Theoretical Parameters to Characterize Antioxidants

Part 2

The Cases of Melatonin and Carvedilol

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This theoretical study focuses on two indole derivatives, melatonin (**1**) and carvedilol (**8**), with the objective of improving our understanding of the molecular mechanisms underlying their radical-scavenging activity. Quantum-mechanical calculations were carried out using the AM1 semi-empirical method, some results being confirmed by *ab initio* (3-21G) calculations. The quantum-chemical descriptor ΔH_{ox} (relative adiabatic oxidation potential) and the shape of the SOMO (singly occupied molecular orbital) indicate that the stabilization of its radical cation can partially explain the well-documented antioxidant efficacy of melatonin. This stabilization may result from electrostatic interactions and from a hyperconjugative effect existing in a family of conformers of the melatonin radical cation having the side chain almost perpendicular to the plane of the aromatic rings. Furthermore, 6-hydroxymelatonin (**7**) appears to be a better free-radical scavenger than melatonin (**1**) in agreement with experimental results. According to the theoretical parameters ΔH_{ox} and ΔH_{abs} (relative bond dissociation enthalpy), carvedilol (**8**) is not a good antioxidant, in contrast to its ring-hydroxylated metabolites whose powerful antioxidant effects are explained by the formation of an oxyl radical stabilized by delocalization over the carbazole nucleus.

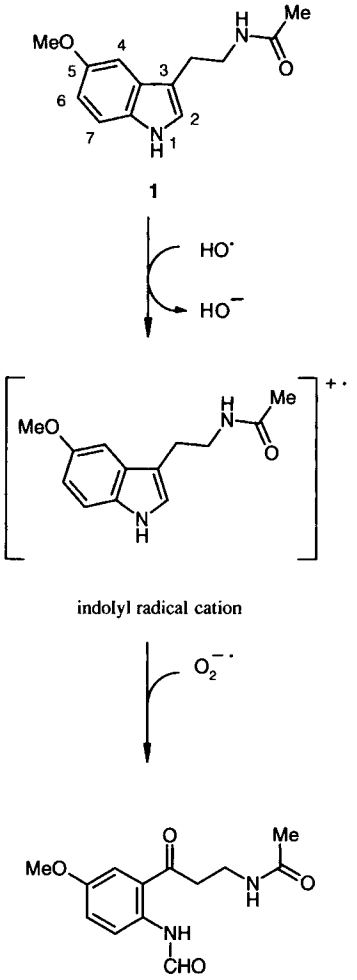
1. Introduction. – There is much evidence that biochemical damages mediated by free radicals underlie numerous human pathologies [1][2]. To design successful antioxidant drugs, it is thus important to understand the involvement of radicals in such disorders as well as the mechanisms by which antioxidants exert their effects.

In a previous study, we focused our attention on simple quantum-chemical descriptors characterizing free-radical scavenging activity in a series of vitamin E analogs [3]. We now extend our investigations to two indole derivatives of great medicinal relevance, namely melatonin and carvedilol. The two compounds are known antioxidants as discussed below, but their molecular mechanisms and conditions of action remain to be better elucidated.

The antioxidant activity of melatonin (**1** in *Scheme*) has been demonstrated in a variety of experimental tests and appears to explain its protective biological roles [4][5]. Due to its lipophilicity (calculated log $P = 0.88$) [6], melatonin (**1**) can easily reach various biochemical targets in a number of subcellular compartments [5][7]. In contrast to other intracellular antioxidants such as vitamin C and vitamin E, which act primarily in the cytosol and membranes, respectively, **1** has shown ubiquitous antioxidant effects in membranes, cytosol, and nuclei [5]. This broad distribution of sites of action makes melatonin (**1**) a compound of particular interest, not to mention its receptor-mediated effects.

Some chemical investigations have concluded to the powerful hydroxyl-radical scavenging and efficient peroxyl-radical scavenging activity of melatonin (**1**) [8–10]. Howev-

Scheme. *Postulated Mechanism of Direct Free-Radical Trapping by Melatonin* (**1**) (adapted from *Hardeland et al.*) [17]



*N*1-acetyl-*N*2-formyl-5-methoxykynuramine

er, careful *in vitro* biological evaluations have revealed a peculiar antioxidant profile for **1** [11][12], which was indeed an excellent scavenger of peroxyl radicals in biological media, but was only moderately active against other oxidants such as $O_2^{-\cdot}$ and HClO. Unlike 5-hydroxyindolamines and the metabolite 6-hydroxymelatonin, melatonin and other 5-methoxyindolamines have no prooxidant effect [12]. Furthermore, melatonin (**1**) is able to scavenge free radicals produced during the autoxidation of catecholamines, possibly playing a neuroprotective role [13]. Numerous biological experiments have confirmed the antioxidant properties of melatonin, *e.g.*, its protective role against safrole-induced DNA damage [14], buthionine sulfoximine-induced cataractogenesis [15], and paraquat-induced peroxidation of membrane lipids [16].

