Full Articles

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

2.* The structure of monosodium salts of echinochrome A and their reactions with the hydroperoxyl radical

V. P. Glazunov,* **D. V. Berdyshev, and V. L. Novikov**

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences,
159 prosp. 100 let Vladivostoku, 690022 Vladivostok, Russian Federation.
Fax: +7 (423 2) 31 4050. E-mail: glazunov@piboc.dvo.ru

The relative gas-phase acidities of all OH groups of 7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A, **1**) were evaluated by the B3LYP/6-311G(d) and B3LYP/6-311G(d,p) methods. Calculations predict that β-OH groups at the C(2) and C(6) atoms are the most acidic in molecule **1** and their acidity is higher than that of *o*-nitrophenol. Conformational analysis of undissociated monosodium salts of **1** and their radicals was performed. It was shown that gas-phase quenching reactions of the hydroperoxyl radical by monosodium salts of **1** are exothermic.

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

Earlier,[1] we have reported a B3LYP/6-311G(d) study of the antioxidant properties of 7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A, (Et)NZ(OH)$_3$ (**1**), where NZ is naphthazarin (5,8-dihydroxy-1,4-naphthoquinone)), the active principle of a domestic drug Histochrome®, taking its interaction with the hydroperoxyl radical HOO$^•$ as an example. It was shown that quenching of HOO$^•$ proceeds most efficiently if compound **1** reacts in the form of a monoanion, *i.e.*, when the O—H bond in a β-OH group undergoes heterolysis.

From pK_a data obtained for OH groups of compound **1** by thin-layer voltammetry (a polarographic technique)[2] and potentiometric titration[3] it follows that at physiological pH values of the medium, molecule **1** dissociates to mono- and dianions. However, the structures of these anions and their antioxidant properties remained unclear.

In the quinonoid tautomers **A** and **C** (see below), three β-OH groups of compound **1** are structural fragments of vinylogous carboxylic groups or a polyphenol, while both

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* For Part 1, see Ref. 1.

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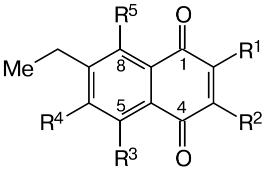
α-OH groups of this compound are only polyphenol fragments. Experimental determination of pK_a values (which is the measure of acidity of carboxyl and hydroxyl groups) of polycarboxylic acids and polyphenols is a rather complicated task. The relative acidities can be evaluated by quantum chemical calculations of the heterolytic dissociation energies of the O—H bonds in the functional groups in question

$$\text{RO—H} \longrightarrow \text{RO}^- + \text{H}^+. \qquad (1)$$

As a quantitative measure of the acidity of OH-acids in the gas phase (hereafter, acidity), we consider the Gibbs free energy (ΔG_H) and enthalpy (ΔH_H) of type-(1) reactions, which characterize the proton affinitiy of the RO^- anion. These quantities, which are determined at 298 K, characterize the acidity.[4] In the present study, the acidity of the OH groups of compound **1** was estimated based on ΔG_H values.

Computational methods including the density functional theory permit efficient prediction of the pathways of deprotonation of polycarboxylic acids or protonation of polybases.[5—8] It should be noted that errors in gas-phase calculations of ΔH_H and ΔG_H for such compounds are at most 1%.[9—14] Recently,[15,16] this approach has been used to evaluate the acidity of some aliphatic and aromatic carboxylic acids and phenols and became rather useful in interpreting experimental data. It was also shown that the results of acidity calculations in the gas phase and with inclusion of solvent effects are in qualitative agreement with experimental data for solutions.[13,17]



