7-Ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A): a DFT study of the antioxidant mechanism. 1. Interaction of echinochrome A with hydroperoxyl radical

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The molecular geometry and electronic structure of hydroxy-substituted naphthazarin (NZ) - 7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A, (Et)NZ(β-OH)$_3$, **1**) were calculated by the B3LYP/6-311G(d) method. The influence of the (i) character of the β-OH groups dissociation and (ii) conformational mobility of molecule **1** and the anions, radicals, and radical anions derived from **1** on the energy of their reactions with hydroperoxyl radical was studied by the (U)B3LYP/6-31G and (U)B3LYP/6-311G(d) methods. The enol-enolic tautomerism due to the transfer of hydrogen atoms of α-OH groups and rotational isomerism of the β-OH groups at the C(2) and C(3) atoms and of the α-OH groups at the C(5) and C(8) atoms were studied. The equilibrium in the gas-phase reaction **1** + $^{\bullet}$OOH ⇄ (Et)(HO-β)$_2$NZ(β-O$^{\bullet}$) + HOOH (1) (quenching of hydroperoxyl radical) is shifted to the separated reagents. Heterolysis of the O—H bond in one of the three β-hydroxy groups considerably reduces the energy of subsequent O—H bond homolysis in either of the two remaining β-hydroxy groups. As a consequence, the reaction (Et)(HO-β)$_2$NZ(β-O$^-$) + $^{\bullet}$OOH ⇄ (Et)(HO-β,$^-$O-β)NZ(β-O$^{\bullet}$) + HOOH (2) (quenching of hydroperoxyl radical) becomes exothermic and the equilibrium is shifted to the formation of hydrogen peroxide. The Gibbs energy gain in reaction (2) varies from −6.4 to −10.9 kcal mol^{-1} depending on which β-hydroxy group is involved in the O—H bond homolysis.

Key words: density functional theory, conformational analysis, polyhydroxy-1,4-naphthoquinones, naphthazarins, echinochrome A, antioxidant, hydroperoxyl radical, bond dissociation energy, homolysis, heterolysis.

Echinochrome A (7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone, **1**), the sea urchin pigment from the Sea of Japan, and related β-hydroxy-substituted derivatives of 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin, NZ) are efficient antioxidants and antiradical agents.[1—4] Compound **1** possessing the highest antioxidant activity serves the substance of an efficient domestic drug HISTOCROME® designed for treatment of acute myocardial infarction and ishemic heart disease.[5,6]

Compound **1** and β-hydroxy-substituted naphthazarins form a new structural class of antioxidants. Formally, molecule **1** comprises a benzenoid moiety and a quinonoid fragment; at the same time it can also be considered as a polyphenol and a hydroxy-substituted *p*-quinone. Phenols are a well-known class of antioxidants and their antioxidant and antiradical action has been a subject of numerous experimental and theoretical studies,[7—12] but no such properties of hydroxy-substituted *p*-quinones were not reported so far. However, the key feature of the cyclic system of molecule **1** is that, similarly to the molecules of other β-hydroxy-substituted naphthazarins, this species experiences reversible isomerism (prototropic, enol-enolic tautomerism).[13,14] As the NZ molecule,[15] molecule **1** can theoretically exist as a mixture of four tautomeric forms due to fast asynchronous or concerted transfer of the bridging protons of the six-membered chelate rings from oxygen atoms of the hydroquinonoid (benzenoid) fragment to the carbonyl oxygens of the quinonoid fragment (Scheme 1). Among them, the forms **1A** and **1C** are of the 1,4-naphthoquinonoid type while the other two forms, **1B** and **1D**, are of the 1,5-naphthoquinonoid ("semiquinonoid") type. Conversion **1A** ⇌ **1C** causes the nature of the rings to be changed, namely, the benzenoid ring of molecule **1** becomes a quinonoid one and *vice versa*. Because the classical phenolic antioxidants do not behave in such a fashion, the aforesaid gives reason to consider compound **1** and related β-hydroxy-substituted derivatives of naphthazarin as a new structural type of antioxidants.

Treating compound **1** as polyphenol, it is difficult to explain its high antioxidative efficiency.[1—3] The hydroxyl groups in α-positions of the naphthalene nucleus almost

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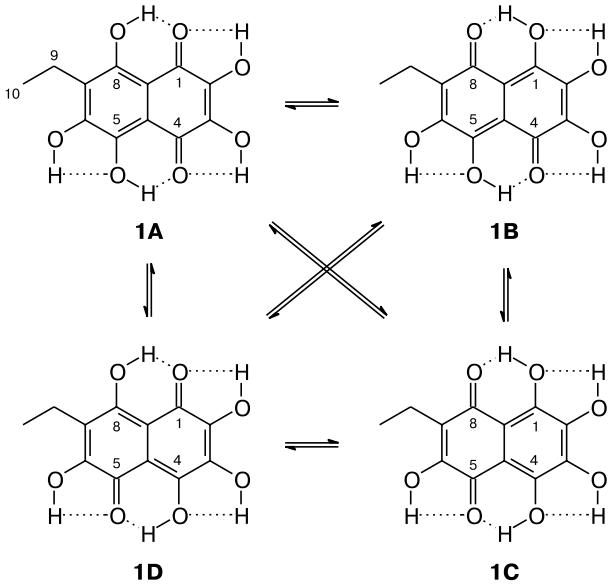
Scheme 1



do not contribute to the antioxidant action of compound **1** because the phenols whose OH groups are involved in the formation of strong intramolecular hydrogen bonds (IMHB) do not show the antioxidant effect.[7] (According to our B3LYP/6-31G(d) evaluation, the energies of the IMHB involving the α-OH groups at the C(5) and C(8) atoms in molecule **1** are 12.73 and 12.62 kcal mol^{-1}, respectively). Neither NZ nor its benzo analog 1,4-dihydroxy-9,10-anthraquinone (quinizarin) possess the antioxidative activity[1,2] and the number of reactive OH groups in molecule **1** is thus reduced to one in **1A** (simple phenol) and two in **1C** (1,2-dihydroxybenzene, pyrocatechol). Nevertheless, experiments showed that compound **1** is an efficient antioxidant.[1–4]

The ishemized myocard is characterized by enhanced lipid peroxidation and reduced activity of antioxidant-protective enzymes. The data accumulated to date suggest an oxygen free-radical mechanism of autoaggression in the case of ishemic heart disease and acute myocardial infarction and involvement of the active forms of oxygen (AFO) in cardiovascular pathology.[16]

The AFO include various reduction products of molecular oxygen, such as the superoxide radical anion $O_2^{\bullet -}$, its protonated form (hydroperoxyl radical $^{\bullet}OOH$), singlet oxygen ($O^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$), and hydrogen peroxide (H_2O_2). Among all the AFO listed above, the least stable (most reactive) is the hydroxyl radical and the most stable is hydrogen peroxide. Based on the data of the *in vitro* experiments, one can assume that one of the mechanisms of the anti-ishemic action of compound **1** in living organism will be efficient inhibition of AFO. To understand the mechanism of the antioxidant action of compound **1** *in vivo*, it is important to allow for some features of its physicochemical properties.

In the circulatory system of a human organism, compound **1** inevitably interacts with serum blood proteins that deliver the substance to the ishemized myocard. Among these proteins, the most active is human serum albumin (HSA). One of the main functions of HSA is non-specific transport of low-molecular-weight physiological metabolites including pharmaceuticals.[17] The molecular mechanisms of the HSA—ligand binding play an important role, because this process is accompanied by changes in the physicochemical properties of the protein and the ligands, which can lead to either a less or more pronounced action of the pharmaceuticals in organisms.

We established that HSA forms a tightly bound complex with compound **1** in a physiological solution. Coincidence of the electronic absorption spectrum of the complex HSA·**1** (1 : 1 mole ratio) in the visible region with the absorption spectrum of sodium salt of echinochrome A (**1** + $NaHCO_3$, 1 : 1 mole ratio)* shows that the binding of compound **1** with albumin is accompanied by heterolysis of the O—H bond in one β-OH group. One can assume that this heterolytic bond dissociation causes significant changes in both the molecular geometry and characteristics of the corresponding potential energy surface (PES). The assumption that compound **1** mainly exists as a monoanion at the physiological pH value of the medium can be confirmed by the fact that the pK_a value of one β-OH group in molecule **1** is equal to 4.90 (see Ref. 18) or 5.30 (see Ref. 3).

Since the mechanisms of the antioxidant action of compound **1** *in vivo* are still unclear, the aforesaid makes theoretical investigations of various channels of its interaction with AFO topical and quite interesting.

The aim of this work was carry out a theoretical study of possible mechanisms of the reaction of compound **1** with hydroperoxyl radical (analog of lipid peroxyl radicals $LOO^{\bullet}$ [19]). To this end, we calculated the homolytic dissociation energies (D_{OH}) of O—H bonds in β-OH groups in the neutral molecule **1** and in the deprotonated forms derived from **1** in the framework of the density functional theory (DFT).

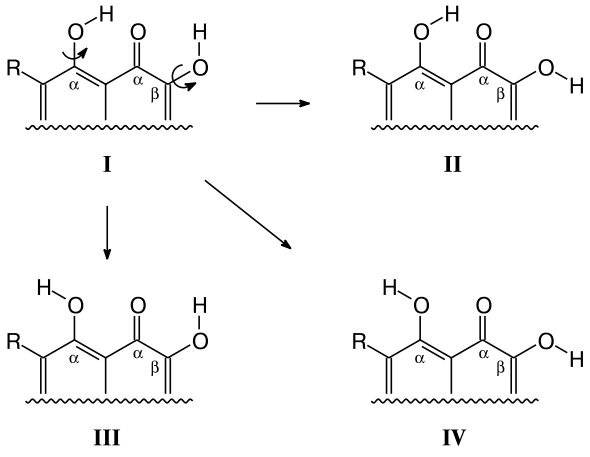
Recently,[20] the energy characteristics of quenching of hydroperoxyl radical with phenol and toluene were calculated using the DFT approach. In addition, systematic investigations of the applicability of various exchange-correlation functionals to calculations of the enthalpies of O—H bond homolytic dissociation in phenol, pyrocatechol, hydrogen peroxide, methanol, and water revealed the possibility of quantitative prediction of the energy characteristics of such reactions by quantum chemistry methods.[21–23]

* Compound **1** is present in the HISTOCHROME® drug in this form.

Results and Discussion

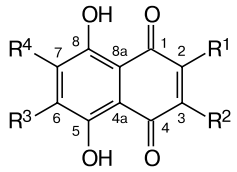
All five hydroxyl protons in molecule **1** are bonded by rather strong IMHB. The α-OH groups are involved in fast tautomeric transitions, which predetermines the possibility for molecule **1** to exist as a mixture of four tautomers **1A—1D** (see Scheme 1). In turn, each tautomer of molecule **1** can exist as a mixture of rotamers (type-**I** (**II**, **III**, and **IV**) rotamers, Scheme 2) with respect to internal rotation of the β-OH and α-OH groups, or to simultaneous rotation of these groups about corresponding C—O bonds (type-**I**, type-**II**, and mixed-type rotational isomerism, respectively). Notations of the possible types of rotamers involving the β-OH and α-OH groups in molecule **1** and related molecules and in products of their transformations will be considered in detail below.