Although the molecular mechanism of antioxidant action of melatonin (**1**) is not fully established, accumulated data suggest that it could play a key role in protecting organisms from damages initiated by free radicals. One hypothesis is that melatonin acts as an electron donor to yield a stable indolyl radical cation which could be further oxidized to N^1-acetyl-N^2-formyl-5-methoxykynuramine (*Scheme*) [17].

The β-adrenoceptor antagonist carvedilol (**8**, see below) is another indole derivative of interest. Its unique cardioprotective properties appear to result from non-receptor-mediated activities [18], in agreement with the finding that the antioxidant effects of the drug or some metabolite(s) should account for a high efficacy in preventing cardiac ischaemia [18–21]. This pathology involves injuries caused by oxygen radicals [22], hence the hypothesis that the free-radical scavenging properties of carvedilol contribute significantly to its cardioprotective action [18].

Various physicochemical, biochemical, cellular, and *in vivo* studies have documented the antioxidant properties of carvedilol (**8**) and mostly of its 1-hydroxylated (**9**) and 3-hydroxylated (**10**) metabolites [19–21][23–25]. Structure-activity relationship studies have clarified that the antioxidant activity of **8** resides mainly in the carbazole moiety [23]. Carbazole itself has a high oxidation potential (1250 mV in MeCN) [23] and acts as an *in vitro* antioxidant only in the presence of the hydroxyl radical HO$^\cdot$ [23][26]. In contrast, carbazol-3-amine and carbazol-3-ol have a relatively low oxidation potential [23] and are potent free-radical scavengers in lipid peroxidation tests [26]. The presence of a OH or NH_2 function on the carbazole moiety thus appears as a condition for free-radical scavenging activity. Indeed, the two hydroxylated metabolites are 10- to 40-fold more effective as antioxidants than carvedilol (**8**) itself [18] and may, therefore, contribute significantly to its therapeutic effects [27][28].

In summary, many physicochemical and biochemical results are compatible with the known *in vivo* antioxidant effects of melatonin (**1**) and carvedilol (**8**). What remains to be further clarified, however, are the molecular mechanisms underlying such effects. Here, we present a quantum-mechanical study that addresses these questions and examines the electronic properties and reactivity of melatonin, carvedilol, and analogs. The results point to an unexpected stabilization of the melatonin radical cation, and confirm the antioxidant potential of the phenolic metabolites of carvedilol.

2. Theoretical Parameters. – Facility of electron transfer and stability of the radical formed by H-atom abstraction are two characteristics considered essential for a good radical scavenger antioxidant [3][29]. Hence, some simple theoretical parameters were calculated here to quantitatively express these characteristics, namely the relative adiabatic ionization potential (ΔH_{ox}), and the relative O–H bond dissociation enthalpy (ΔH_{abs}) [3].

Among the simple theoretical parameters available, we chose ΔH_{ox} and ΔH_{abs}, because they proved to be the most informative parameters in the study of vitamin E [3].

The parameter ΔH_{ox} is defined in *Eqn. 2*, itself obtained from the isodesmic *Reaction 1*. This parameter describes the capacity of a compound RH (relative to α-tocopherol (α-Toc–OH)) to donate an electron and is directly related to the adiabatic ionization potential. The latter, which is calculated as the difference between the heat of formation of the optimized radical cation and that of the corresponding neutral species, is an index

of the facility of single-electron transfer (SET) from the antioxidant molecule to the free radical [3].

$$RH + \alpha\text{-Toc}-OH^{+\cdot} \rightleftarrows RH^{+\cdot} + \alpha\text{-Toc}-OH \quad (1)$$

$$\begin{aligned} \Delta H_{ox} = {} & \Delta H_f(\alpha\text{-Toc}-OH) + \Delta H_f(RH^{+\cdot}) \\ & - \Delta H_f(\alpha\text{-Toc}-OH^{+\cdot}) - \Delta H_f(RH) \end{aligned} \quad (2)$$

The relative O–H bond dissociation enthalpy ΔH_{abs} is defined by *Eqn. 4*, itself obtained from the isodesmic *Reaction 3*. This parameter describes the capacity of a compound RH (relative to α-tocopherol) to donate a H-atom, and provides a quantitative estimate of the stability of the neutral radical species R· [3].