1—31

Compound	R1	R2	R3	R4	R5
1	OH	OH	OH	OH	OH
2	O^-	OH	OH	OH	OH
3	OH	O^-	OH	OH	OH
4	OH	OH	O^-	OH	OH
5	OH	OH	OH	O^-	OH
6	OH	OH	OH	OH	O^-
7	ONa	OH	OH	OH	OH
8	OH	ONa	OH	OH	OH
9	OH	OH	ONa	OH	OH
10	OH	OH	OH	ONa	OH
11	OH	OH	OH	OH	ONa
12	ONa	$O^{\bullet}$	OH	OH	OH
13	ONa	OH	$O^{\bullet}$	OH	OH
14	ONa	OH	OH	$O^{\bullet}$	OH
15	ONa	OH	OH	OH	$O^{\bullet}$
16	$O^{\bullet}$	ONa	OH	OH	OH
17	OH	ONa	$O^{\bullet}$	OH	OH
18	OH	ONa	OH	$O^{\bullet}$	OH
19	OH	ONa	OH	OH	$O^{\bullet}$
20	$O^{\bullet}$	OH	ONa	OH	OH
21	OH	$O^{\bullet}$	ONa	OH	OH
22	OH	OH	ONa	$O^{\bullet}$	OH
23	OH	OH	ONa	OH	$O^{\bullet}$
24	$O^{\bullet}$	OH	OH	ONa	OH
25	OH	$O^{\bullet}$	OH	ONa	OH
26	OH	OH	$O^{\bullet}$	ONa	OH
27	OH	OH	OH	ONa	$O^{\bullet}$
28	$O^{\bullet}$	OH	OH	OH	ONa
29	OH	$O^{\bullet}$	OH	OH	ONa
30	OH	OH	$O^{\bullet}$	OH	ONa
31	OH	OH	OH	$O^{\bullet}$	ONa

Since echinochrome A is used in drugs of the Histochrome® series in the form of sodium salts, it was interesting to elucidate the pathways of reactions of its OH groups with NaOH in relation to acidity of OH groups (heat balance of neutralization reaction), the structure of undissociated monosodium salts of compound **1**, and possible efficiency of these salts as antiradical agents compared to monoanions of **1**,[1] which are formed in heterolytic dissociation of its β-OH groups.

The aim of this work is to make a theoretical (quantum chemical) evaluation, by the density functional theory, of the relative acidity of all OH groups of echinochrome A (monoanions **2—6**), to determine the energy characteristics of reactions of these OH groups with NaOH, the structure of undissociated monosodium salts of this compound (salts **7—11**), and to study their antiradical activity in the reactions with $HOO^{\bullet}$ (compounds **12—31**).

Experimental

Quantum chemical calculations were carried out in the framework of the density functional theory with the (U)B3LYP exchange-correlation functional[18] and the 6-31G(d), 6-311G(d), and 6-311G(d,p) basis sets using the GAUSSIAN 03 program.[19] Full geometry optimization was performed in the 6-311G(d) and 6-311G(d,p) basis sets for the major isomers* of monosodium salts of echinochrome A and in the 6-311G(d) basis set for the major isomers of their radicals.

The temperature corrections, G_T and H_T, were calculated with the same basis sets as those used for geometry optimization. The Gibbs energies G and enthalpies H were determined with allowance for all electronic, translational, rotational, and vibrational degrees of freedom for $T = 298.15$ K. The statistical weight of the ith isomer of compound X (g_{X_i}) was calculated using the relation

$$g_{X_i} = [\exp(-\Delta G_{X_i}/RT)]/[\Sigma_i \exp(-\Delta G_{X_i}/RT)],$$

where summation is performed over all isomeric forms of the compound X; $\Delta G_{X_i} = G_{X_i} - G_{X_M}$ (G_{X_M} is the Gibbs free energy of the major isomer of compound X). The percentage of the ith isomer (g_i) was calculated as $g_{X_i} \cdot 100\%$.

The ground-state wave functions of compounds **1—11**, H_2O, NaOH, HOOH, *o*-nitrophenol, 2,4-dinitrophenol, and spino-

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* The major isomer of this compound is characterized by the lowest Gibbs free energy.

chrome D were calculated in the one-determinant approximation by the spin-restricted B3LYP method with the 6-31G(d), 6-311G(d), and 6-311G(d,p) basis sets. For compounds **12—31** and radicals $HOO^{\cdot}$ and $H^{\cdot}$, the UB3LYP spin-unrestricted method and the 6-31G(d) and 6-311G(d) basis sets were used.