Scheme 2



R = OH, Et, O^-, $O^{\bullet}$

The energetically most favorable state of molecule **1** is tautomer **1A** (see Scheme 1). Rotation of any β-OH and moreover α-OH group about a C—O bond leads to a considerable increase in system´s energy. As to the products **2—11** * obtained by different transformations of molecule **1** (see below), rotational isomerism of the β-OH groups at the C(2) and C(3) atoms (in some cases, also rotational isomerism of the α-OH groups at the C(5) and C(8) atoms) contributes largely to the total energies (*E*), enthalpies (*H*), and Gibbs energies (*G*)**. In turn, this affects the energies of the reactions of $^{\bullet}$OOH radical with these compounds, resulting in quenching of the former.



1—14

Compound	R^1	R^2	R^3	R^4	Compound	R^1	R^2	R^3	R^4
1	OH	OH	OH	Et	**8**	OH	OH	O^-	Et
2	$O^{\bullet}$	OH	OH	Et	**9**	O^-	$O^{\bullet}$	OH	Et
3	OH	$O^{\bullet}$	OH	Et	**10**	O^-	OH	$O^{\bullet}$	Et
4	OH	OH	$O^{\bullet}$	Et	**11**	OH	O^-	$O^{\bullet}$	Et
5	OH	OH	OH	MeCH*	**12**	$O^{\bullet}$	O^-	OH	Et
6	O^-	OH	OH	Et	**13**	$O^{\bullet}$	OH	O^-	Et
7	OH	O^-	OH	Et	**14**	OH	$O^{\bullet}$	O^-	Et

* $C(10)H_3—C^{\bullet}(9)H$.

Isomeric transformations lead to a considerable increase in the number of states in which molecule **1** and its derivatives can react with free radicals and thus manifest their antioxidant properties. We have preliminarily calculated the quenching of $^{\bullet}$OOH radical, which proceeds by the bimolecular mechanism and involves approach of the $^{\bullet}$OOH radical to the β-OH group at the C(2) or C(3) atom of molecule **1**, formation of a pre-reaction complex, and subsequent transfer of H atom. According to our calculations, the reaction is vitally associated with a cascade of conformational transitions (changes in the spatial molecular structure) initiated by this collision, which involve protons of both β-OH and α-OH groups. At the instant of formation of the pre-reaction complex, the IMHB between the carbonyl group and the β-OH group adjacent to that involved in the reaction (see Scheme 1) is characterized by a one-well ($R_{O...O}$ = 2.37—2.40 Å) rather than asymmetrical two-well potential, as before the collision.

From this it follows that the antioxidant properties of compound **1** and its derivatives (deprotonated and radical forms) can depend on their conformational mobilities, which is due to tautomerism involving the α-OH groups and to rotational isomerism involving both the β-OH and α-OH groups.

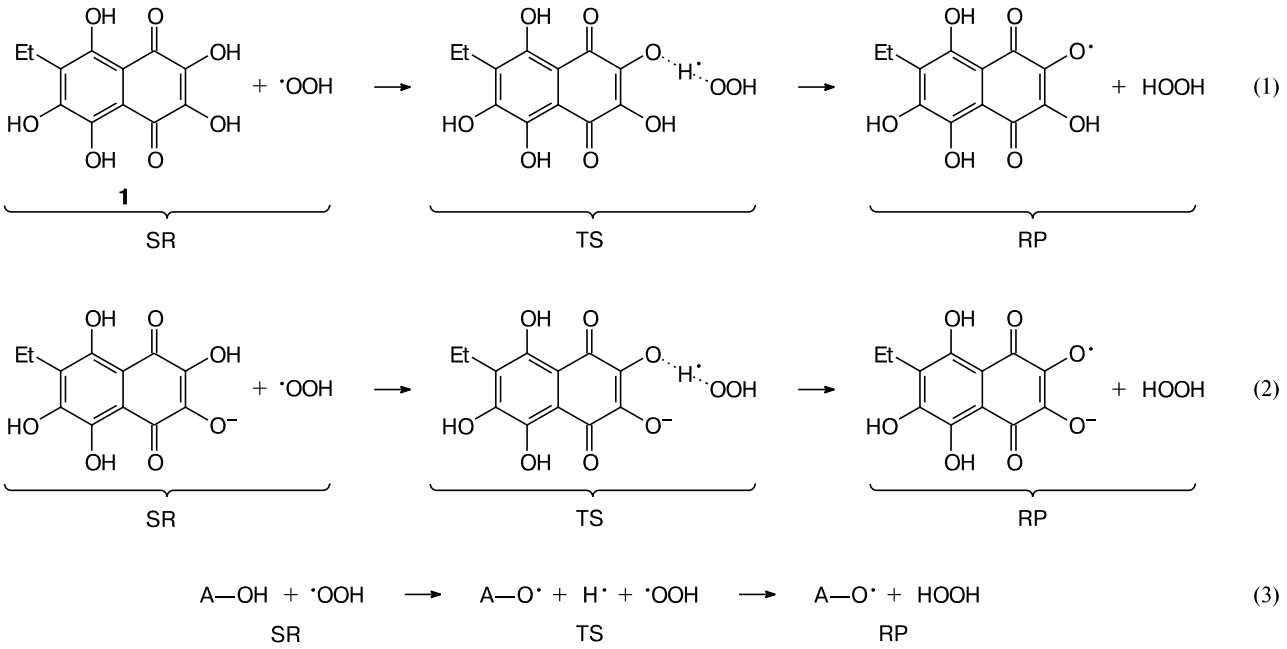
We evaluated the energy balance (energies) of the reactions of molecule **1** and deprotonated forms of this species with $^{\bullet}$OOH radical (Scheme 3, reactions (1) and (2), quenching of $^{\bullet}$OOH radical). In both cases only the initial states (starting reactants, SR) and the final states (reaction products, RP) were considered. The collisional stage of the bimolecular reactions, which involves drawing the reactants closer together, formation of pre-reaction complexes, and transfer of H atom from molecule **1** and anions derived from it to the hydroperoxyl radical, was not modeled.

Hydrogen atom transfer was treated as a two-step process (see Scheme 3, reaction (3)).

* The simple numbering of compounds by figures only means that each compound is a mixture of all possible isomeric forms taken in the corresponding percentages.

** $E = E_0$ + ZPE, where E_0 is the electronic energy, ZPE is the zero-point vibration energy; $H = E_0 + H_T$, where H_T is the temperature correction to the total enthalpy; $G = E_0 + G_T$, where G_T is the temperature correction to the total Gibbs energy.

Scheme 3



In this case, the transition state (TS) is a state in which the reaction system is comprised of three noninteracting radical species, which are conditionally placed at infinite distances from one another.

The energy characteristics of each stage of reaction (3) can be determined using calculations of the homolytic dissociation energies of the O—H bond in a β-OH group in molecule **1** or in the anions derived from molecule **1** (for the first stage) and an O—H bond in the hydrogen peroxide molecule (for the second stage). Because both mechanisms, *i.e.*, the two-step mechanism (reaction (3)) and one-step collisional mechanism (reactions (1) and (2)), lead to the separated products as the final states, they are characterized by the same energy effect. Detailed treatment of the collisional mechanism of the reaction with ·OOH radical (quenching of ·OOH radical) will be reported elsewhere. In this work the emphasis is placed on the study of the effect of the conformational mobility of molecule **1** and its derivatives on the O—H bond dissociation energies of different β-OH groups and on the energy balance of the reduction reactions of hydroperoxyl radical.

Products **2—11** were obtained by formal O—H bond heterolysis or homolysis in the β-OH groups of molecule **1**. Only radical **5** (its role will be considered below) was obtained by homolysis of the C—H bond at the C(9) atom in the methylene unit of the Et substituent.

The atomic numbering scheme shown for tautomer **1A** was used for all isomers (tautomers and rotamers) of compounds **1—11**.

According to classical concepts of organic chemistry, in the case of O—H bond homolysis the unpaired O2p-electron remains localized on the O atom, whereas in the case of O—H bond heterolysis an electron is transferred to the O atom from the 1s-AO of hydrogen. This approach was used for conditional representation of the structure of compounds **2—11**. The spin density distribution in radicals **2—5** and radical anions **9—11** obtained from quantum chemical calculations is shown in Fig. 1.

The starting and final states of reaction (3), *i.e.*, reduction of hydroperoxyl radical with molecule **1** and its derivatives, were modeled assuming that all relaxation processes in the molecules are completed and the reaction system in each stage is in thermodynamic equilibrium (only stationary states of the molecules were considered). In this case the states of radical anions $\mathbf{1}^{\bullet -}$ are insensitive to the simulation procedure. For instance, heterolysis and homolysis of the O—H bonds in the β-OH groups at the C(2) and C(3) atoms of molecule **1** can occur in different fashion, namely, 1) O—H bond heterolysis in the C(2)—OH group followed by O—H bond homolysis in the C(3)—OH group, 2) O—H bond homolysis in the C(3)—OH group followed by O—H bond heterolysis in the C(2)—OH group, 3) O—H bond heterolysis in the C(3)—OH group followed by O—H bond homolysis in the C(2)—OH group, and 4) O—H bond homolysis in the C(2)—OH group followed by O—H bond heterolysis in the C(3)—OH group. These four combinations of O—H bond dissociation in the β-OH groups correspond to the same final state with distributed spin and electron densities (compounds **9** and **12**). This equally holds for the pairs of compounds **10** and **13**, **11** and **14**; therefore, in the text below we present only the results of calcula-