$$RH + \alpha\text{-Toc}-O^{\cdot} \rightleftarrows R^{\cdot} + \alpha\text{-Toc}-OH \quad (3)$$

$$\begin{aligned} \Delta H_{abs} = {} & \Delta H_f(\alpha\text{-Toc}-OH) + \Delta H_f(R^{\cdot}) \\ & - \Delta H_f(\alpha\text{-Toc}-O^{\cdot}) - \Delta H_f(RH) \end{aligned} \quad (4)$$

3. Methods. – All calculations were performed on *Silicon Graphics Indy R4400* and *O2 R5000* workstations. To locate the main valleys in the conformational space of melatonin, quenched molecular dynamics (MD) simulation [30–32] were performed at 2000 K using SYBYL 6.3 [33]. The simulations were run for 100 ps with steps of 1.0 fs. The frame data were stored every 0.05 ps, giving 2000 frames. The starting velocity of each atom was calculated from a *Boltzmann* distribution. Finally, 10% of all conformations were randomly selected and saved in a database ultimately containing 200 conformations. All the conformations in the database were then minimized using the *Tripos* force field [34] with *Gasteiger-Marsili* atomic charges [35]. The *Powell* minimization method was applied with the gradient value of 0.001 to test for convergence. The maximum number of iterations was set at 3000. The conformers were then classified according to increasing energy.

The conformational similarity of the 200 conformers was investigated by comparing all pairs of conformers. The two criteria of comparison were the force-field energy and the RMS distance difference calculated by the option MATCH in SYBYL over all heavy atoms and polar H-atoms. A *Fortran* program then calculated the mean and standard deviations of the root mean squares (RMS) values. Two conformers were considered identical when their energy difference was $\leq$ 3 kcal/mol, and their RMS distance difference $\leq$ the RMS mean minus the standard deviation. When this was the case, the conformer with higher energy was eliminated from the database.

All retained conformers were minimized a second time at the semi-empirical level. Semi-empirical calculations were performed using the *Austin* Model 1 (AM1) *Hamiltonian* as implemented in SPARTAN 5.0 [36]. Closed-shell species were optimized using the restricted *Hartree-Fock* (RHF) approximation, whereas the open-shell species were calculated using the UHF approximation. To avoid large spin contamination ($\langle S^2 \rangle$ greater than 0.9) a single-point restricted open-shell *Hartree-Fock* (ROHF) calculation was systematically performed using the MOPAC 5.0 with the keyword PRECISE [37].

Some *ab initio* calculations were also carried out using SPARTAN 5.0 at the RHF/3-21G level for closed-shell species, and at the unrestricted *Hartree-Fock* (UHF)/3-21G level for open-shell species. Single-point ROHF/3-21G calculations were also performed with *Gaussian 94* [38] on all radicals because of the different degrees of spin contamination. Indeed, failure to correct for a large degree of spin contamination would lead to seemingly lower energies which do not reflect a real improvement of the results, but are the result of a computational artifact [39].

4. Results and Discussion. – 4.1. *Melatonin* (**1**). 4.1.1. *Stabilization of the Melatonin Radical Cation.* The hypothesis that melatonin (**1**) acts as antioxidant by single-electron transfer producing a stable radical cation has been presented (see *Scheme*) [17]. To confirm this mechanistic hypothesis, also supported by some experimental results [11][12], quantum-chemical descriptors were evaluated using vitamin E as a reference [3].

All conformers identified by a quenched MD exploration were further optimized at a semi-empirical level using the RHF approximation. An electron was then removed

from all conformers, yielding the radical cationic conformers of melatonin (open-shell species), which were subjected to a full geometry optimization using the UHF approximation, followed by a single-point RHF calculation.

Six low-energy conformers of the melatonin radical cation (designated **1Au**$^{+\cdot}$, **1Ad**$^{+\cdot}$, **1Bu**$^{+\cdot}$, **1Bd**$^{+\cdot}$, **1Cu**$^{+\cdot}$, and **1Cd**$^{+\cdot}$) were identified. They differed significantly in the geometry of the lateral chain and in the conformation (up or down) of the MeO group (*Fig. 1*). The relative adiabatic ionization potential (ΔH_{ox}) of the six conformers was small (see *Table 1*), indicating a relatively easy formation and suggesting that the antioxidant properties of melatonin (**1**) are due to the stability of its radical cation. This hypothesis is validated by the high capacity of **1** to scavenge the trichloromethylperoxyl