In studying tautomerism of the α-OH groups and rotamerism of the α-OH and β-OH groups of molecule **1**, monoanions, and undissociated monosodium salts, the geometry optimization and normal-mode vibrational analysis were carried out in the framework of the density functional theory with the B3LYP exchange-correlation functional and the 6-31G(d), 6-311G(d), and 6-311G(d,p) basis sets. In the case of radicals of monosodium salts, the UB3LYP exchange-correlation functional and the 6-31G(d) and 6-311G(d) basis sets were used. The condition $|\mathrm{grad}| \le 10^{-6}$ au Å^{-1} served a criterion for termination of the geometry optimization procedure.

The assignment of the stationary points on the potential energy surface (PES) to minima and saddle points was based on the results of normal-mode vibrational analysis (a stationary point is a saddle point if there is one imaginary frequency in the theoretical spectrum and a minimum if no imaginary frequencies were found). If a tautomer corresponded to a point on a plateau on the PES or to flat portions of the repulsive potential, 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 (digits in parentheses denote the numbers of the C atoms to which these O atoms are bound).

Results and Discussion

Earlier,[1] conformational analysis of undissociated molecule **1** and its monoanions **2**, **3**, and **5** using the B3LYP exchange-correlation functional has made it possible to find the major isomers. Also, the heterolytic O—H bond dissociation energies (ΔG_{H}) of the β-hydroxyls at the C(2), C(3), and C(6) atoms of molecule **1** were estimated as the Gibbs free energy differences between the major isomers of the corresponding monoanion and undissociated molecule

$$\Delta G_{\mathrm{H}} = G((\mathrm{Et})(\beta\text{-O}^-)\mathrm{NZ}(\beta\text{-OH})_2) - G((\mathrm{Et})\mathrm{NZ}(\beta\text{-OH})_3) + G_{\mathrm{tr,H^+}}, \quad (2)$$

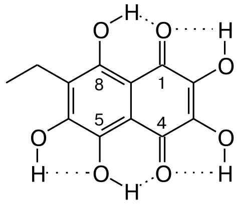
where $G = E_0 + G_{\mathrm{el}} + G_{\mathrm{tr}} + G_{\mathrm{rot}} + G_{\mathrm{vibr}}$; E_0 is the ground-state energy calculated with the 6-311G(d) basis set; G_{el}, G_{tr}, G_{rot}, and G_{vibr} are, respectively, the electronic, translational, rotational, and vibrational components of the Gibbs free energy calculated with the 6-31G basis set and the geometric parameters optimized with the same basis set; and $G_{\mathrm{tr,H^+}}$ is the Gibbs free energy of free proton.

For monoanions **2** (heterolysis of the β-OH group at the C(2) atom; hereafter, 2β-OH group) and **5** (heterolysis of the β-OH group at the C(6) atom; hereafter, 6β-OH group), the ΔG_{H} values calculated with the 6-311G(d) basis set are nearly the same (307.9 and 307.5 kcal mol^{-1}, respectively; Table 1). The value obtained for monoanion **3** (heterolysis of the β-OH group at the C(3) atom; hereafter, 3β-OH group) is 4.2—4.5 kcal mol^{-1} higher (312.0 kcal mol^{-1}).

Table 1. Heterolytic dissociation energies (ΔG_{H}) of the α-OH and β-OH groups in different tautomers and rotamers of molecule **1** (calculated using Eq. (2))

Compound	Computational method	ΔG_{H} /kcal mol^{-1}
2C$_{(3)}$	B3LYP/6-311G(d)	307.9
	B3LYP/6-311G(d,p)	311.5
3C$_{(2)}$	B3LYP/6-311G(d)	312.0
	B3LYP/6-311G(d,p)	315.7
4A	B3LYP/6-31G(d)	314.6
	B3LYP/6-311G(d)	310.1
	B3LYP/6-311G(d,p)	313.9
4C	B3LYP/6-31G(d)	317.0
5A$_{(5)}$	B3LYP/6-311G(d)	307.5
	B3LYP/6-311G(d,p)	311.3
6A	B3LYP/6-31G(d)	324.8
6C	B3LYP/6-31G(d)	324.2
	B3LYP/6-311G(d)	319.1
	B3LYP/6-311G(d,p)	323.0

Based on the fact that the 2β-OH and 3β-OH groups are in the quinonoid nucleus of the major tautomeric form **1A**, this result is quite unexpected because both groups are fragments of vinylogous carboxyl groups (conjugated with the carbonyl groups in positions 4 and 1, respectively). At the same time, the 6β-OH group in **1A** is in the benzenoid nucleus and should formally be considered as an OH group of the triatomic phenol. Therefore, the 3β-OH group should be more acidic than the 6β-OH group, *i.e.*, $\Delta G_{\mathrm{H}}(3\beta\text{-OH}) < \Delta G_{\mathrm{H}}(6\beta\text{-OH})$.