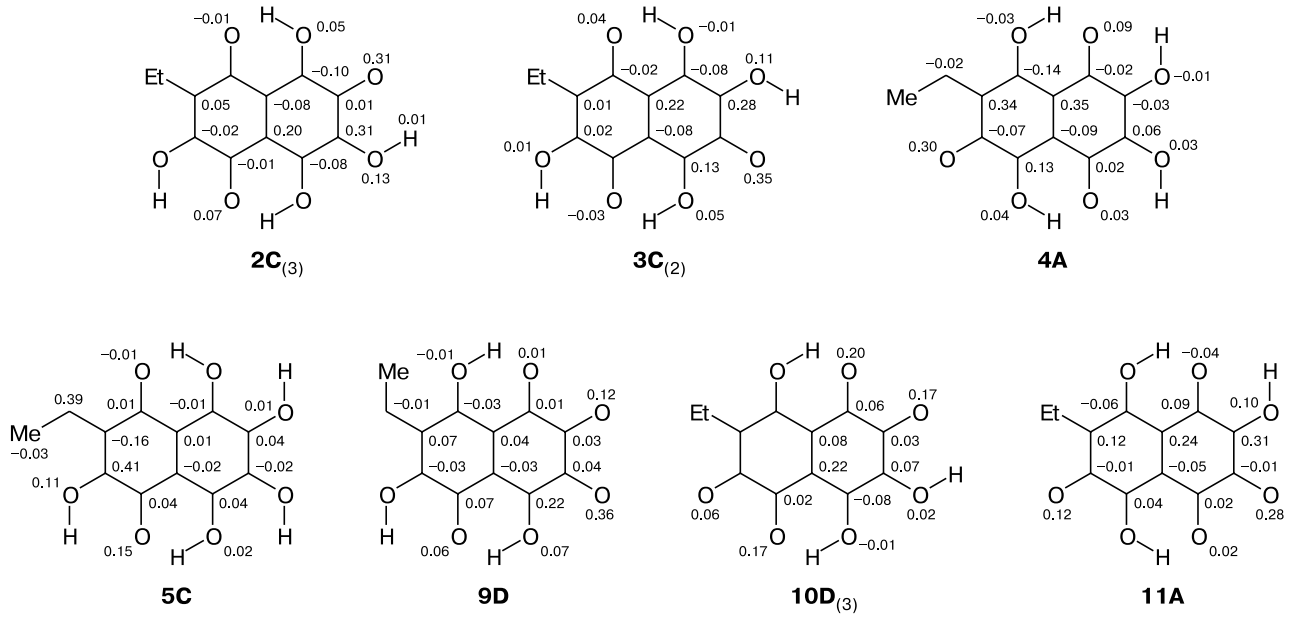


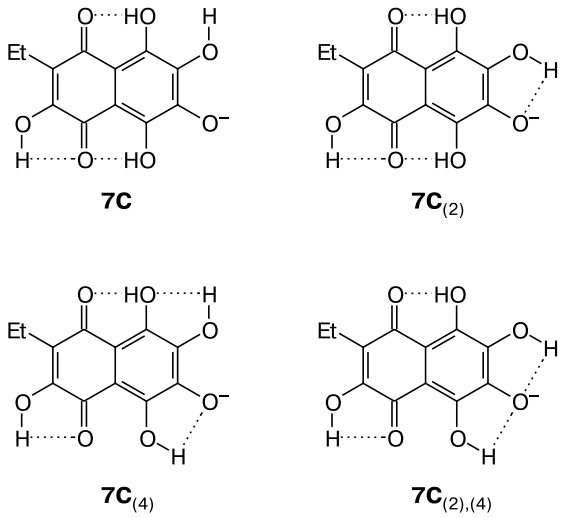
Fig. 1. Spin density distribution in radicals **2**—**5** and radical anions **9**—**11**.

tions obtained for compounds **9**—**11**. In this connection the centers on which the unpaired electron and charge is localized in radicals, anions, and radical anions (see above) should be treated conditionally, because they only indicate particular bond dissociation types. This is also valid for the compounds discussed below.

As the parent molecule **1**, each of the products **2**—**11** can also theoretically exist as a mixture of four tautomers **A**, **B**, **C**, and **D** (see Scheme 1). Moreover, each tautomer can in turn be a mixture of type-**I** (**II**, **III**, and **IV**) rotamers with respect to internal rotation of the β-OH and α-OH groups about corresponding C—O bond (see Scheme 2). We will denote the rotamers with respect to rotation of the β-OH groups at the C(2) and C(3) atoms, using the subscripts "(2)" and "(3)", respectively. The rotamers with respect to rotation of the α-OH groups at the C(1), C(4), and C(5) atoms will be denoted analogously using the subscripts "(1)", "(4)", and "(5)", respectively.

Now we will illustrate the notations of isomers taking compound **7** as an example. In particular, the notation "**7C**$_{(2),(4)}$" (mixed-type (type-**IV**) rotamer, see Scheme 2) denotes the type-**C** tautomer (type-**I** rotamer, see Scheme 2) of compound **7**, the subscript "(2)" denotes the rotamer with respect to rotation of the β-OH group at the C(2) atom (type-**II** rotamer with respect to **7C**), and the subscript "(4)" denotes the rotamer with respect to rotation of the α-OH group at the C(4) atom (type-**III** rotamer with respect to **7C**$_{(2)}$).

Based on the results of the Gibbs energy calculations using the (U)B3LYP/6-31G and (U)B3LYP/6-311G(d) methods, we carried out the conformational analysis of



molecule **1** and its derivatives with allowance for tautomerism involving the α-OH groups and rotational isomerism involving the α-OH and β-OH groups. The primary screening of all tautomers and rotamers of compounds **1**—**11** by the (U)B3LYP/6-31G method (Table 1) was followed by full optimization of the geometries of the isomers characterized by the percentages of 3% and higher (Fig. 2) by the (U)B3LYP/6-311G(d) method (Table 2). Only these isomers of compounds **1**—**11** were taken into account in considering the spin density distributions in the molecules and the energy balances of reactions (1) and (2).

The rotamers with respect to rotation of β-OH groups in molecules **1**, **5**, and **9** do not contribute to reactions (1)

Table 1. Gibbs energies (G/a.u.)[a] and statistical weights ($g_{X_{i,j}}$)[b] of different tautomers and rotamers of molecule **1** and its derivatives (radicals, anions, and radical anions) obtained from (U)B3LYP/6-31G calculations

Compound	$-G/g$			
	A	**B**	**C**	**D**
1	989.416402/0.93454	989.407296/0.00006	989.413890/0.06534	989.411460/0.00006
2	988.789773/0.24080	988.781717/0.00005	988.785143/0.00180	988.786041/0.00460
$\mathbf{2}_{(3)}$[c]	988.790397/0.46450	988.783616/0.00040	988.789075/0.11450	988.789073/0.17340
3	988.790475/0.52521	988.783454/0.00031	988.786277/0.00616	988.785708/0.00337
$\mathbf{3}_{(2)}$	988.790028/0.32713	988.786056/0.00487	988.789154/0.12963	988.785693/0.00332
4	988.784510/0.91349	988.775331/0.00005	988.780087/0.00844	988.782187/0.07802
5	988.791665/0.01103	988.792111/0.01769	988.795890/0.96989	988.788874/0.00057
$\mathbf{5}_{(6)}$	988.775564/$<1\cdot10^{-5}$	988.7755697/$<1\cdot10^{-5}$	988.776883/$<1\cdot10^{-5}$	988.789222/0.00083
6	988.902579/0.00153	988.895649/$<1\cdot10^{-5}$	988.901486/0.00048	988.903539/0.00424
$\mathbf{6}_{(3)}$	988.904807/0.01623	988.907465[d]/0.27095	988.907486/0.27705	988.907900/0.42952
7	988.898376/0.03113	988.894861/0.00076	988.894586/0.00057	988.893694/0.00022
$\mathbf{7}_{(2)}$	988.900514/0.30135	988.898449/0.03382	988.901197/0.62120	988.897372/0.01079
8	988.906998/0.76149	988.900277[d]/0.00062	988.902928/0.01022	988.905858/0.22767
9	988.295881/0.53450	988.290530/0.00190	988.295743[d]/0.46180	988.290544/0.00190
10	988.284882/0.00018	988.281282/$<1\cdot10^{-5}$	988.283382/0.00004	988.289375/0.02094
$\mathbf{10}_{(3)}$	988.287313/0.00236	988.283365/0.00004	988.286462/0.00096	988.293002/0.97550
11	988.293393/0.76800	988.287520[d]/0.00153	988.289652/0.01461	988.291158/0.07200
$\mathbf{11}_{(2)}$	988.291171/0.07292	988.288740/0.00556	988.290965/0.05900	988.288866/0.00638

[a] $G = E_0 + G_{el} + G_{tr} + G_{rot} + G_{vibr}$, where E_0 is the ground-state electronic energy and G_{el}, G_{tr}, G_{rot}, and G_{vibr} are respectively the electronic, translational, rotational, and vibrational components of the Gibbs energy.
[b] X is the notation of the compound, i and j are the subscripts denoting the tautomer (**A**, **B**, **C** or **D**) and rotamer of compound X.
[c] This notation denotes that all four tautomers ($\mathbf{2A}_{(3)}$, $\mathbf{2B}_{(3)}$, $\mathbf{2C}_{(3)}$, and $\mathbf{2D}_{(3)}$) were considered for this rotamer of compound **2**. Notations for other compounds have the same sense.
[d] Corresponds to the mid-point of the plateau on the PES.

and (2), because the total energies and the total Gibbs energies of these species are more than 6 kcal mol^{-1} higher than the corresponding energy characteristics of the starting compounds in which the OH group in question is involved in the IMHB.[24] The content of such rotamers is at most 0.01%. Rotational isomerism of the β-OH group at the C(6) atom in compounds **2**, **3**, and **5—7** leads to cleavage of the IMHB O(6)—H...O(5)* and is characterized by a similar increase in the Gibbs energies.

According to B3LYP and MP2 calculations with the 6-31G, 6-31G(d), 6-311G(d), and cc-pvTZ basis sets, the energetically most favorable tautomer of molecule **1** is **1A**. The total energies of other tautomers of molecule **1** estimated by different methods in different basis sets lie in the ranges

$$E(\mathbf{1A}) + 5.0 < E(\mathbf{1B}) < E(\mathbf{1A}) + 9.0 \text{ (kcal mol}^{-1}),$$
$$E(\mathbf{1A}) + 0.7 < E(\mathbf{1C}) < E(\mathbf{1A}) + 2.0 \text{ (kcal mol}^{-1}),$$
$$E(\mathbf{1A}) + 3.0 < E(\mathbf{1D}) < E(\mathbf{1A}) + 7.0 \text{ (kcal mol}^{-1}).$$

Similar relationships were also obtained for the Gibbs energies:

$$G(\mathbf{1A}) + 5.7 < G(\mathbf{1B}) < G(\mathbf{1A}) + 6.9 \text{ (kcal mol}^{-1}),$$
$$G(\mathbf{1A}) + 1.6 < G(\mathbf{1C}) < G(\mathbf{1A}) + 2.0 \text{ (kcal mol}^{-1}),$$
$$G(\mathbf{1A}) + 3.1 < G(\mathbf{1D}) < G(\mathbf{1A}) + 3.2 \text{ (kcal mol}^{-1}).$$

Because of this molecules **1** exist in the gas phase as a mixture of tautomers **1A** (93—98%) and **1C** (2—7%).

Similarly to molecule **1**, the major isomeric forms of radical **4**, anion **8**, and radical anion **11** are the tautomer **A** and the type-**I** (for **4** and **11**) and type-**III** rotamers (for **8**), namely, **1A** (98%), **4A** (98%), $\mathbf{8A}_{(5)}$ (53%), and **11A** (82%) (see Table 2). The major rotamer of radical anion **9** is also a type-**I** species, but in another tautomeric form (**D**). The "semiquinonoid" (4,8-quinonoid) tautomer **8B** of compound **8** can not exist in principle because it corresponds to a plateau on the PES with energies that are ~4.5—5.5 kcal mol^{-1} higher than the energy of the major isomer. This also holds for tautomer **11B** of molecule **11**, which corresponds to a plateau with energies $G(\mathbf{11B}) \approx G(\mathbf{11A}) + (3.5—4.5)$ kcal mol^{-1} on the PES.