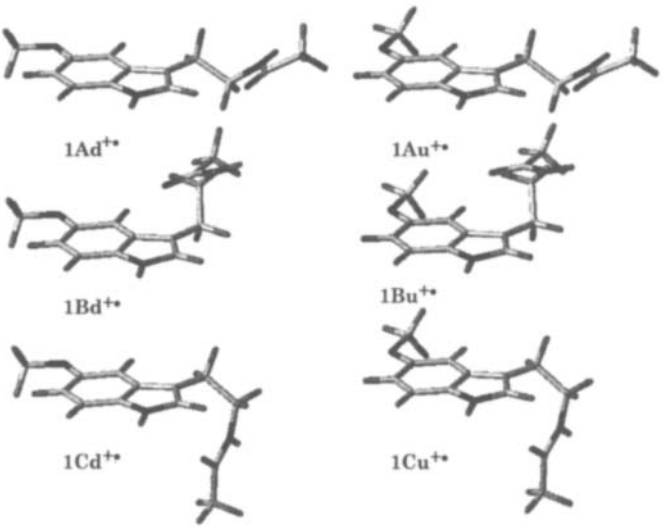


Fig. 1. *The most stable conformers of the melatonin radical cation* (**1Ad**$^{+\cdot}$, **1Au**$^{+\cdot}$, **1Bd**$^{+\cdot}$, **1Bu**$^{+\cdot}$, **1Cd**$^{+\cdot}$, and **1Cu**$^{+\cdot}$; see *Table 1* for heats of formation). A, B, and C refer to side-chain conformation; d and u refer to the conformation of the MeO group.

Table 1. *Theoretical Parameters of Melatonin* (**1**)[a]) *and Its Adiabatic Ionization Potentials Relative to α-Tocopherol*[b])

Chemical species[c])	ΔH_{RC}^{RHF}[d])	ΔH_{ox}^{AM1}[e])	Chemical species[c])	ΔH_{RC}^{RHF}[d])	ΔH_{ox}^{AM1}[e])
1Ad$^{+\cdot}$	140.07	−0.33	**1Bu**$^{+\cdot}$[g])	138.74	−1.67
1Au$^{+\cdot}$	141.21	0.80	**1Cd**$^{+\cdot}$	139.40	−1.01
1Bd$^{+\cdot}$[f])	141.31	0.90	**1Cu**$^{+\cdot}$	140.45	0.04

[a]) Heat of formation of the most stable neutral species $\Delta H_{NE} = -33.61$ kcal/mol and $E_{NE} = -756.01282$ hartree according to 3-21G calculations. [b]) The values for α-tocopherol are: $\Delta H_{NE} = -91.54$ kcal/mol, $\Delta H_{RC}^{RHF} = 82.48$ kcal/mol, $\Delta H_{RN}^{RHF} = -60.65$ kcal/mol, $E_{NE} = -687.71367$ hartree, $E_{RC}^{ROHF} = -687.485002$ hartree. [c]) Conformers of the melatonin radical cation, see *Fig. 1*. [d]) Heat of formation (in kcal/mol) of the radical cation species using the RHF approximation. [e]) Enthalpy of the isodesmic *Reaction 1* (in kcal/mol) according to AM1 calculations. [f]) Total energy of the radical cation species $E_{RC}^{ROHF} = -755.78318$ hartree and enthalpy of the isodesmic *Reaction 1* $\Delta H_{ox}^{3\text{-}21G} = 0.62$ kcal/mol according to 3-21G calculations. [g]) Total energy of the radical cation species $E_{RC}^{ROHF} = -755.79021$ hartree and enthalpy of the isodesmic *Reaction 1* $\Delta H_{ox}^{3\text{-}21G} = -3.79$ kcal/mol according to 3-21G calculations.

radical ($Cl_3COO^{\cdot}$) [12]. This alkylperoxyl radical is highly electron-deficient and oxidizes most of its substrates by electron transfer [40].

Which are the molecular factors accounting for this relative stability of the radical cation? The geometry and charge distribution of the six conformers point to the stabilizing effect of through-space electrostatic interactions between the carbonyl O-atom of the *N*-Ac group and electropositive atoms in the indole ring (*Fig. 1*). In particular, the electrostatic stability of the conformers **1Ad**$^{+\cdot}$ and **1Au**$^{+\cdot}$ may come from the interaction between the carbonyl O-atom of the *N*-Ac group and the H-atom at C(2) of the indole nucleus, as well as of the conformers **1Cd**$^{+\cdot}$ and **1Cu**$^{+\cdot}$ which are also stabilized by the interaction between the carbonyl O-atom of the *N*-Ac group and the H-atom at C(1).

