

# Theoretical investigation of melatonin and its hydroxy isomers

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## Abstract

The structural and electronic properties of melatonin and its six hydroxy isomers have been investigated theoretically by performing semi-empirical and ab initio molecular orbital theory calculations. The geometry of the systems has been optimized considering the semi-empirical molecular orbital theory at the level of AM1, and the electronic properties of the systems have been calculated by ab initio RHF including full MP2 correlation correction in their ground state. Conclusions were drawn by comparing with experimental results. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Melatonin is a product of the pineal gland, where it is synthesized from amino acid tryptophan, which is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase. In addition to the pineal, it is also produced in retina, bacteria, plants, ovary, lens and the gastrointestinal tract. Some body fluids contain very high levels of melatonin. This wide distribution and being an ancient and highly conserved molecule justify its potent antioxidant, radioprotector and antitumoral properties and its involvement in light-mediated processes in the retina and biological clock function [1].

The enzyme 5-hydroxytryptophan decarboxylase acts on 5-hydroxytryptophan to form 5-hydroxytryptamine (5-HT, serotonin) which is converted to *N*-acetylserotonin by the action of *N*-acetyl-transferase.

The *N*-acetylserotonin produced is *O*-methylated by hydroxindole-*O*-methyltransferase (HIOMT) to form *N*-acetyl-5-methoxytryptamine (melatonin) [2,3]. Melatonin is then metabolized in the liver to 6-hydroxymelatonin by melatonin hydroxylase and converted to a sulfate or to a glucuronide for urinary excretion [2,4,5].

Under normal conditions, melatonin levels foreshadow the sleep cycle, usually increasing rapidly from the late evening until midnight, then decreasing as morning approaches. In this way melatonin helps regulate circadian rhythm, the body's 24 h day light clock that governs the timing of hormone production, sleep body temperature and more [6–9]. Not surprisingly, people with high levels of melatonin usually sleep longer than those with a deficiency. The elderly who produce less melatonin than the young and middle aged are typically more susceptible to insomnia [9]. Given its ability to regulate body rhythms, promote normal sleep, melatonin has proven useful not only for insomnia; but for jet lag and seasonal affective disorder [6,9–12].

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Some researchers speculate that chronically low melatonin levels may be linked with cancer, especially in the breast, skin or prostate gland. They note that many cancer patients have a poorly functioning pineal gland and show low levels of melatonin [13,14]. Melatonin can also hold back the spread of cancer, and can delay the onset of aging, lower cholesterol levels and reduce high blood pressure [5,11,14–16].

Hydroxyl radical ( $\text{OH}\cdot$ ), produced in biological systems from superoxide radicals, is highly reactive and toxic, readily damages proteins, DNA and lipids in the cell. The attack of the  $\text{OH}\cdot$  is totally random and site specific; damage site is close to generation site. However,  $\text{OH}\cdot$  is not enzymatically detoxified within cells (unlike its precursors  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$ ). Thus it can only be neutralized by direct free radical scavengers such as melatonin, mannitol and glutathione [1]. Considering the most damaging of all free radicals generated in organisms, hydroxyl radicals are often responsible for DNA damage caused by ionizing radiation. Melatonin and similar indoleamines have been shown to have protective effects against oxidative stress caused by ionizing radiation both in vitro and in vivo by stimulating the activities of antioxidant enzymes and scavenging free radicals directly or indirectly [17–20].

Bromme et al. [21] used the alloxan/GSH-reaction in the presence of ferrous ions to generate  $\text{OH}\cdot$  and showed that melatonin has effectively scavenging ability of  $\text{OH}\cdot$ . However, the potential of melatonin to prevent lipid peroxidation was considerably less pronounced. Recent research efforts have concentrated on whether melatonin can offer in vitro protection against LDL oxidative modification as an antioxidant in atherogenesis [22]. To this end Gozzo et al. [22] studied the structure–activity relationships of the amide group, of the methoxy group, and of the aromatic ring on the antioxidant activity of melatonin in the LDL oxidation model.

The level of 8-hydroxy-deoxyguanosine is measured as a product of oxidatively damaged DNA. The elevated levels of 8-hydroxy-deoxyguanosine in rats treated with kainic acid, an activator of excitatory amino acid receptors triggering the formation of reactive oxygen species, were significantly reduced in animals that were co-treated with melatonin. The authors attributed melatonin's ability to reduce

mediators of DNA damage to its direct free radicals scavenging activity and general antioxidative actions [23].