The energetically most favorable isomeric forms of radicals **2** and **3** and anions **6** and **7** are the tautomer **C** and type-**II** rotamers $\mathbf{2C}_{(3)}$, $\mathbf{3C}_{(2)}$, $\mathbf{6C}_{(3)}$, and $\mathbf{7C}_{(2)}$,

* Figure in parentheses at an oxygen atom denotes the number of the carbon atom to which the oxygen atom is bonded.

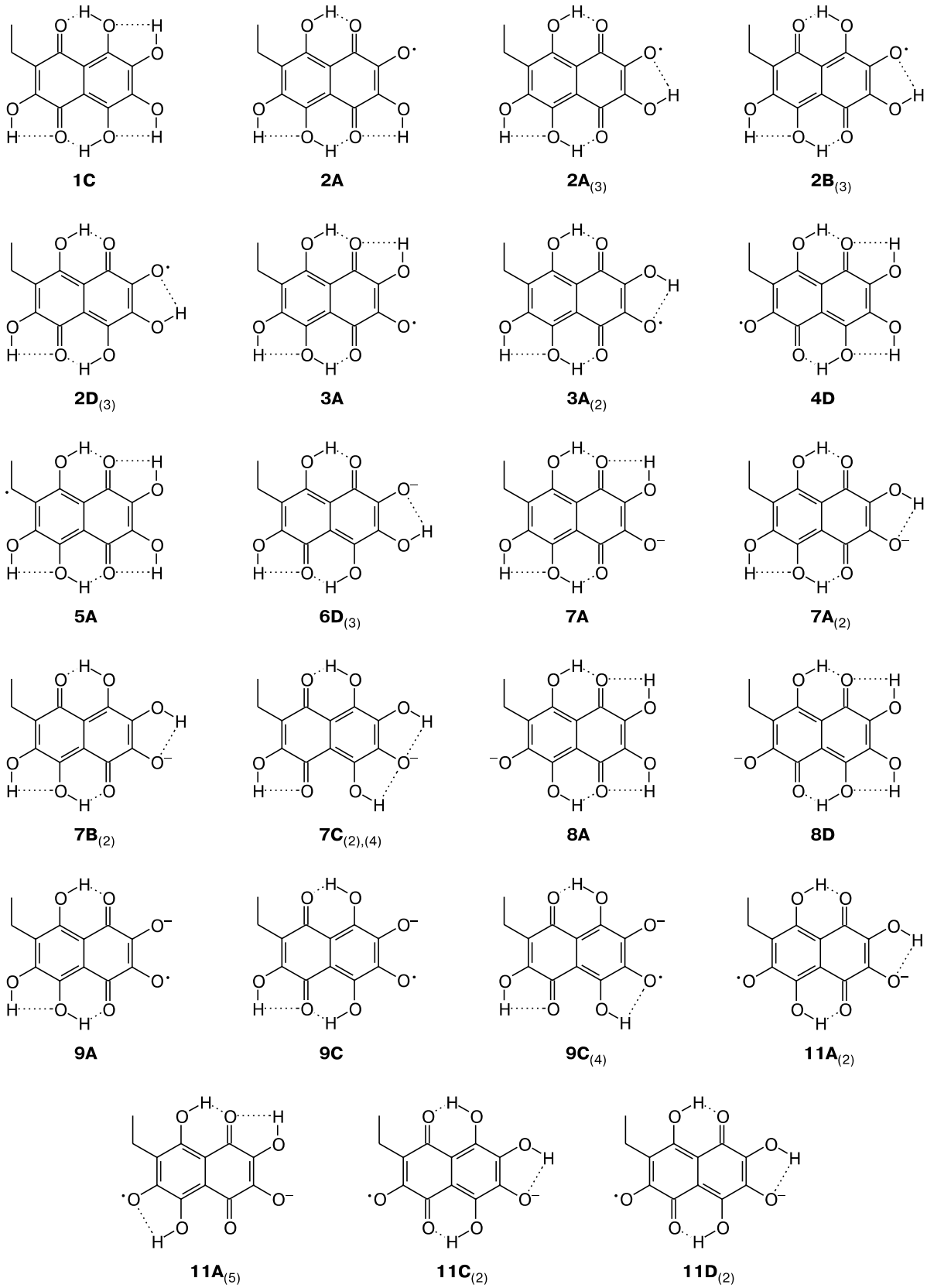


Fig. 2. Rotamers of compounds **1—11** (except the energetically most favorable isomers) whose percentages exceed 3%.

Table 2. Parameters $G^*(X)$/kcal mol^{-1} = $G_X^{eff} - G_{XM}$, electronic energies (E_0/a.u.), temperature corrections to the total Gibbs energy (G_T/a.u.), and total Gibbs energies (G/a.u. = $E_0 + G_T$) of molecule **1** and its derivatives and their percentages (g) obtained from (U)B3LYP/6-311G(d) and (U)B3LYP/6-31G) calculations

Compound	G^* /kcal mol^{-1}	Conformer	$-E_0$	G_T	$-G$	g (%)
1	0.05	**1A**	990.140307	0.169572	989.970735	97.61
		1C	990.136397	0.169161	989.967236	2.39
2	0.23	**2A**	989.502345	0.156263	989.346082	9.79
		$\mathbf{2A}_{(3)}$	989.503113	0.156473	989.346640	17.52
		$\mathbf{2B}_{(3)}$	989.495346	0.155538	989.339808	0.01
		$\mathbf{2C}_{(3)}$	989.503617	0.156154	989.347463	41.88
		$\mathbf{2D}_{(3)}$	989.503248	0.156072	989.347177	30.90
3	0.27	**3A**	989.503386	0.156976	989.346410	21.09
		$\mathbf{3A}_{(2)}$	989.502593	0.156468	989.346125	15.60
		$\mathbf{3C}_{(2)}$	989.503851	0.156403	989.347448	63.32
4	0.04	**4A**	989.498201	0.155465	989.342736	98.22
		4D	989.493396	0.154448	989.338948	1.78
5	0.08	**5A**	989.342131	0.155440	989.342131	4.87
		5C	989.500503	0.155577	989.344926	95.12
6	0.16	$\mathbf{6C}_{(3)}$	989.619614	0.156538	989.463076	80.65
		$\mathbf{6D}_{(3)}$	989.617674	0.156046	989.461628	19.35
7	0.19	**7A**	989.605960	0.155304	989.450656	0.14
		$\mathbf{7A}_{(2)}$	989.608160	0.155479	989.452681	1.16
		$\mathbf{7B}_{(2)}$	989.605486	0.154995	989.450491	0.11
		$\mathbf{7C}_{(2)}$	989.613038	0.156476	989.456562	70.71
		$\mathbf{7C}_{(2),(4)}$	989.612005	0.156321	989.455684	27.89
8	0.04	**8A**	989.619931	0.156593	989.463338	46.69
		$\mathbf{8A}_{(5)}$	989.619625	0.156174	989.463451	52.52
		8D	989.615122	0.155616	989.459506	0.79
9	0.18	**9A**	988.990368	0.142348	988.848020	1.75
		9C	988.993954	0.142896	988.851058	43.85
		$\mathbf{9C}_{(4)}$	988.992230	0.143067	988.849163	5.88
		9D	988.993679	0.142293	988.851156	48.52
10	0.00	$\mathbf{10D}_{(3)}$	988.993463	0.142375	988.851088	100.0
11	0.28	**11A**	988.993658	0.142199	988.851459	82.34
		$\mathbf{11A}_{(2)}$	988.991041	0.141886	988.849155	7.17
		$\mathbf{11A}_{(5)}$	988.990203	0.141776	988.848427	3.31
		$\mathbf{11C}_{(2)}$	988.990977	0.141844	988.849133	7.00
		11D	998.983637	0.141971	988.841666	0.00
		$\mathbf{11D}_{(2)}$	988.986494	0.140838	988.845656	0.18

respectively (Fig. 3). Here, on going from $\mathbf{2C}_{(3)}$ to $\mathbf{6C}_{(3)}$ the percentage of tautomer **C** increases from ~42 to 81% (see Table 2). In the case of radical anion **10** the energetically most favorable and the only tautomer is the "semiquinonoid" form **D** (type-**II** rotamer), namely, $\mathbf{10D}_{(3)}$ (100% percentage). Similarly to compounds **4** and **8**, the semiquinonoid tautomer **6B** of compound **6** can not exist.

All isomers of compounds **1—11** listed in Table 2 rather than only the energetically most favorable isomers contribute to the energy balances of reactions (1) and (2). The contribution of the conformational mobility of the compounds under study to the energy balance of reactions can be included using the effective Gibbs energies $G_X^{eff} = \Sigma_i(g_{X_i} G_{X_i})$, where G_X^{eff} is the effective Gibbs energy of compound X; g_{X_i} is the statistical weight of the ith isomer (rotameric-tautomeric form) of compound X; i is the index corresponding to all types of the isomeric forms of compound X listed in Table 2; and G_{X_i} is the Gibbs energy of the ith isomer of compound X. According to (U)B3LYP/6-311G(d) calculations for compounds **1—11**, the differences $G^*(X) = G_X^{eff} - G_{X_M}$, where G_{X_M} is the Gibbs energy of the major (M) isomer of compound X, lie between 0 and 0.28 kcal mol^{-1} for compounds **11** and **10**, respectively (see Table 2).

Comparison of the data listed in Tables 1 and 2 shows that the calculated percentages of different isomers of the same compound strongly depend on the quality of the basis set used in quantum chemical calculations. For instance, calculations of compound **2** led to two different