1A

This result[1] is not a consequence of the small size of the 6-31G basis set used in our calculations of the rovibrational contribution to the Gibbs free energy (see Eq. (2)), because calculations with larger basis sets (B3LYP/6-311G(d) and B3LYP/6-311G(d,p)) give for the difference $\Delta G_{\mathrm{H}}(6\beta\text{-OH}) - \Delta G_{\mathrm{H}}(2\beta\text{-OH})$ (heterolytic dissociation energy difference between isomers **5A**$_{(5)}$ and **2C**$_{(3)}$* of compounds **5** and **2**, respectively) al-

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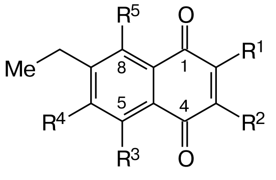
* For detailed description of notations of isomers (rotamers) with respect to internal rotation of the β-OH and α-OH groups in molecule **1** and in products of heterolytic or homolytic dissociation of the O—H bonds, see Ref. 1. The subscript in parentheses denotes the number of carbon atom to which the OH group rotated by 180° is bound. For all OH groups, the initial position (no subscript) corresponds to their positions in the major isomer of molecule **1** (**1A**). **A**—**D** are possible tautomeric forms of the compounds under study.

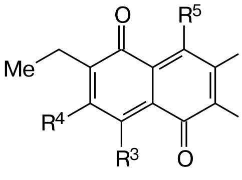
most equal values lying within ±0.30 kcal mol^{-1} (see Table 1). Thus, the acidities of the 2β-OH and 6β-OH groups of molecule **1** predicted by B3LYP/6-311G(d,p) calculations are close (311.5 and 311.3 kcal mol^{-1}, respectively) and higher than that of the 3β-OH group (315.7 kcal mol^{-1}).

Probably, the main reason for the higher acidity of the 6β-OH group compared to that of the 3β-OH group in so highly conjugated system, as molecule **1**, is the effect of the nature of substituents in the *ortho*-position rather than formal assignment to fragments of a polyphenol or a vinylogous carboxylic acid.

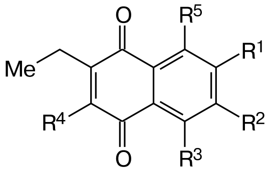
It is interesting to compare the acidities of the α-OH and β-OH groups of compound **1**. We performed a detailed conformational analysis of monoanions **4** and **6** formed upon heterolysis of the α-OH groups at the C(5) and C(8) atoms (hereafter, 5α-OH and 8α-OH groups, respectively) similarly to that done for compounds **2**, **3**, and **5**, which can exist in tautomeric forms **A**—**D**, which can in turn have a large number of rotamers each.[1]