Melatonin's actions in protection against oxidative damage and DNA damage have been reviewed with possible routes for molecular mechanisms in several reviews [1,19,24].

The site(s) on the melatonin molecule scavenging the  $\text{OH}\cdot$  have been investigated. Both in vitro and in vivo the experimentally generated hydroxyl radical resulted in the production of 3-hydroxymelatonin (3-OHM) as a melatonin metabolite. The metabolite was identified in the urine of rats and humans using spectroscopic analysis and proposed as a direct scavenging route of the radical by melatonin [25,26].

Recently the reactions of melatonin with free radicals [27] and conformational properties of melatonin [28,29] were investigated theoretically, proposing a dominant role of the indole ring in the free radical scavenging mechanism. Considering the importance of endogenous melatonin in free radical scavenging and antioxidant actions and therapeutic potential, we have undertaken this study to further elucidate melatonin's probable bonding site(s) of  $\text{OH}\cdot$  scavenging. In this study, we have investigated the structural and electronic properties of isolated melatonin and its hydroxy isomers theoretically by performing semi-empirical molecular orbital and ab initio calculations because of its biological and medical importance.

## 2. Method of calculation

In the present study, the melatonin molecule has been considered theoretically by performing both semi-empirical molecular orbital theory and ab initio calculations. Preoptimization has been performed by applying the molecular-mechanics method [30] using MM + force field [31]; this makes easier to perform full optimization by extended methods. The Austin Model 1 (AM1) semi-empirical method [32] within the restricted Hartree–Fock (RHF) [33] formalism has been considered to optimize fully the geometry of the systems considered.

Geometry optimization is carried out by using a conjugate gradient method (Polak–Ribiere algorithm [34]), then the electronic structure of the system has been calculated by applying the ab initio RHF

Table 1

Some of the molecular properties of the melatonin molecule (M) and its hydroxy isomers (Mx-OH; x = 1–6) in their ground state (ab initio results). All the six isomers have the same molecular properties

Quantity	M	Mx-OH
Number of electrons	124	132
Number of doubly occupied levels	62	66
Number of total orbitals	101	106
Number of primitive Gaussians	303	318
Multiplicity	Singlet	Singlet
Molecular point group	C <sub>1</sub>	C <sub>1</sub>

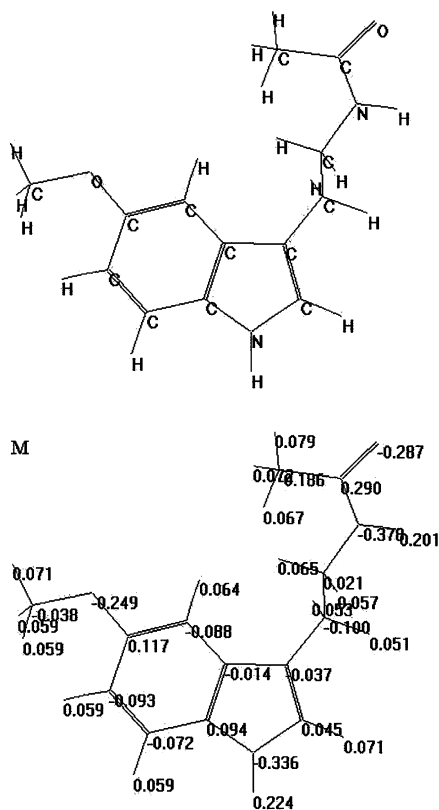


Fig. 1. The optimized structure (upper picture) of melatonin molecule (M). The structure of M is nonplanar with C<sub>1</sub> symmetry in its ground state; optimization has been performed by AM1 method. Calculated excess charge on the atoms of M (lower picture); charges have been calculated by ab initio method.

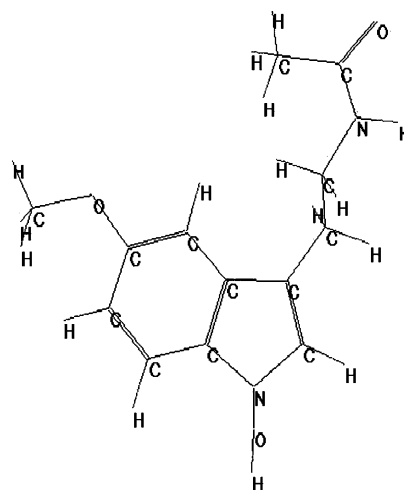


Fig. 2. Same as Fig. 1 but for melatonin hydroxy isomer, model-1, M1-OH.

including full MP2 correlation correction [35] in the ground state. The minimum basis set (STO-3G) [36] has been used in the calculations, which may give qualitative but reliable information about the systems considered. The SCF convergency is set to 0.00001 kcal/mol in the calculations. We have performed all the calculations by using the HyperChem-5.1 packet.