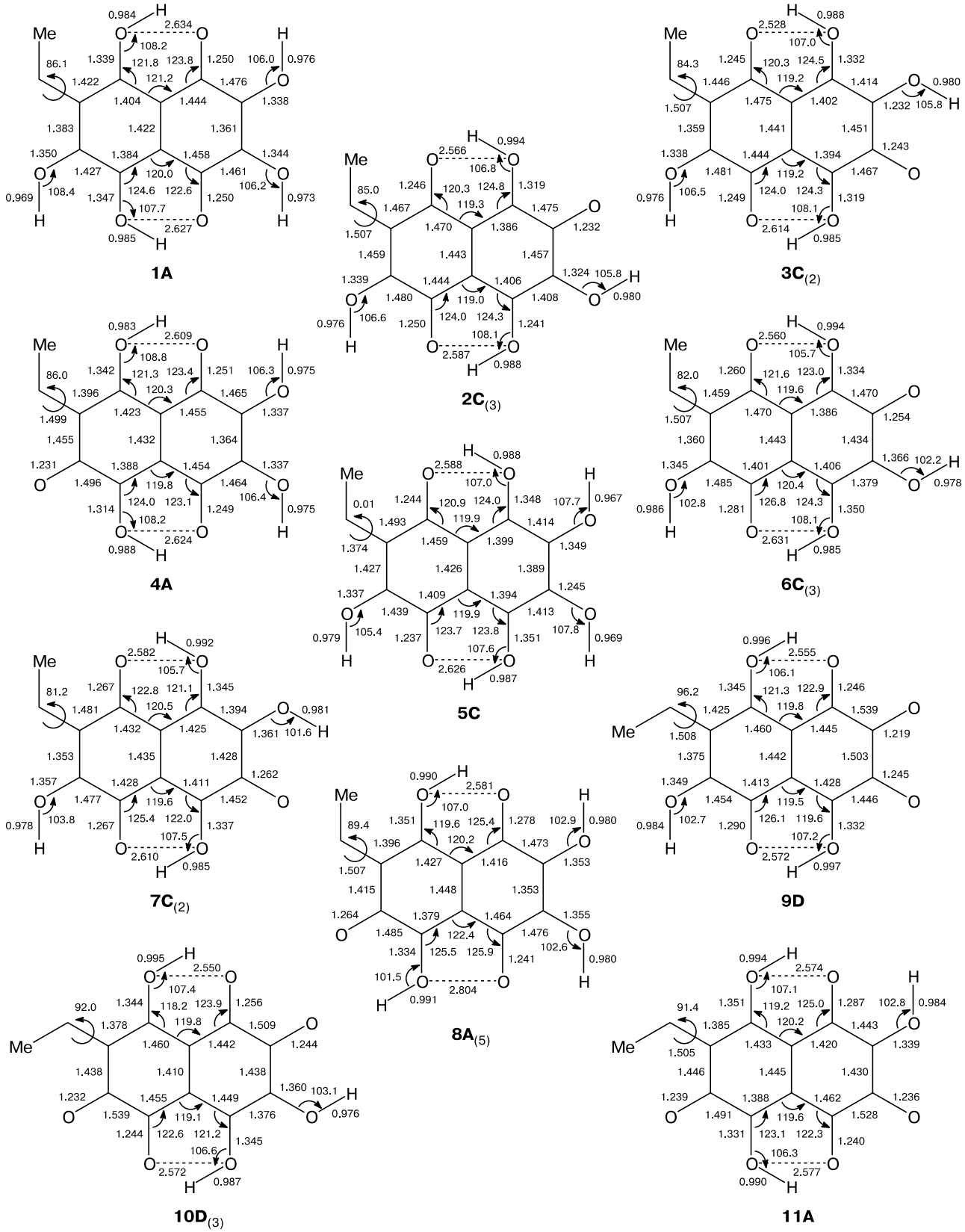


Fig. 3. Energetically most favorable isomers of compounds **1**—**11**; the bond lengths (Å) and bond angles (in degrees) were obtained from (U)B3LYP/6-311G(d) calculations.

species, namely, $\mathbf{2A}_{(3)}$ (B3LYP/6-31G) and $\mathbf{2C}_{(3)}$ ((U)B3LYP/6-311G(d)) as the major isomers. Similarly, calculations of compounds **3** and **9** with the 6-31G basis set predict that the major isomers are **3A** and **9A** (*cf.* $\mathbf{3C}_{(2)}$ and **9D**, respectively, obtained form calculations with the 6-311G(d) basis set).

However, because the statistical weights of isomers exponentially depend on the difference $\Delta G(X) = G_{X_i} - G_{X_M}$, the $G^*(X)$ values calculated in different basis sets differ by at most 0.15 kcal mol^{-1}.

The geometric parameters of the energetically most favorable isomers of molecule **1** and its derivatives **2—11** obtained from (U)B3LYP/6-311G(d) calculations are shown in Fig. 3. The results obtained for the naphthazarin system of nuclei in molecule **1** are in good agreement with the X-ray analysis data for crystalline complex of **1** with dioxane.[25] Noteworthy is the difference between the calculated geometric parameters of two six-membered chelate rings, ...H—O(8)—C(8)=C(8a)—C(1)=O(1)... and ...H—O(5)—C(5)=C(4a)—C(4)=O(4).... In particular, the R(O(1)...O(8)) distance is 2.634 Å, whereas the R(O(4)...O(5)) distance is 2.627 Å. The H_α—O(8)—O(1) and H_α—O(5)—O(4) bond angles are 22.1 and 23.6°, respectively; as a consequence, the distance between the proton of the α-OH group at the C(8) and O(1) atoms, equal to 1.762 Å, is shorter than R(O(5)—H_α...O(4)) = 1.768 Å. Differences in the electronic and dynamic structure of both six-membered chelate rings lead to different chemical shifts of protons of the α-OH groups in molecule **1** in the experimental ^{1}H NMR spectrum (according to our data, $\Delta\delta = 0.2$ in solution in $CDCl_3$).

Dissociation of O—H bonds in the β-OH groups in molecule **1** predetermines significant changes in the molecular geometry. In this respect, the most sensitive are the chemical bonds in the atomic groups forming the six-membered chelate rings (*e.g.*, ...H—O—C(8)=C(8a)—C(1)=O...) and all the chemical bonds of the C atoms bonded to those β-OH groups in which the O—H bonds undergo homolysis or heterolysis (see Fig. 3). For instance, after dissociation of corresponding β-OH group the C—O bond becomes shorter than the C—O bonds in carbonyl groups.

The spin densities (SD) on the atoms of the energetically most favorable isomers of radicals and radical anions of compound **1** are shown in Fig. 1 (only the SD values equal to at least 0.01 in absolute value are presented). The SD values on the atoms of all other isomers of radicals **2—5** and radical anions **9—11** are listed in Table 3.

Homolysis of the O—H bond in a β-OH group in molecule **1** causes the appearance of a high positive spin density SD = 0.28—0.35 on the O atom of the homolysed β-OH group and on the C atom adjacent to the carbon atom to which this β-OH group is bonded (see Fig. 1, isomers $\mathbf{2C}_{(3)}$, $\mathbf{3C}_{(2)}$, and **4A**). A high negative spin density of −0.14 is also localized on the C(8) atom in isomer **4A**.

C—H Bond homolysis in the methylene unit of the ethyl substituent in molecule **1** causes the appearance of

Table 3. Spin densities (SD) on the atoms of radicals and radical anions of compound **1** obtained from UB3LYP/6-311G(d) calculations

Atom*	SD												
	2A	$\mathbf{2A}_{(3)}$	$\mathbf{2B}_{(3)}$	$\mathbf{2D}_{(3)}$	**3A**	$\mathbf{3A}_{(2)}$	**4D**	**5A**	**9A**	**9C**	$\mathbf{11A}_{(2)}$	**11D**	$\mathbf{11C}_{(2)}$
C(1)	0.00	0.00	0.01	0.02	−0.01	−0.03	−0.03	0.02	0.02	0.06	−0.08	−0.14	−0.09
C(2)	−0.07	−0.03	−0.02	0.01	0.44	0.42	−0.03	0.05	0.05	0.03	0.20	0.27	0.15
C(3)	0.45	0.45	0.44	0.30	−0.06	−0.02	0.06	−0.03	0.07	0.04	0.04	−0.01	0.04
C(4)	0.01	−0.02	−0.01	0.11	0.01	0.01	0.02	0.02	0.11	0.22	0.02	0.03	0.14
C(4a)	0.01	0.02	0.02	0.22	0.01	0.01	−0.09	0.20	−0.02	−0.02	−0.06	−0.06	−0.08
C(5)	0.01	0.01	−0.04	0.03	−0.01	−0.02	0.12	−0.05	0.02	0.03	0.06	0.07	0.01
C(6)	−0.01	0.00	0.02	0.01	0.03	0.02	−0.07	0.29	−0.01	−0.02	−0.01	−0.01	−0.02
C(7)	0.04	0.03	0.00	0.00	0.00	0.00	0.34	−0.16	0.04	0.05	0.15	0.08	0.22
C(8)	−0.02	−0.02	−0.01	0.05	0.01	0.00	−0.14	0.04	−0.01	−0.02	−0.08	−0.06	−0.07
C(8a)	0.03	0.03	0.04	0.05	0.04	0.05	0.35	−0.05	0.04	0.00	0.30	0.35	0.29
O(1)	0.00	0.00	0.01	0.03	0.08	0.08	0.09	0.03	0.02	0.02	−0.06	−0.03	−0.01
O(2)	0.25	0.26	0.25	0.23	0.17	0.17	−0.07	0.02	0.22	0.18	0.07	0.07	0.05
O(3)	0.18	0.18	0.18	0.12	0.29	0.30	0.03	0.00	0.35	0.35	0.24	0.25	0.28
O(4)	0.12	0.12	0.16	0.02	0.12	0.01	0.03	0.10	0.11	0.07	0.03	0.04	0.05
O(5)	0.00	0.00	−0.01	0.17	0.00	−0.01	0.05	0.00	0.00	0.02	0.02	0.02	−0.02
O(6)	0.00	0.00	0.00	0.00	0.01	0.01	0.30	0.00	0.00	0.00	0.15	0.13	0.12
O(8)	0.00	0.01	−0.02	0.02	0.01	0.01	−0.02	0.00	0.00	−0.02	−0.01	0.00	−0.04
C(9)	−0.02	0.00	0.00	0.00	−0.02	0.00	−0.02	0.51	0.00	0.00	−0.01	−0.01	0.02
C(10)	0.00	0.00	0.00	0.00	0.00	0.00	0.02	−0.04	0.00	0.00	0.01	0.00	0.01

* Figure in parentheses at the O atom denotes the number of the C atom to which this oxygen atom is bonded.

a high positive spin density of 0.39 and 0.41 on the C(9) and C(6) atoms, respectively, of 0.11 and 0.15 on the O(6) and O(5) atoms, respectively, and a negative spin density (SD = –0.16) on the C(7) atom (isomer **5C** of compound **5**).

Radical anions **9—11** are obtained by heterolysis and subsequent homolysis (or *vice versa*). Here, high positive spin density is localized on the O(3), C(4), and O(2) atoms of isomer **9D** (SD = 0.36, 0.22, and 0.12, respectively), on the C(4a), O(1), O(2), and O(5) atoms of isomer $\mathbf{10D}_{(3)}$ (SD = 0.22, 0.20, 0.17, and 0.17, respectively), and on the C(2), O(3), C(8a), O(6), and C(7) atoms of isomer **11A** (SD = 0.31, 0.28, 0.24, 0.12, and 0.12, respectively) (see Fig. 1).

It should be noted that the high spin densities are only localized on those nuclei in the structures of the energetically most favorable isomers of radicals **2—5**, in which O—H or C—H bond homolysis occurred. The spin density distribution in radical anion **9D** is similar to that determined for the isomers of radicals **2—5**. At the same time, calculations of the energetically most favorable isomers $\mathbf{10D}_{(3)}$ and **11A** predict localization of high spin density on both nuclei in these structures.

The barriers, $\Delta V^{\#}$, to intramolecular α-OH proton transfer in the O—H...O fragments and to internal rotation of β-OH groups are of the order of 2—7 kcal mol^{-1}. As a consequence, the corresponding rate constants are rather high ($k \approx 6.22 \cdot 10^{12}\gamma\exp[-\Delta V^{\#}/(RT)]$, where $\gamma \geq 1.0$ is the factor characterizing the possibility for tunneling reactions to occur). The conformational transformations of compounds **2—4** and **9—11** (see above) are accompanied by corresponding changes in the spin densities on their atoms, *i.e.*, a specific kind of spin density "beats". This basically differs β-hydroxy-substituted derivatives of naphthazarin from typical phenolic antioxidants (tocopherol, ionol, dibunol, *etc.*) in which the conformational mobility (tautomerism and rotational isomerism involving aromatic OH groups) is either absent or hindered.