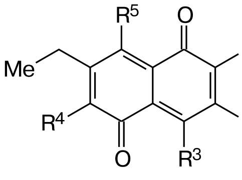
A



B



C

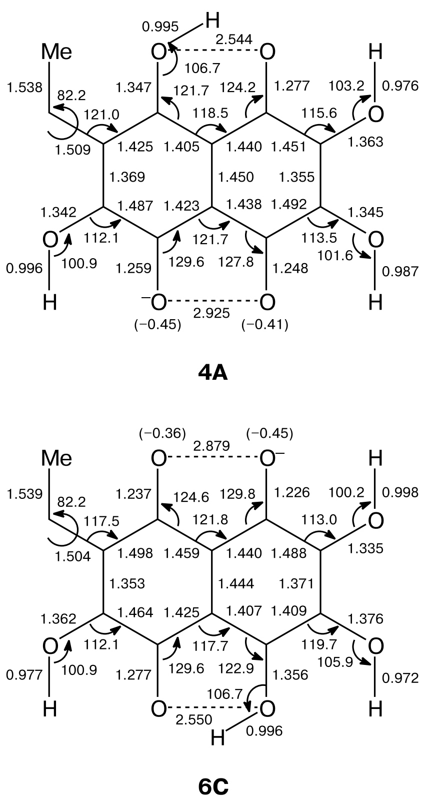


D

1—3, 5: R^1, R^2, R^4 = OH, O^-; R^3 = R^5 = OH;
4: R^1, R^2, R^4, R^5 = OH, R^3 = O^-; **6:** R^1—R^4 = OH, R^5 = O^-

It should be noted that not all tautomeric forms possible for undissociated molecule **1** are realized for monoanions **4**, **6**, monosodium salts **9**, **11**, and their radicals **17**, **19**. For instance, each pair of tautomers **A**, **D** and **B**, **C** of monoanion **4** correspond to one minimum on the global PES of the ground electronic state; this also holds for each pair of tautomers **A**, **B** and **C**, **D** of monoanion **6**. For the monosodium salts **9**, **11** and their radicals **20**—**23** and **28**—**31**, the number of possible tautomeric forms decreases owing to the single-well character of the potential (see below) in which the sodium cation moves; no tautomerism was revealed at all for compounds **23** and **30**.

Each tautomeric form of compounds **4** and **6** can exist as a mixture of rotamers with respect to internal rotation of the β-OH and α-OH groups about the corresponding C—O bonds. Primary screening of all possible isomers of monoanions **4** and **6** was carried out by the B3LYP/6-31G(d) method (see Table 1). It was established that the major isomers of these compounds are the isomeric (tautomeric-rotameric) forms **4A** and **6C**. In the ground electronic state of monoanion **4A** or **6C**, abstraction of a proton from the α-OH group is followed by delocalization of the remaining electron over the entire system. As a result, the electron density on the O(4) and O(1) atoms decreases to −0.45 e (in neutral molecule **1**, these atoms bear equal charges* of −0.59 e) rather than increases as could be expected.



4A

6C

Fig. 1. Structures of the major isomers **4A** and **6C** of monoanions **4** and **6**, respectively, obtained from B3LYP/6-311G(d) calculations. The "minus" sign at the O atom denotes the OH group that undergoes heterolysis. Atomic charges are given in parentheses. Here and in Figs 2 and 7 shown are the bond lengths (in Å) and bond angles (in degrees).

The structures of the major isomers **4A** and **6C** are shown in Fig. 1. The ΔG_H values estimated at the B3LYP/6-311G(d,p) level are 313.9 kcal mol^{-1} for the 5α-OH group of monoanion **4A** (see Table 1) and 323.0 kcal mol^{-1} for the 8α-OH group of monoanion **6C**, thus differing by about 9 kcal mol^{-1}. In addition, the acidity of the 5α-OH group is higher than that of the 3β-OH group. Thus, ΔG_H calculations predict a decrease in the acidity of OH groups of molecule **1** in the following order: 2β-OH ≥ 6β-OH > > 5α-OH > 3β-OH > 8α-OH.

The acidities of the β-OH groups of molecule **1** are comparable with those of the OH groups in mono-, di-, and trinitrophenols whose pK_a values vary from 7.25 (*o*-nitro-

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* Atomic charges were calculated according to Mulliken.

phenol) to 0.4 (2,4,6-trinitrophenol).[20] A comparison of the B3LYP/6-311G(d) estimated heterolytic dissociation energies of the O—H bonds in *o*-nitro- and 2,4-dinitrophenols (325.5 and 307.7 kcal mol^{-1}, respectively) and of the 2β-OH and 6β-OH groups in **1** (307.9 and 307.5 kcal mol^{-1}, respectively) shows that the acidity of the latter is higher than that of the OH groups in *o*-nitrophenol and equals to that of the OH groups in 2,4-dinitrophenol.

Monosodium salts of compound 1. From the aforesaid it follows that the reaction of compound **1** with NaOH should result in five monosodium salts **7**—**11**. It was assumed that the probability of formation, or the order in which each salt is formed, will depend on the acidity of particular OH group. Similarly to monoanions **2**—**6**, undissociated monosodium salts **7**—**11** formed upon neutralization of the OH groups of molecule **1** by sodium hydroxide

$$\text{(Et)NZ(OH)}_3 + \text{NaOH} \longrightarrow \text{(Et)(OH)}_2\text{NZ(O}^-\text{Na}^+\text{)} + \text{H}_2\text{O}, \quad (3)$$

can exist in different isomeric states. In addition, each possible tautomeric form **A**—**D** of these compounds can exist as a mixture of rotamers with respect to internal rotation of β-OH and ONa groups about the corresponding C—O bonds.