The interaction between the carbonyl O-atom of the *N*-Ac group and the H-atom at C(4) is partially responsible of the stabilization of the conformers **1Bd**$^{+\cdot}$ and **1Bu**$^{+\cdot}$. The somewhat greater stabilization of the **1Bu**$^{+\cdot}$ relative to the **1Bd**$^{+\cdot}$ conformer is explained by a further electrostatic interaction between the *N*-Ac O-atom and the MeO substituent in the 'up' conformation. Remarkably, the two conformers (**1Bu**$^{+\cdot}$ and **1Bd**$^{+\cdot}$) have an unexpected side-chain conformation, with the CH_2-CH_2 bond in a plane almost perpendicular to the plane of the aromatic rings. This suggests that, in addition to through-space electrostatic stabilization described above, a through-bond stabilization [41][42] of the radical cation by hyperconjugation (known as the frangomeric effect) could also be involved [43]. This effect requires a good alignment of the π and/or n electrons in the amide group, the CH_2-CH_2 σ bond, and the π orbital of the indole ring. The latter condition is fulfilled with an almost perpendicular orientation of the CH_2-CH_2 σ bond with respect to the indole ring (*Fig. 2*). Because of the flexibility of the side chain, the stabilization due to hyperconjugation is difficult to distinguish from the stabilization due to electrostatic interactions.

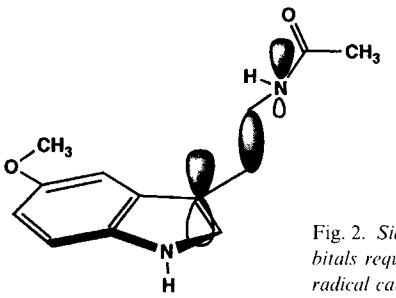


Fig. 2. *Side-chain geometry and alignment of orbitals required for stabilization of the melatonin radical cation by the frangomeric effect*

Ab initio 3-21-G calculations confirmed that the two conformers **1Bu**$^{+\cdot}$ and **1Bd**$^{+\cdot}$ (*Table 1*) experience an additional stabilization which may be due to the hyperconjugative interaction ($E_{RC}^{ROHF} = -755.78318$ and -755.79021 hartree, $\Delta H_{ox}^{3\text{-}21G} = 0.62$ and -3.79 kcal/mol for conformers **1Bd**$^{+\cdot}$ and **1Bu**$^{+\cdot}$, resp.). The geometry of the two conformers obtained at the *ab initio* and semi-empirical methods was similar (RMS = 0.083 and 0.094, considering all heavy atoms in conformers **1Bd**$^{+\cdot}$ and **1Bu**$^{+\cdot}$, resp.).

Moreover, the shape of the singly occupied molecular orbital (SOMO) of the conformer **1Bu**$^{+\cdot}$ (*Fig. 3*) indicates the possible occurrence of a hyperconjugative effect.

We have further investigated the possibility for melatonin (**1**) to form a radical by H-atom abstraction from the N-atom of the indole moiety. The most stable radical has

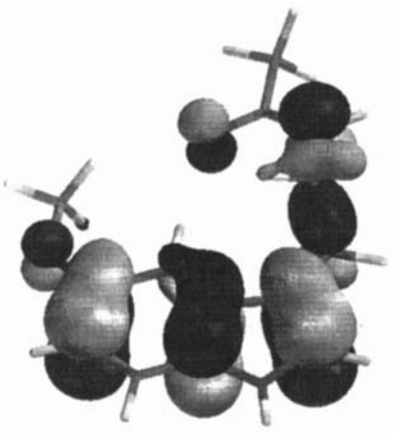
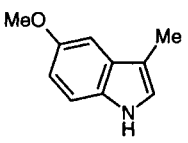


Fig. 3. *SOMO of the melatonin radical cation* (**1Bu**$^{+\cdot}$) *according to AM1 calculations*

a heat of formation of $\Delta H_{RN}^{RHF} = 6.55$ kcal/mol which implies a value of $\Delta H_{abs} = 9.27$ kcal/mol according to AM1 calculations. The high energy necessary to obtain a radical from **1** in comparison to vitamin E, suggests that the direct H-atom abstraction mechanism from the N-atom of the indole moiety is largely improbable.

4.1.2. *Melatonin Analogs.* To better understand the origin of the stabilization of the melatonin radical cation, we also examined a number of melatonin analogs **2**–**7** differing in chain length, *N*-substitution and/or ring substitution. When the side chain was reduced to a Me group (**2**) or shortened by one CH_2 unit (**3**), a decrease in the ease of one-electron oxidation could be deduced from the energy levels shown in *Table 2*. Indeed, the shortening of the side chain prevents the *N*-Ac group from participating in a through-space electrostatic stabilization of the radical cation, and it also renders impossible a stabilization by a through-bond interaction between the remote amide group and the π system.