The electronic energies and the Gibbs energies (see Table 2) were used in energy balance calculations for reactions (1) and (2) (ΔE_r(SR → RP), Table 4) using the transition state modeling procedure described for reaction (3) (see Scheme 3).

Initially, we estimated the energy balance of the one-step type-(1) reactions (4_M)—(7_M) (Scheme 4) and of the type-(2) reactions (8_M)—(13_M) (Scheme 5) where both SR and RP were only the energetically most favorable (M) isomers (see Table 4) that are to the greatest extent responsible for the energy of the reaction (ΔE_r). The Gibbs energies of SR and TS (they were used for calculating the dissociation energies of the O—H bonds in the β-OH groups) and RP for reduction (quenching) of $^{\bullet}$OOH radical with molecule **1** and its derivatives **2—14** are listed in Table 4. Here, the energy balances of reactions (ΔE_r) were estimated using not only the total Gibbs energies (ΔG), but also the total electronic energies (ΔE) and the total enthalpies (ΔH).

O—H Bond homolysis in the hydroxyl groups at the C(2) and C(3) atoms is characterized by the lowest bond dissociation energies (D_{OH}), namely, $D_{OH(2)} \approx D_{OH(3)} =$ 69.32 kcal mol^{-1} and $D_{OH(6)} = D_{OH(3)} + 2.96$ kcal mol^{-1}.* It should be noted that the C—H bond homolytic dissociation energy in the methylene unit of the ethyl substituent in molecule **1** ($D_{CH} = 70.90$ kcal mol^{-1}) is similar to the D_{OH} energies for the β-OH groups of this molecule. It follows that in some cases the ethyl substituent, as the reaction center, can theoretically compete with β-OH groups in the reactions with hydroperoxyl radical. Detailed study of the mechanism of the interaction of radical **5** and its derivatives (radical anions) with hydroperoxyl radical will be reported elsewhere.

The ground-state O—H bond homolytic dissociation energy in the hydrogen peroxide molecule, D_{OH}(HOOH), is 68.86 kcal mol^{-1}. The equilibrium constants (K) for reactions (4_M)—(7_M) (see Table 4) were calculated using the relation $K = \exp[-\Delta G_r/(k_B T)]$, where $\Delta G_r = G(\text{RP}) - G(\text{SR}) = D_{OH}(\beta\text{-OH}) - D_{OH}(\text{HOOH})$, k_B is the Boltzmann constant, and T is the absolute temperature. Reactions (4_M)—(6_M) of molecule **1** involving O—H bond homolysis are characterized by $K < 1$ irrespective of which β-OH group undergoes homolysis. Similarly, the calculated equilibrium constant of the reaction (7_M) between molecule **1** and hydroperoxyl radical resulting in a radical due to C—H bond homolysis in the ethyl substituent is also less than unity, being at the same time higher than the equilibrium constant of reaction (6_M).

According to B3LYP/6-311G(d) calculations, the O—H bond heterolytic dissociation energies in the β-OH groups at the C(2), C(3), and C(6) atoms are 318.62, 322.65 and 318.28 kcal mol^{-1}, respectively. Thus, the O—H bond heterolytic dissociation energies in the β-OH groups at the C(2) and C(6) atoms are similar, being ~4 kcal mol^{-1} lower than the O—H bond dissociation energy in the β-OH group at the C(3) atom. At the same time the O—H bond homolytic dissociation energies in the β-OH groups at the C(2) and C(3) atoms (69.32 kcal mol^{-1}) are lower than the O—H bond dissociation energy in the β-OH group at the C(6) atom (72.28 kcal mol^{-1}).

O—H Bond heterolysis in one β-OH group in molecule **1** causes a substantial decrease in the energy of subsequent O—H bond homolysis in any of the two remaining β-OH groups, which becomes 6.38—10.94 kcal mol^{-1} lower than D_{OH}(HOOH). Reactions (8_M)—(13_M) (see Table 4) become exothermic, the enthalpies (ΔH) of these reactions become negative, and the corresponding equilibrium constants become much

* Figure in parentheses at a D_{OH} value denotes the position of the β-OH group in molecule **1**.

Table 4. Gibbs energies (G/a.u.)[a] of molecule **1** and its derivatives, starting reactants (SR), transition states (TS) and products (RP) of the type-(1) and type-(2) reactions, O—H bond dissociation energies (D_{OH}(SR → TS)/kcal mol^{-1})[b] in β-OH groups, and the energy balance (ΔE_r/kcal mol^{-1})[c] of the reduction of hydroperoxyl radical with neutral molecule **1** and with its deprotonated forms obtained from (U)B3LYP/6-311G(d) calculations

Reaction	$-G$(A—OH)	$-G$(SR)	$-G$(A—O$^{\bullet}$)	$-G$(TS)	$-G$(RP)	D_{OH}	ΔE_r(SR → RP)		
							ΔE	ΔG	ΔH
1A → **2C**$_{(3)}$ (4_M)	989.970734	1140.925333	989.347463	1140.814872	1140.924604	69.32	1.06	0.46 (0.64)[d]	1.12
1A → **3C**$_{(2)}$ (5_M)	989.970734	1140.925333	989.347448	1140.814857	1140.924589	69.32	0.91	0.47 (0.69)	0.97
1A → **4A** (6_M)	989.970734	1140.925333	989.342746	1140.810155	1140.919887	72.28	4.10	3.42 (3.41)	4.27
1A → **5C** (7_M)	989.970734	1140.925333	989.344926	1140.812335	1140.922067	70.90	2.54	2.05 (2.08)	2.96
6C$_{(3)}$ → **9D** (8_M)	989.463076	1140.417675	988.851156	1140.318467	1140.428297	62.25	−5.99	−6.60 (−6.58)	−5.82
6C$_{(3)}$ →**10D**$_{(3)}$ (9_M)	989.463076	1140.417675	988.851088	1140.318497	1140.428229	62.24	−5.53	−6.62 (−6.78)	−5.24
7C$_{(2)}$ → **9D** (10_M)	989.456564	1140.411163	988.851156	1140.318467	1140.428199	58.17	−9.55	−10.69 (−10.71)	−9.51
7C$_{(2)}$ → **11A** (11_M)	989.456564	1140.411163	988.851459	1140.318868	1140.428600	57.92	−9.91	−10.94 (−10.85)	−9.68
8A$_{(5)}$ → **10D**$_{(3)}$ (12_M)	989.463451	1140.418050	988.851088	1140.318497	1140.428229	62.47	−5.42	−6.39 (−6.38)	−5.46
8A$_{(5)}$ → **11A** (13_M)	989.463451	1140.418050	988.851459	1140.318868	1140.428600	62.24	−5.72	−6.62 (−6.34)	−5.54
15 → **16** (14)	457.937003	608.891602	457.328108	608.795517	608.905249	60.29	−8.49	−8.56	−8.75

[a] $G = E_0 + G_{el} + G_{tr} + G_{rot} + G_{vibr}$, where E_0 is the ground-state electronic energy and G_{el}, G_{tr}, G_{rot} and G_{vibr} are respectively the electronic, translational, rotational, and vibrational components of the Gibbs energy. The energies E_0 of the A—OH and A—O$^{\bullet}$ systems were calculated by the (U)B3LYP/6-311G(d) method and the ZPE, G_{el}, G_{tr}, G_{rot}, G_{vibr} H_{el}, H_{tr}, H_{rot}, and H_{vibr} values were calculated by the (U)B3LYP/6-31G//(U)B3LYP/6-31G method. The Gibbs energies, enthalpies, and total energies ($E = E_0$ + ZPE) of hydroperoxyl radical, H atom, and hydrogen peroxide obtained from (U)B3LYP/6-311G(d)//(U)B3LYP/6-311G(d) calculations are as follows: E($^{\bullet}$H) = −0.502156 a.u., E($^{\bullet}$OOH) = −150.932454 a.u., E(HOOH) = −151.554760 a.u., G($^{\bullet}$H) = −0.512810 a.u., G($^{\bullet}$OOH) = −150.954599 a.u., G(HOOH) = −151.577141 a.u., H($^{\bullet}$H) = −0.499795 a.u., H($^{\bullet}$OOH) = −150.928626 a.u., and H(HOOH) = −151.550507 a.u.

[b] The homolytic bond dissociation energies were calculated as D_{OH}(SR → TS) = G(TS) − G(SR).

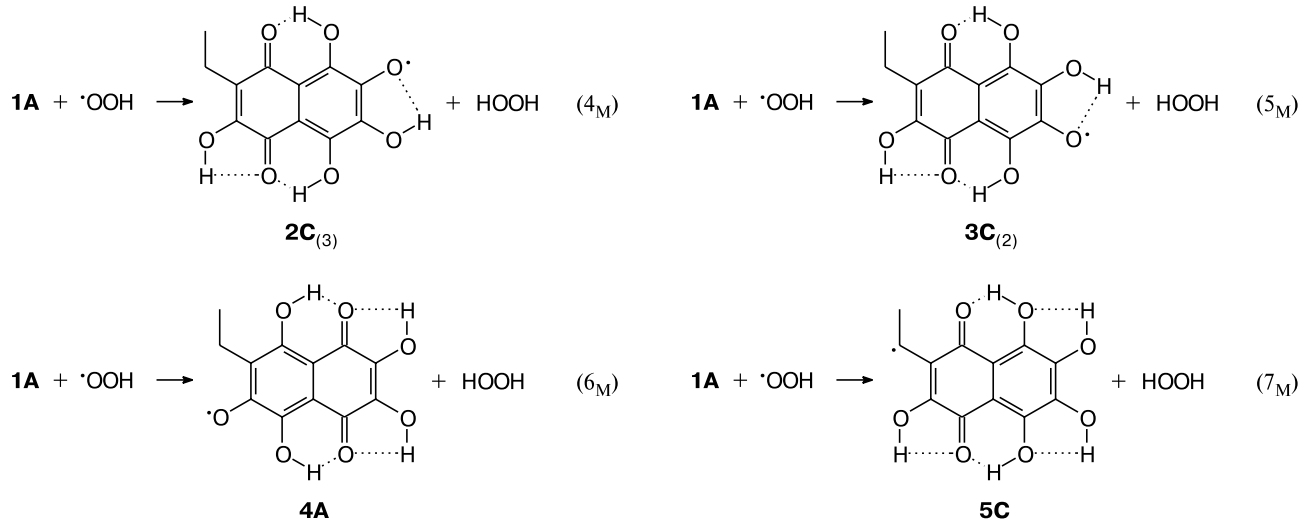
[c] The energy balance of reactions ΔE_r(SR → RP) was calculated as ΔE(SR → RP) = E(RP) − E(SR); ΔG(SR → RP) = G(RP) − G(SR); ΔH(SR → RP) = H(RP) − H(SR).