### 3. Results and discussion

The closed formula of the melatonin molecule (hereafter referred to as M) is in the form C<sub>13</sub>H<sub>16</sub>O<sub>2</sub>N<sub>2</sub>. The AM1 geometry optimization yields

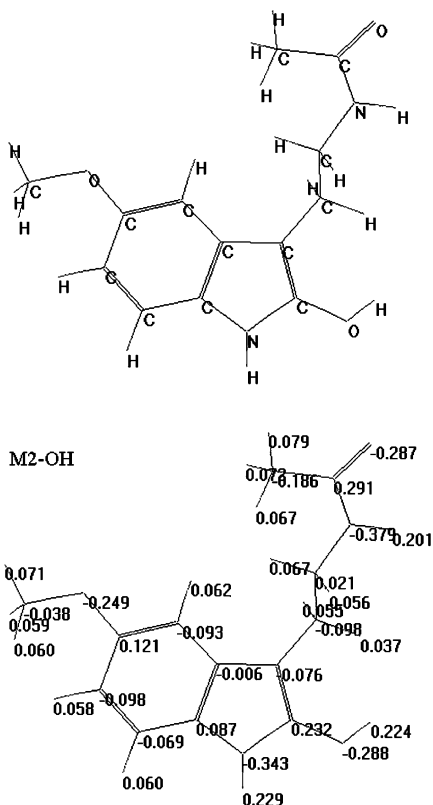


Fig. 3. Same as Fig. 1 but for melatonin hydroxy isomer, model-2, M2-OH.

a nonplanar structure as the stable form of isolated melatonin molecule and its hydroxy isomers. Hydroxyl radical ( $\text{OH}$ ) can be bound at different sites of melatonin, the so called melatonin hydroxy isomers (hereafter referred to as  $\text{M}_x\text{-OH}$ ) may show various properties and stability depending on the bonding site. We have considered six possible hydroxyl bonding sites, the closed formula of the melatonin hydroxyl is in the form  $\text{C}_{13}\text{H}_{16}\text{O}_3\text{N}_2$ . Some of the molecular properties of the systems considered are given in Table 1. The optimized structures (by the AM1 method) and the calculated excess charge on the atoms (by the ab initio method) of the systems considered in this work are given in Figs. 1–7.

The calculated energy values (by both AM1 and ab initio methods) of the systems studied are given in Table 2. The highest occupied and the lowest unoccupied molecular orbital energies (HOMO and LUMO, respectively) and the interfrontier molecular orbital

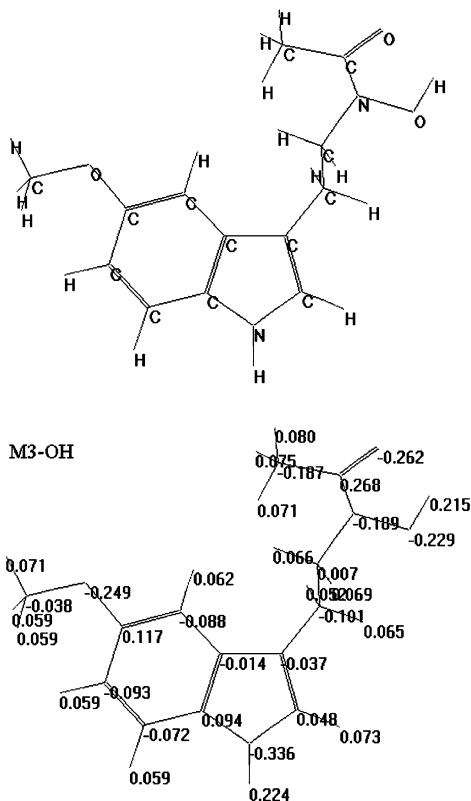


Fig. 4. Same as Fig. 1 but for melatonin hydroxy isomer, model-3, M3-OH.

energy gap (LUMO–HOMO energy difference,  $\Delta E$ ) with the lowest and highest level energy values are also given in Table 2. The calculated dipole moment values of the systems considered are also given in Table 2.