Monosodium salts **9** and **11** are formed upon neutralization of the α-OH groups of compound **1**. Here, the energetically favorable isomers are **9A** and **11C**, in which the sodium cation forms a coordination bond with the corresponding oxygen atom of carbonyl group in position 4 or 1 to give a six-membered chelate ring (Fig. 2). It should be noted that the ionic and coordination bonds formed by the sodium atom in salts **9** and **11** (see below) are indistinguishable within the limits of accuracy of the methods employed. Therefore, these bonds are denoted in schemes and figures by identical arrows. For instance, in isomer **9A** the Na—O(5) bond* is only ~0.01 Å shorter than the Na—O(4) bond, while in isomer **11C** the lengths of these bonds are equal to each other (see Fig. 2; obtained from 6-311G(d) calculations). Figure 3 shows the one-dimensional (1D) potential $\Delta E = f(\Delta r)$ for the motion of Na^+ cation in isomer **9A**. Here one has $\Delta E = E - E_{\text{min}}$ and $\Delta r = R_{\text{O(4)...O(5)}}/2 - R_{\text{Na—O(5)}} \cdot \cos\alpha$ (α is the bond angle Na—O(5)—O(4)); the latter parameter is the displacement of the Na^+ cation along the O(4)...O(5) axis. For displacements of the Na^+ cation from the equilibrium po-