[d] The effective ΔG values averaged over all tautomeric and rotameric forms are given in parentheses: $\Delta G_{XY} = G_Y^{eff}(RP) - G_X^{eff}(SR)$, where $G_Y^{eff}(RP) = \Sigma_i(g_{Y_i}G_{Y_i})$, $G_X^{eff}(SR) = \Sigma_i(g_{X_i}G_{X_i})$, and the subscript i runs over all tautomeric and rotameric forms of compounds X and Y (see Table 1).

higher than unity. The constants vary from the lowest value ($K = 4.5 \cdot 10^4$) for the reaction **8A**$_{(5)}$ + $^{\bullet}$OOH ⇄ ⇄ **10D**$_{(3)}$ + HOOH (12_M) to the maximum value ($K = 1.1 \cdot 10^8$) for the reaction **7C**$_{(2)}$ + $^{\bullet}$OOH ⇄ **11A** + + HOOH (11_M). Therefore, one can assume that molecule **1** involved in the reduction of hydroperoxyl radical will best exhibit its antioxidant properties in those cases where the O—H bond in one β-OH group in this molecule undergoes heterolysis and the product of this transformation enters the reaction with hydroperoxyl radical in the "activated" (deprotonated) state.

The discussed energy balance of the reduction of hydroperoxyl radical was estimated (see above) for the reactions involving only the energetically most favorable isomers of both SR and RP (reactions (4_M)—(13_M)). The conformational analysis of molecule **1**, its derivatives, and products of their reactions with the $^{\bullet}$OOH radical (see above) made it possible to evaluate the effects of all other (not only the energetically most favorable) isomers of these compounds on the energies of the reactions under study. Tautomerism and rotational isomerism of molecule **1** allows a variety of states of the SR, intermediates, and RP to occur. This does not change the qualitative picture of the course of the gas-phase reactions (1) and (2) but leads to large variations in their energy characteristics. For instance, the ΔG_r values for reactions (8)—(12) estimated for the case of conservation of the isomeric form of reactants can differ by more than 4 kcal mol^{-1}. Aver-

Scheme 4



aging over all conformations of the reactants shows (see Tables 2 and 4) different contributions of the $\Delta G_r^* = G^*(RP) - G^*(SR)$ terms to the ΔG_r^{eff} values of reactions (1) and (2). For reactions (4_M)—(7_M) the ratio $(\Delta G_r^*/\Delta G_r)\cdot 100\%$ reaches a value of 45%, being equal to at most 3% for reactions (8_M)—(13_M). However, in all cases the absolute values of ΔG_r^* are at most ~0.3 kcal mol^{-1}.

Table 5 lists the energy characteristics for a particular case of the reduction of hydroperoxyl radical occurring with conservation of the isomeric form of the antioxidant molecule. Namely, both compound **1** and its derivatives are in the same tautomeric form **A** (endothermic reactions (4a)—(7a) and exothermic reactions (8a)—(13a)). These modeling conditions were chosen because about 98% of molecules **1** exist in this tautomeric form (see Table 2). These reactions are the initial stages of a possible channel of multistage endothermic reactions (4)—(7) and exothermic reactions (8)—(13). Reaction (4) chosen as an example of the type-(1) reactions is shown in Scheme 6, which includes only those conformational transitions that result in the intermediates characterized by a percentage of 1% and higher.

From the energy characteristics of the reactions listed in Table 5 it follows that the ΔG values of reactions (4a), (5a), and (7a) are 0.66—1.69 kcal mol^{-1} larger than the ΔG^{eff} values estimated with allowance for isomerism for the reactions (4), (5), and (7), respectively. Thus, isomerism causes a decrease in the endothermicity of these reactions. The ΔG and ΔG^{eff} values of reactions (6a) and (6) differ by only 0.02 kcal mol^{-1} because the percentage of the minor isomeric forms of the SR and RP is less than 1%.

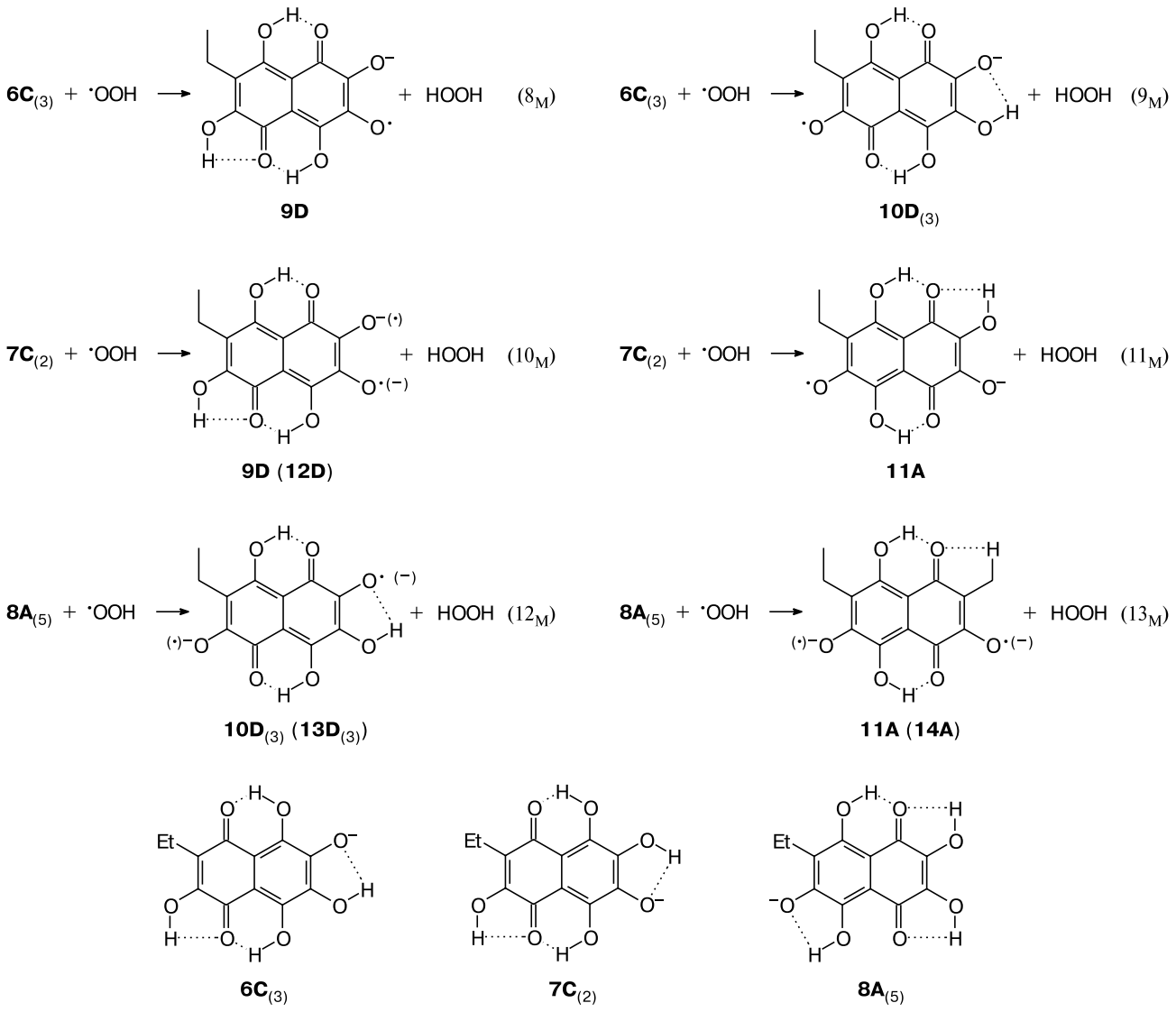
From the definition of the ΔG^{eff} value it follows that the energy effects of the multistage reactions (4)—(7) and one-stage reactions (4_M)—(7_M) (see Tables 5 and 4, respectively) differ by $\Delta G^* = \Delta G^{eff} - \Delta G_M \approx$ 0.02—0.21 kcal mol^{-1}.

Table 5. Energy characteristics of the reduction of hydroperoxyl radical estimated with (ΔG^{eff}/kcal mol^{-1}) and without (ΔG/kcal mol^{-1}) inclusion of isomerism of molecule **1** and its derivatives

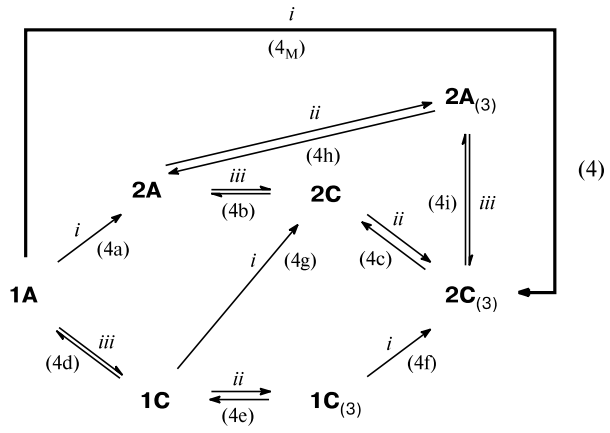
Reaction	ΔG	Reaction	ΔG^{eff} [a]	ΔG(RP)[b]
1A → **2A** (4a)	1.33	**1** → **2** (4)	0.64 (−0.66)	−0.01[c]
1A → **3A** (5a)	1.12	**1** → **3** (5)	0.69 (−0.40)	—
1A → **4A** (6a)	3.43	**1** → **4** (6)	3.41 (0.01)	—
1A → **5A** (7a)	3.80	**1** → **5** (7)	2.08 (−1.69)	—
6A → **9A** (8a)	−9.87	**6** → **9** (8)	−6.58 (3.39)	0.02[d]
6A → **10A** (9a)	−7.53	**6** → **10** (9)	−6.78 (0.75)	—
7A → **9A** (10a)	−12.49	**7** → **9** (10)	−10.70 (1.79)	0.25[e]
7A → **11A** (11a)	−14.65	**7** → **11** (11)	−10.85 (3.80)	—
8A → **10A** (12a)	−2.07	**8** → **10** (12)	−6.38 (−4.31)	0.23[f]
8A → **11A** (13b)	−6.58	**8** → **11** (13)	−6.34 (0.24)	—

[a] The $\Delta G^{eff} - \Delta G$ values are given in parentheses.
[b] The total Gibbs energy difference between the major isomers of products of the multistage reactions with the same SR and different RP.
[c] $G(\mathbf{2C}_{(3)}) - G(\mathbf{3C}_{(2)})$.
[d] $G(\mathbf{9D}) - G(\mathbf{10D}_{(3)})$.
[e] $G(\mathbf{9D}) - G(\mathbf{11A})$.
[f] $G(\mathbf{10D}_{(3)}) - G(\mathbf{11A})$.

