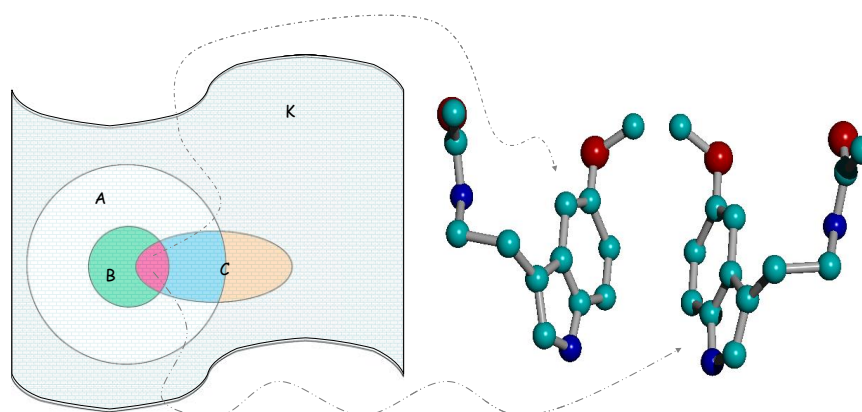


Figure 6. The Venn-diagram of Implicit Models, as well as Conformers Belonging to Intersection. K - Conformational Space Obtained by HF/6-31G* method. A (Sicsic), B (Marot) and C (Sastre) - Subspaces Defined by Different Conditions.

V. Publication list

Publications directly related to the topic of thesis:

- I. Tasi G, Mizukami F, Pálinkó I, **Csontos J**, Győrffy W, Nair P, Maeda K, Toba M, Niwa S, Kiyozumi Y and Kiricsi I. Enumeration of the conformers of unbranched aliphatic alkanes. *Journal of Physical Chemistry A* **1998**, *102*, 7698-7703. [IF: 2,630]
- II. Tasi G, Mizukami F, **Csontos J**, Győrffy W and Pálinkó I. Quantum algebraic-combinatoric study of the conformational properties of n-alkanes. *Journal of Mathematical Chemistry* **2000**, *27*, 9-17. [IF:0,752]
- III. **Csontos J**, Tasi G and Kálmán M. Conformational analysis of the hormone of darkness. *European Journal of Biochemistry* **2002**, *269*, 94. Supplement I. [IF: 2,849]
- IV. **Csontos J**, Kálmán M and Tasi G. Conformational analysis of melatonin at Hartree–Fock ab initio level. *Journal of Molecular Structure (Theochem)* **2003**, *640*, 69-77. [IF: 0,919]
- V. **Csontos J**, Kálmán M and Tasi G. The stereochemistry of the chemical expression of darkness. *Journal of Molecular Structure (Theochem)* **2003**, *666-667*, 515-520. [IF: 0,919]

Publications not directly related to thesis:

- VI. Hajós G, Tasi G, **Csontos J**, Győrffy W, Riedl Z, Timári G and Messmer A. MEP maps as useful tools for prediction of selectivity of alkylations of fused N-heteroaromatics. *Journal of Molecular Structure (Theochem)* **1998**, 455, 191-198. [IF: 0,919]
- VII. Stralis-Pavese N, Sessitsch A, Weilharter A, Reichenauer T, Riesing J, **Csontos J**, Murrell CJ and Bodrossy L. Optimisation of diagnostic microarray for application in analyzing landfill methanotroph communities under different plant covers. *Environmental Microbiology* **2004**, 6, 347-363. [IF: 3,276]