According to AM1 calculation, binding energy of the melatonin molecule is about  $-3433$  kcal/mol, heat of formation of melatonin is about  $-33$  kcal/mol and it is exothermic. According to ab initio calculation HOMO–LUMO gap of the melatonin molecule is about 12 eV.  $\Delta E$  values of the hydroxy isomers ( $\text{M}_x\text{-OH}$ ) are also around 12 eV; the bonding site of  $\text{OH}$  does not change  $\Delta E$  considerably. Furthermore, both HOMO and LUMO are mainly localized on the hexagonal and pentagonal rings. These findings are in agreement with previous calculations for melatonin [28]. The melatonin molecule has relatively larger dipole moment, about 5 Debye. One may conclude that melatonin is a highly polar molecule, therefore it

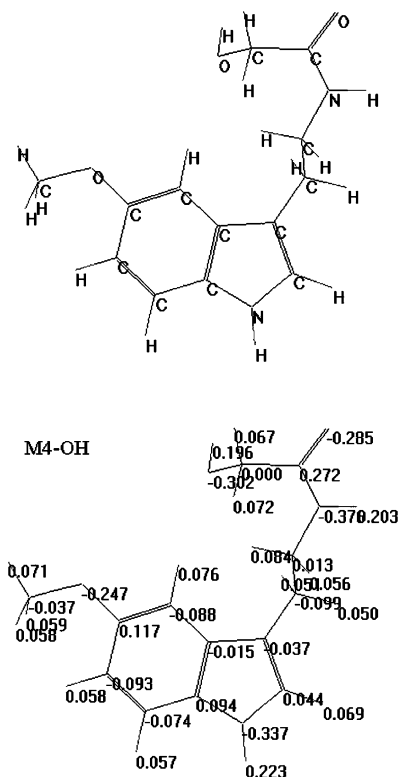


Fig. 5. Same as Fig. 1 but for melatonin hydroxy isomer, model-4, M4-OH.

may interact with its environment, especially other polar molecules in the cell more strongly. Hydroxyl radical can easily be scavenged by melatonin; there are various possibilities (sites) for bonding of 'OH to M. In the case of single 'OH scavenging, there are six possible sites (there may be even more) on M. The probable sites (single 'OH bonding models) are shown in Figs. 2–7.

According to AM1 calculations, model-5 (M5-OH) has largest binding energy with the value of about  $-3548$  kcal/mol. The heat of formation of this model has also the largest value among all the models considered, which has a value of about  $-88$  kcal/mol. In recent experimental works of Reiter et al. [1,25] it was identified that one of the possibilities of 'OH scavenging of M is the same as model-6 in the present work. On the other hand, in the recent theoretical work of Turjanski et al. [27] it was proposed that a possible pathway for reaction of melatonin with 'OH is the same as model-2 in the present work.

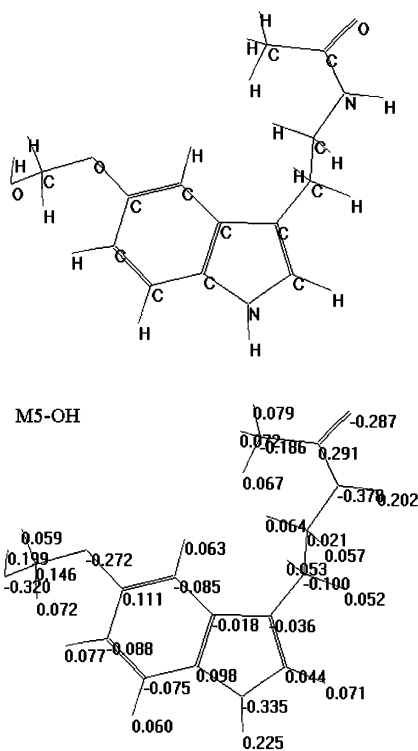


Fig. 6. Same as Fig. 1 but for melatonin hydroxy isomer, model-5, M5-OH.

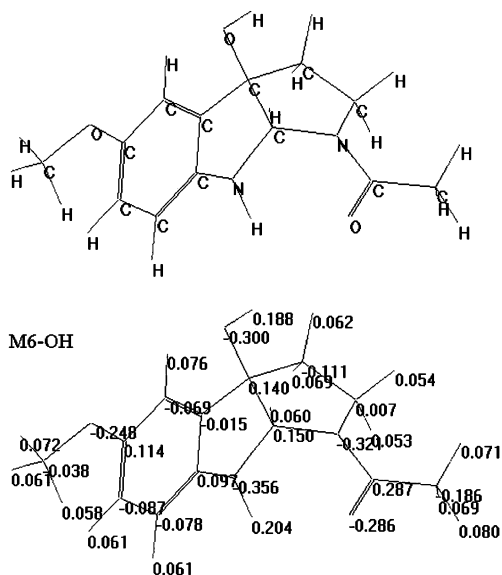


Fig. 7. Same as Fig. 1 but for melatonin hydroxy isomer, model-6, M6-OH.