Scheme 5



Scheme 6

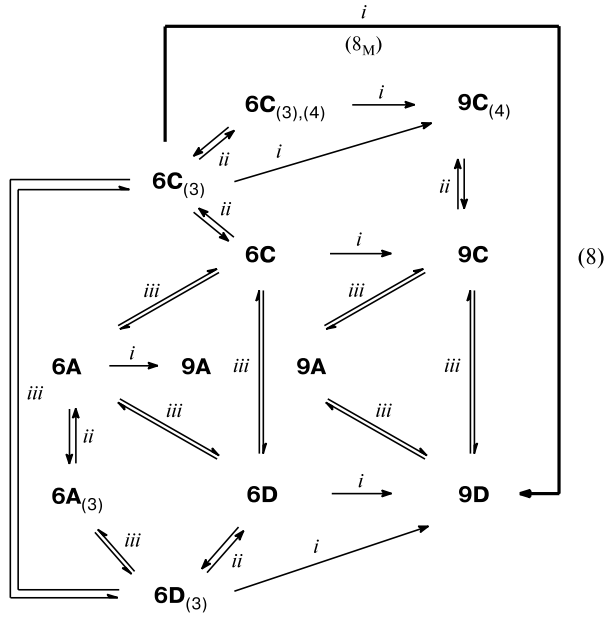


i. Homolysis; *ii*. Rotational isomerism; *iii*. Tautomerism.

Scheme 7 shows the possible pathways of the multistage reaction (8) belonging to type-(2) reactions, which include the types of conformational transitions considered in this work.

If molecule **1** is involved in the reduction of hydroperoxyl radical in the form of monoanion, the account for the isomerism in reactions (8)—(11) and (13) does not change their exothermicities but causes reduction of the corresponding ΔG values by 0.18—3.80 kcal mol^{-1} in absolute value. Only for reaction (12) the energy gain calculated with allowance for isomerism increases by 4.31 kcal mol^{-1}. At the same time comparison of the ΔG_M and ΔG^{eff} values for reactions (8), (10), and (12) shows that $|\Delta G^{eff}| = |\Delta G_M| + (0.01—0.05)$ kcal mol^{-1}. For reaction (9) one gets $|\Delta G^{eff}| = |\Delta G_M| + 0.16$ kcal mol^{-1} while for reactions (11) and (13) one has $|\Delta G^{eff}| = |\Delta G_M| - (0.14—0.24)$ kcal mol^{-1}.

Scheme 7

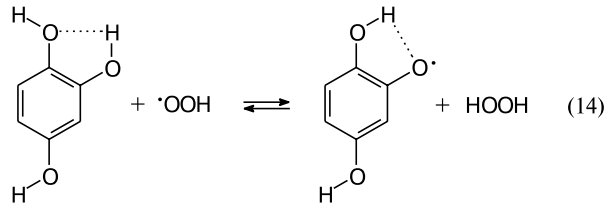


(8)

i. Homolysis; *ii*. Rotational isomerism; *iii*. Tautomerism.

Table 5 also lists the total Gibbs energy differences (ΔG_{RP}) between the energetically most favorable isomers of the RP obtained *via* O—H bond homolysis of different OH groups of the same SR. For all reactions, except reaction (6), the ΔG_{RP} values are at most 0.25 kcal mol^{-1}, thus being no greater than 4% of the ΔG_r value.

Based on the results obtained, one can qualitatively compare the possible mechanisms of the antioxidant action of compound **1** and certain polyphenols. The main difference between them in the reaction with hydroperoxyl radical consists in that deprotonation of one β-OH group in molecule **1** by O—H bond heterolysis considerably reduces the homolytic dissociation energies of the remaining β-OH groups and compound **1** will act much more efficiently in the ionized rather than neutral form. Contrary to this, polyphenols efficiently react with hydroperoxyl radical in the neutral rather than ionized form. For instance, our (U)B3LYP/6-311G(d) calculations of 1,2,4-trihydroxybenzene **15** showed that reaction (14) is exothermic with $\Delta H_r = -8.49$ kcal mol^{-1} ($\Delta G_r = -8.56$ kcal mol^{-1}, see Table 4).



(14)

15 **16**

Since the OH groups in polyphenols are characterized by p$K_a > 9$, these compounds do not undergo deprotonation under physiological conditions (pH ≈ 7). Therefore, it is quite probable that the mechanism of the antioxidant action of polyphenols through pre-heterolysis of one hydroxyl group will not be realized.

* * *

The conformational analysis of molecule **1** and its derivatives showed that, unlike the parent molecule, the energetically most favorable isomers of radicals **2** and **3** and anions **6** and **7** (here, we deal with homolysis or heterolysis of the β-OH groups at the C(2) or C(3) atoms, respectively) are the species **2C**$_{(3)}$, **3C**$_{(2)}$, **6C**$_{(3)}$ and **7C**$_{(2)}$, respectively. In the case of radical **4** and anion **8** (β-OH group at the C(6) atom undergoes homolysis or heterolysis) these are tautomers **4A** and **8A**$_{(5)}$, respectively. A feature of radical anion **9** is simultaneous homolysis and heterolysis of two β-OH groups in the same nucleus of the naphthazarin structure of molecule **1**; here the energetically most favorable tautomer is **9D**. In radical anions **10** and **11**, two β-OH groups in different nuclei of structure **1** undergo homolysis and heterolysis; this leads to the 1,5-semiquinonoid tautomeric-rotameric form **10D**$_{(3)}$ and 1,4-quinonoid tautomeric form **11A**, respectively, as the energetically most favorable isomers. Note that the Gibbs energies of the energetically most favorable isomers of products of the same-type transformations of molecule **1** are similar. Indeed, they differ by less than 0.01 kcal mol^{-1} for **2C**$_{(3)}$ and **3C**$_{(2)}$ and less than by 0.3 kcal mol^{-1} for **9D**, **10D**$_{(3)}$, and **11A**.

The results obtained suggest that all OH groups in molecule **1** and in its derivatives **2—11** are involved in the IMHB. All compounds **1—11** are characterized by significantly different PES and the Gibbs energy surfaces. As a result, the AFO reduction reactions of molecule **1** and its monoanions are accompanied by conformational rearrangements in these species, which are driven by the participation of the corresponding OH groups in the formation of the energetically more favorable IMHB.

The variety of tautomers of molecule **1** and tautomeric-rotameric forms of radicals and radical anions that can be produced by the O—H and C—H bond homolysis or heterolysis in the β-substituents in this molecule increases the number of states in which these molecules can participate in the AFO quenching reactions. Each β-OH group and even the Et group in molecules **1—11** can act as a reaction center in the reactions with free radicals, thus making this structural type of antioxidants more efficient in these reactions (or, in other words, thus increasing the effective cross-section of the reaction).

To estimate the reduction energy of hydroperoxyl radical with molecule **1** and anions derived from the parent molecule, it is important to perform a preliminary con-

formational analysis, which makes it possible to determine the major isomers of the reactant molecules and corresponding reaction products. According to the results of conformational analysis, it is these isomers that are mainly responsible for the energies of the reactions under study.

O—H Bond heterolysis in one β-OH group in molecule **1** causes the energies of subsequent O—H bond homolysis in any of the two remaining β-OH groups to decrease by 7—14 kcal mol^{-1} compared to the D_0 values for these bonds in neutral molecule **1**. This process "activates" the molecule as a free radical trap and the deprotonated form of compound **1** should react with hydroperoxyl radical as efficient reducing agent. The results obtained in our study suggest that compound **1** should possess the maximum antioxidant activity in living organism where it is present in the activated (deprotonated) form.

It is quite probable that the mechanism of the antioxidant action of compound **1** proposed in this work based on the results of theoretical calculations can also be realized in the case of other natural and synthetic polyhydroxy-substituted 1,4-naphthoquinones and such an efficient natural antioxidant as ascorbic acid.

Experimental

The main method of quantum chemical modeling of the type-(1) and type-(2) reactions was the density functional theory with the B3LYP exchange-correlation functional. The total electronic energies E_0 were determined after full geometry optimization in the 6-311G(d) basis set. The zero-point vibration energy corrections (ZPE) and the temperature corrections H_T and G_T were calculated with the 6-31G basis set using the geometric parameters optimized with the same basis set. The Gibbs energies, G, and the enthalpies, H, were calculated with allowance for all electronic, translational, rotational, and vibrational degrees of freedom for $T = 298.15$ K. The statistical weight (g_{X_i}) of the ith isomer of compound X was calculated by the relation $g_{X_i} = \exp[-\Delta G_{X_i}/(k_B T)]/\Sigma_i \exp[-\Delta G_{X_i}/(k_B T)]$, where summation is performed over all isomeric forms of compound X, and $\Delta G_{X_i} = G_{X_i} - G_{X_M}$ (G_{X_M} is the total Gibbs energy of the major (M) isomer of compound X). The percentage of the ith isomer was calculated as $g_{X_i} \cdot 100\%$. All computations were carried out using the PC GAMESS program complex.[26]

The ground-state wave functions were calculated in the one-determinant approximations by the spin-restricted B3LYP method for compounds **1**, **6—8**, HOOH, and 1,2,4-trihydroxybenzene and by the spin-unrestricted UB3LYP method for compounds **2—5**, **9—12**, and the $^{\bullet}$OOH and H$^{\bullet}$ radicals.

In studying tautomerism and rotational isomerism of α-OH and β-OH groups in molecule **1** the geometry optimization and normal vibrational mode analysis were performed using the density functional theory with the B3LYP exchange-correlation functional and by the spin-restricted Hartree—Fock method with inclusion of electron correlation at the second-order Møller—Plesset (MP2) of perturbation theory with the 6-31G, 6-311G(d), and cc-pVTZ basis sets.

Geometric parameters of various tautomers and rotamers of radicals, anions, and radical anions derived from molecule **1** were optimized using the density functional theory with the B3LYP functional in the 6-31G and 6-311G(d) basis sets.

If a tautomer corresponded to a plateau on the PES or to a flat portion of the potential wall, the PES was scanned over the geometric parameter $Q_1 \equiv R(\mathrm{O(1)—H}) \cdot \cos(\alpha_1)$ or $Q_2 \equiv R(\mathrm{O(4)—H}) \cdot \cos(\alpha_2)$, where α_1 and α_2 are the angles H—O(1)...O(8) and H—O(4)...O(5), respectively; figures in parentheses denote the numbers of the carbon atoms to which these O atoms are bonded.

The absorption spectra of aqueous solutions of (i) complex **1** with HSA and (ii) sodium salt of echinochrome A were recorded on a Shimadzu UV-1601PC spectrophotometer in quartz cells 1 cm thick. The complex of **1** with HSA was prepared by adding aliquot of a concentrated solution of **1** in EtOH to an aqueous HSA solution (~5 mg mL^{-1}) until a **1** : HSA mole ratio of 1 : 1. The concentration of EtOH in the aqueous solution of the complex was at most 2%. The aqueous solution of sodium salt of echinochrome A was prepared by mixing equimolar amounts of aqueous solutions of **1** (~10^{-5} mol L^{-1}) and $NaHCO_3$. HSA (Reanal) with a molecular weight of 64 kDa was used.

^{1}H NMR spectra were recorded on a Bruker Avance DPX-300 spectrometer (300.13 MHz) in $CDCl_3$ (with Me_4Si as internal reference). Compound **1** was synthesized following a known procedure.[27]

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