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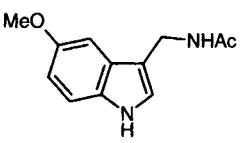

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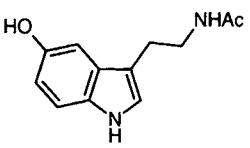

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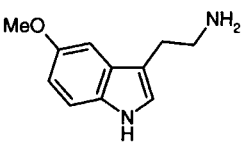

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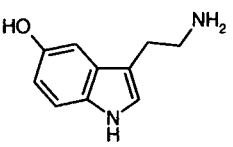

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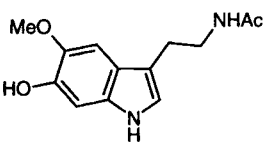

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Table 2. *Theoretical Parameters of Melatonin Analogs Obtained by AM1 Calculations, Relative to α-Tocopherol*[a])

Chemical species[b])	ΔH_{NE}[c])	ΔH_{RC}^{RHF}[d])	ΔH_{ox}[e])	Chemical species[b])	ΔH_{NE}[c])	ΔH_{RC}^{RHF}[d])	ΔH_{ox}[e])
2d, **2d**$^{+\cdot}$		187.48	3.52	**5Au**$^{+\cdot}$		182.48	2.18
2u, **2u**$^{+\cdot}$	9.94	188.30	4.34	**5Bd**$^{+\cdot}$		185.35	5.05
3d, **3d**$^{+\cdot}$		152.35	4.84	**5Bu**, **5Bu**$^{+\cdot}$	6.28	186.08	5.77
3u, **3u**$^{+\cdot}$	−26.52	154.39	6.88	**5Cd**$^{+\cdot}$		181.41	1.11
4Ad$^{+\cdot}$[f])		135.26	1.27	**5Cu**$^{+\cdot}$		181.48	1.18
4Au, **4Au**$^{+\cdot}$	−40.03	136.30	2.30	**6Ad**$^{+\cdot}$[g])		176.64	2.72
4Bd$^{+\cdot}$		137.68	3.69	**6Au**$^{+\cdot}$		177.92	3.99
4Bu$^{+\cdot}$		135.99	2.00	**6Bd**$^{+\cdot}$		181.41	7.49
4Cd$^{+\cdot}$		134.50	0.51	**6Bu**, **6Bu**$^{+\cdot}$	−0.10	182.91	8.98
4Cu$^{+\cdot}$		135.46	1.46	**6Cd**$^{+\cdot}$		176.64	2.72
5Ad$^{+\cdot}$		181.42	1.12	**6Cu**$^{+\cdot}$		177.61	3.69

[a]) For the values of α-tocopherol, see *Table 1*. [b]) See *Fig. 1* for the designation of conformers. [c]) Heat of formation of the neutral species (in kcal/mol). [d]) Heat of formation of the radical cation species using the RHF approximation (in kcal/mol). [e]) Enthalpy of the isodesmic *Reaction 1* in kcal/mol. [f]) ΔH_{abs} = 3.52 kcal/mol being ΔH_{RN}^{RHF} = 5.62 kcal/mol for **4Ad**$^{\cdot}$. [g]) ΔH_{abs} = 3.90 kcal/mol being ΔH_{RN}^{RHF} = 34.69 kcal/mol for **6Ad**$^{\cdot}$.

The other analogs of melatonin investigated were its precursors *N*-acetylserotonin (**4**) and serotonin (**6**), and its metabolite 5-methoxytryptamine (**5**). For each, six conformers of the radical cation were investigated whose side-chain conformation were close to those shown in *Fig. 1*. The higher values of ΔH_{ox} (*Table 2*) indicate a reduced electrostatic stabilization in all the conformers and a reduced hyperconjugative stabilization in the conformers **4Bu**$^{+\cdot}$, **4Bb**$^{+\cdot}$, **5Bu**$^{+\cdot}$, **5Bd**$^{+\cdot}$, **6Bu**$^{+\cdot}$, and **6Bd**$^{+\cdot}$ compared to **1Bu**$^{+\cdot}$ and **1Bd**$^{+\cdot}$. The shape of the SOMO of **5Bu**$^{+\cdot}$ (*Fig. 4*) is in agreement with this reduced stabilization. These results suggest that the three compounds have a lower free-radical scavenging activity than melatonin (**1**), as indeed seen experimentally for serotonin [11]. Furthermore, *ab initio* calculations with the 3-21G basis set confirmed a reduction of the hyperconjugative stabilization in conformers **5Bd**$^{+\cdot}$ and **6Bd**$^{+\cdot}$ (ΔH_{ox} = 7.02 and 13.58 kcal/mol, resp.). The reason for this reduced stabilization is unknown, since the structural requisites for a frangomeric effect in radical cations are poorly known.