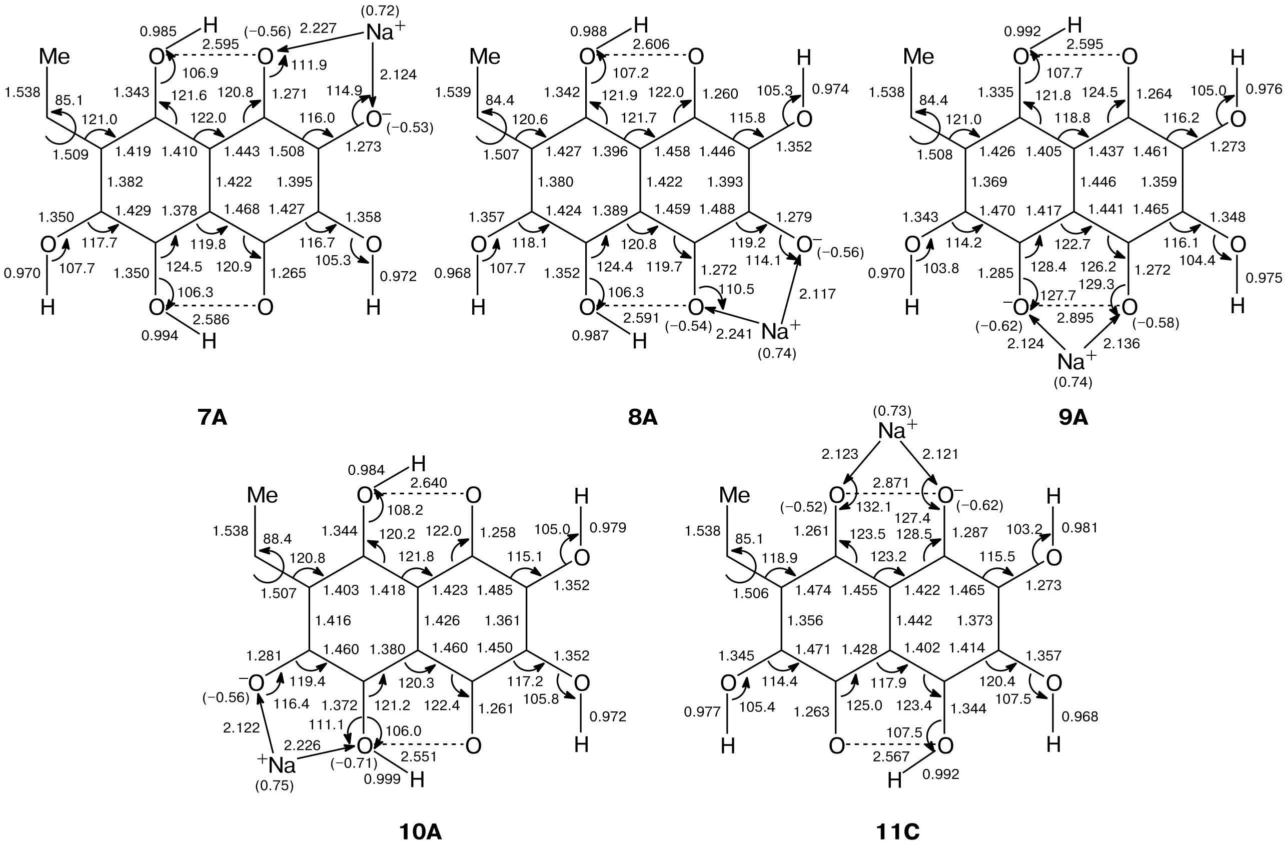

Fig. 2. Structures of the major isomers **7A**—**10A** and **11C** of monosodium salts **7**—**11** obtained from B3LYP/6-311G(d) calculations. The "minus" sign at the O atom denotes the OH group that undergoes neutralization with NaOH. Atomic charges are given in parentheses.

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* Hereafter the digit in parentheses near an O atom denotes the number of the C atom bound to this O atom.

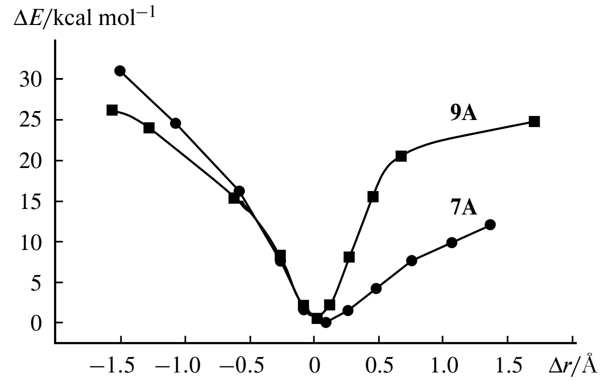


Fig. 3. Relative energy (ΔE) *vs.* Na^+ displacement from the equilibrium position (Δr) plots obtained for isomers **7A** and **9A** of monosodium salts **7** and **9**, respectively.

sition within ±0.5 Å, the potential is symmetrical. The single-well character and symmetrical shape of the potential (at $\Delta E \le 10$ kcal mol^{-1}) indicate an equally strong interaction of Na^+ cation with both O atoms.

Similarly to salts **9** and **11**, the energetically favorable isomers of monosodium salts **7**, **8**, and **10**, which are formed upon neutralization of the β-OH groups, are those that contain two bonds formed by Na^+ with O atoms of the ionized β-OH group and the neighboring carbonyl group. The corresponding distances R(Na—O) are close, but unlike isomer **9A**, in isomer **7A** they differ by 0.1 Å (see Fig. 2). In contrast to salts **9** and **11**, the sodium cation in salts **7**, **8**, and **10** forms a five-membered chelate ring.

As for isomer **9A**, the 1D potential in the case of **7A** $\Delta E = f(\Delta r)$ ($\Delta r = R_{O(1)...O(2)}/2 - R_{Na-O(2)} \cdot \cos\alpha$, where α is the angle Na—O(2)—O(1)), has a single-well shape. For Na^+ displacements from the equilibrium position within ±0.5 Å ($\Delta E \le 3$ kcal mol^{-1}), the potential is slightly asymmetrical (see Fig. 3).