Table 2

Some of the calculated energy values and dipole moments ( $\mu$ ) of the melatonin molecule (M) and its hydroxy isomers (Mx-OH) in their ground state with singlet symmetry. TE: total energy; BE: binding energy; IAE: isolated atomic energy; EE: electronic energy; CCI: core–core interaction; HoF: heat of formation; MP2: MP2 correlation contribution; eKeeN: eK, ee and eN energy; NRE: nuclear repulsion energy; LLE: lowest level energy;  $\Delta E$ : HOMO–LUMO gap; HLE: highest level energy. Energies marked with \* are in kcal/mol, those marked with + are in eV and the dipole moments are in Debyes

Quantity	M	M1-OH	M2-OH	M3-OH	M4-OH	M5-OH	M6-OH
AM1							
TE (*)	-67,772.2	-75,121.2	-75,162.4	-75,122.8	-75,166.3	-75,176.9	-75,164.9
BE (*)	-3432.9	-3492.4	-3533.6	-3494.0	-3537.4	-3548.1	-3536.1
IAE (*)	-64,339.3	-71,628.8	-71,628.8	-71,628.8	-71,628.8	-71,628.8	-71,628.8
EE (*)	-411,522.3	-459,403.9	-459,655.1	-461,219.2	-463,920.4	-456,778.3	-479,824.7
CCI (*)	343,750.1	384,282.7	384,492.7	386,096.4	388,754.2	381,601.4	404,659.7
HoF (*)	-32.6	-32.5	-73.7	-34.1	-77.6	-88.2	-76.2
Ab initio							
TE (*)	-471,673.3	-518,001.1	-518,030.6	-518,006.9	-518,018.5	-518,024.6	-518,043.2
MP2 (*)	-588.6	-608.9	-609.2	-610.8	-609.4	-607.6	-594.9
eKeeN (*)	-1,195,090.1	-1,321,504.7	-1,321,630.8	-1,324,593.2	-1,329,151.3	-1,315,252.3	-1,366,456.7
NRE (*)	724,005.3	804,112.5	804,209.5	807,197.2	811,742.2	797,835.2	849,008.4
LLE (+)	-551.955	-553.327	-553.105	-552.409	-551.803	-551.748	-551.992
HOMO (+)	-5.632	-6.050	-5.557	-5.626	-5.494	-5.806	-5.511
LUMO (+)	6.425	6.132	6.567	6.435	6.561	6.421	7.064
$\Delta E$ (+)	12.057	12.182	12.124	12.061	12.055	12.227	12.575
HLE (+)	31.697	31.493	31.721	31.713	31.834	31.601	31.309
$\mu_x$	4.171	3.604	3.237	3.685	2.873	2.623	-0.316
$\mu_y$	2.340	1.004	1.784	1.193	2.348	2.220	-3.149
$\mu_z$	-2.025	-2.000	-1.526	-0.972	-1.001	-1.900	0.274
$\mu$	5.193	4.242	3.998	3.993	3.843	3.927	3.176

Table 3

Calculated net charge (in units of electron charge) on the hexagonal (R6) and pentagonal (R5) ring structures of melatonin (M) and its hydroxy isomers (Mx-OH; x = 1–6) in their ground state (ab initio results)

Molecule	R6	R5	R5(2)
M	-0.056	-0.248	-
M1-OH	-0.050	-0.080	-
M2-OH	-0.058	-0.106	-
M3-OH	-0.056	-0.245	-
M4-OH	-0.059	-0.251	-
M5-OH	-0.057	-0.247	-
M6-OH	-0.038	+0.016	-0.135

OH binding reduces the polarity of M, dipole moment values of Mx-OH isomers are relatively smaller than that of M itself. Binding energies and heats of formations of model-1 and model-3 are close to each other, on the other hand, the same quantities for the rest of the models are relatively larger. Net excess charge on the ring structured part of the systems considered are given in Table 3. These parts have negative excess charge, except the model-6, which has two pentagonal rings; the central ring (pentagonal) has relatively small positive charge. In general, pentagonal ring has larger excess charge with respect to hexagonal ring. The negative excess charge on the ring structured part makes melatonin attractive for radicals; negative charge accumulation on the ring structured part may cause melatonin as a scavenging species for OH radical. In the present study we have considered only single OH binding, of course more than one OH is possible to be bonded to melatonin molecule.

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