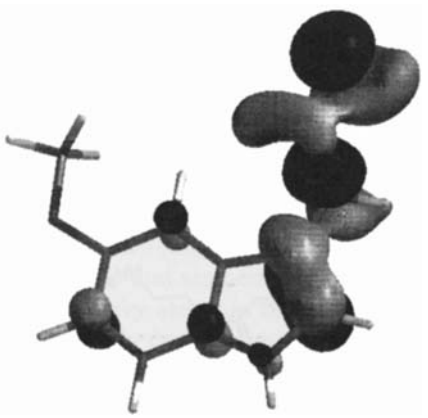


Fig. 4. *SOMO of the radical cation of 5-methoxytryptamine* (**5Bu**$^{+\cdot}$) *according to AM1 calculations*

The possibility of an antioxidant mechanism resulting from direct H-atom abstraction was investigated for *N*-acetylserotonin (**4**) and serotonin (**6**), since these compounds contain a free OH group. The values of ΔH_{abs} for **4** and **6** (ΔH_{abs} = 3.52 and 3.90 kcal/mol, resp., see *Table 2*) suggest that they should indeed be able to scavenge free radicals by a H-atom-transfer mechanism, but not very efficiently.

4.1.3. *6-Hydroxymelatonin* (**7**). A major hepatic metabolite of melatonin is 6-hydroxymelatonin (**7**). This compound exhibits higher *in vitro* antioxidant activities than melatonin (**1**) itself [44]. Indeed, an additional electron-donating group in the aromatic moiety should contribute to a better stabilization of a radical cation. In analogy with **1**, the six most stable conformers of compound **7** were considered (*Table 3*), showing that the radical cation of **7** in conformation **C** (**7Cu**$^{+\cdot}$) is more stable than all conformers of the melatonin radical cation.

Table 3. *Theoretical Parameters of 6-Hydroxymelatonin* (**7**)[a]) *Obtained by AM1 Calculations*

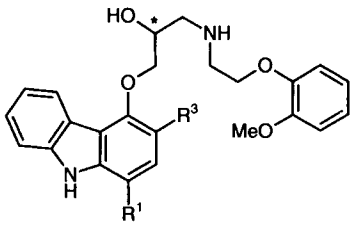
Chemical species[b])	ΔH_{RC}^{RHF}[c])	ΔH_{ox}[d])	ΔH_{RN}^{RHF}[e])	ΔH_{abs}[f])
7Ad$^{+\cdot}$, **7Ad**$^{\cdot}$	98.58	2.38	−42.88	0.38
7Au$^{+\cdot}$, **7Au**$^{\cdot}$	94.01	−2.19	−42.53	0.73
7Bd$^{+\cdot}$, **7Bd**$^{\cdot}$	97.93	1.73	−40.66	2.60
7Bu$^{+\cdot}$, **7Bu**$^{\cdot}$	96.16	−0.05	−42.07	1.19
7Cd$^{+\cdot}$, **7Cd**	97.78	1.58	−42.88	0.38
7Cu$^{+\cdot}$, **7Cu**$^{\cdot}$	93.47	−2.74	−42.53	0.73

[a]) The heat of formation of the most stable neutral species is ΔH_{NE} = − 77.82 kcal/mol. [b]) See *Fig. 1* for the designation of conformers. [c]) Heat of formation of the radical cation species (in kcal/mol) using the RHF approximation after UHF full geometry optimization. [d]) Enthalpy of the isodesmic *Reaction 1* (in kcal/mol). [e]) Heat of formation of the radical species (in kcal/mol) using the RHF approximation after UHF full geometry optimization. [f]) Enthalpy of the isodesmic *Reaction 3* (in kcal/mol) calculated considering the most stable neutral species without intramolecular H-bond, *i.e.*, ΔH_{NE} = − 74.153 kcal/mol.

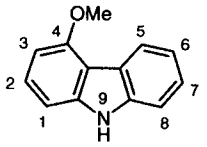
Furthermore, the presence of an OH group increases the possibility of an H-atom transfer. As a consequence, the ΔH_{abs} value indicates a good H-transfer (*i.e.*, 1.58 kcal/mol, see *Table 3*). The possible formation of an oxyl radical is in agreement with the moderate pro-oxidant properties of **7** [9]. Thus, both SET and H-atom abstraction appear as plausible mechanisms to explain the antioxidant activity of **7**.

4.2. *Carvedilol* (**8**). Since the antioxidant capacity of carvedilol (**8**) is believed to reside in its carbazole moiety [23][26], 4-methoxycarbazole (**11**) was used as a model compound for carvedilol (**8**), and compounds **12** and **14** as models for its 1-hydroxylated (**9**) and 3-hydroxylated (**10**) metabolites, respectively. AM1 Semi-empirical calculations indicate that 4-methoxycarbazole (**11**) should not possess high antioxidant activity, since its ΔH_{ox} value is relatively high, and H-atom abstraction from the N-atom of the carbazole moiety requires much more energy than from the OH group of vitamin E (12.73 kcal/mol; see the values of ΔH_{ox} and ΔH_{abs} in *Table 4*). However, direct addition of a radical (especially HO$^{\cdot}$) to the carbazole moiety or to the phenyl ring is a conceivable mechanism by which carvedilol itself could exert antioxidant effects.

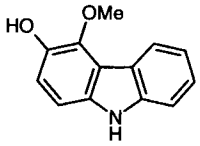
3-Hydroxy-4-methoxycarbazole (**12**) and 1-hydroxy-4-methoxycarbazole (**14**) may have good antioxidant activities based of their ΔH_{abs} values. These calculations suggest that compounds **12** and **14** scavenge free radicals *via* direct H-atom transfer, since their



8 R^1 = H, R^3 = H
9 R^1 = OH, R^3 = H
10 R^1 = H, R^3 = OH



11



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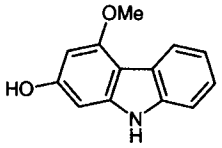

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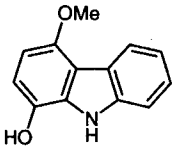

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Table 4. *Theoretical Parameters of Carbazoles Obtained by AM1 Calculations*

Chemical species	ΔH_{NE} [a])	ΔH_{RC}^{RHF} [b])	ΔH_{ox}^{RHF} [c])	ΔH_{RN}^{RHF} [d])	ΔH_{abs}^{RHF} [e])
11, **11**$^{+\cdot}$, **11**$^{\cdot}$	31.01	211.59	6.56	74.63	12.73[f])
12, **12**$^{+\cdot}$, **12**$^{\cdot}$	−11.80	166.05	3.83	20.26	−0.90
13, **13**$^{+\cdot}$, **13**$^{\cdot}$	−13.59	167.80	7.37	24.36	7.06
14, **14**$^{+\cdot}$, **14**$^{\cdot}$	−11.75	169.08	6.81	17.16	−1.99

[a]) Heat of formation of the neutral species (in kcal/mol). [b]) Heat of formation of the radical cation species (in kcal/mol) using the RHF approximation after UHF full geometry optimization. [c]) Enthalpy of the isodesmic *Reaction 1* (in kcal/mol). [d]) Heat of formation of the radical species (in kcal/mol) using the RHF approximation after UHF full geometry optimization. [e]) Enthalpy of the isodesmic *Reaction 3* (in kcal/mol). [f]) Abstraction of the H-atom from the N-atom of the carbazole moiety.

oxyl radical is relatively stable. These two compounds achieve a better delocalization of the unpaired electron compared to compound **13**, since the oxyl radical at C(1) or C(3) of the carbazole moiety (*ortho*/*para* to the NH and MeO groups) is more stable than the one at C(2) (*i.e.*, *meta*).

These results are in agreement with experimental data showing that the two hydroxylated metabolites **9** and **10**, of carvedilol, are better antioxidants than carvedilol (**8**) [21][27][28]. Furthermore, other hydroxylated carbazole derivatives such as carazostatin

and carbazomycin show a considerable antioxidant activity, whereas non-hydroxylated carbazoles are inactive [45][46].

5. Conclusion. – As revealed here by semi-empirical and *ab initio* quantum-mechanical calculations, stabilization of the radical cation of some conformers of melatonin (**1**) due to electrostatic interactions and hyperconjugation could account for its well-documented antioxidant efficacy. In addition, 6-hydroxymelatonin (**7**) appears as a better free-radical scavenger than **1**, again in agreement with experimental results.

For carvedilol (**8**), the theoretical parameters obtained in this study are not consistent with a good antioxidant activity. In contrast, some of its ring-hydroxylated metabolites appear as powerful antioxidants due to the formation of an oxyl radical stabilized by delocalization over the carbazole nucleus.

Quantum-mechanical calculations, even at a semi-empirical level, can thus give insights into the molecular mechanisms by which antioxidants exert their effects.

The authors are indebted to the *Swiss National Science Foundation* for support. Insightful discussions with Prof. *Pierre Vogel* (Institute of Organic Chemistry, University of Lausanne) are warmly acknowledged.

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Received April 23, 1993