The Gibbs free energy of rotamer **7A**$_{(2)}$ with respect to rotation of ONa group about the C(2)—O bond at which Na^+ forms the second coordination bond with the O atom of the 3β-OH group, is 2.2 kcal mol^{-1} higher than that of rotamer **7A** (Table 2). Unlike monoanions of compound

Table 2. Relative Gibbs free energies (ΔG) and percentages (g) of isomers of monosodium salts of compound **1** and their radicals (calculated by the (U)B3LYP/6-31G(d) method)

Compound	ΔG /kcal mol^{-1}	g (%)
7A	5.6	0.008
7B	—[a]	—
7C	7.7	$2.29 \cdot 10^{-4}$
7D	5.9	0.004
7A$_{(2)}$	7.8	$1.85 \cdot 10^{-4}$
7A$_{(3)}$	5.7	0.007
8A	7.6	$2.78 \cdot 10^{-4}$
8B	12.4	$7.72 \cdot 10^{-8}$
8C	11.2	$5.91 \cdot 10^{-7}$
8D	12.6	$5.40 \cdot 10^{-8}$
8D$_{(3)}$	15.2	$1.95 \cdot 10^{-10}$
9A	0.0	98.470
9C	2.5	1.340
10A	6.4	0.002
10B	13.6	$1.14 \cdot 10^{-8}$
10C	7.7	$2.14 \cdot 10^{-4}$
10D	5.9	0.005
11A	4.8	0.030
11C	3.9	0.137
12A	4.5	0.015
12A$_{(2)}$	0.0	31.560
12B	—[b]	—
12B$_{(2)}$	3.8	0.050
12C	8.5	$1.98 \cdot 10^{-5}$
12C$_{(2)}$	0.4	15.070
12D	4.4	0.017
12D$_{(2)}$	1.3	3.288
13A	14.8	$4.86 \cdot 10^{-10}$
13C	16.1	$5.06 \cdot 10^{-11}$
14A	10.8	$3.82 \cdot 10^{-7}$
14B	—[c]	—
14C	18.7	$6.24 \cdot 10^{-13}$
14D	13.2	$6.54 \cdot 10^{-9}$
15A	17.8	$2.62 \cdot 10^{-12}$
15C	12.3	$2.96 \cdot 10^{-8}$
15C$_{(3)}$	11.3	$1.53 \cdot 10^{-7}$
16A	4.4	0.019
16A$_{(3)}$	0.0	31.560
16B	8.4	$2.13 \cdot 10^{-5}$
16B$_{(3)}$	3.8	0.050
16C	10.1	$1.33 \cdot 10^{-6}$
16C$_{(3)}$	0.4	15.070
16D	9.4	$3.87 \cdot 10^{-6}$
16D$_{(3)}$	1.3	3.288
17A	9.5	$3.18 \cdot 10^{-6}$
17C	7.2	$1.54 \cdot 10^{-4}$
17C$_{(2)}$	7.0	$2.27 \cdot 10^{-4}$
18A	11.2	$1.81 \cdot 10^{-7}$
18B	17.0	$1.17 \cdot 10^{-11}$
18C	18.6	$7.52 \cdot 10^{-13}$
18D	17.0	$1.13 \cdot 10^{-11}$
19A(B)	24.6	$2.82 \cdot 10^{-17}$
19C(D)	25.1	$1.33 \cdot 10^{-17}$
20A$_{(3)}$	7.0	$2.52 \cdot 10^{-4}$
20C$_{(3)}$	8.0	$4.30 \cdot 10^{-5}$
21A$_{(2)}$	6.6	$4.68 \cdot 10^{-4}$
21C$_{(2)}$	4.9	0.008
21C$_{(2,4)}$	7.0	$2.27 \cdot 10^{-4}$
22A	6.8	$3.03 \cdot 10^{-4}$
22A$_{(5)}$	6.4	$6.56 \cdot 10^{-4}$
22C	13.3	$5.93 \cdot 10^{-9}$
23	13.7	$3.00 \cdot 10^{-9}$
24A$_{(3)}$	12.2	$3.38 \cdot 10^{-8}$
24B$_{(3)}$	16.3	$3.61 \cdot 10^{-11}$
24C$_{(3)}$	10.4	$7.14 \cdot 10^{-7}$
24D$_{(3)}$	9.4	$4.14 \cdot 10^{-6}$
25A$_{(3)}$	12.6	$1.81 \cdot 10^{-8}$
25B$_{(3)}$	15.1	$2.82 \cdot 10^{-10}$
25C$_{(3)}$	10.1	$1.19 \cdot 10^{-6}$
25D$_{(3)}$	11.8	$5.80 \cdot 10^{-8}$
26A	6.4	$6.55 \cdot 10^{-4}$
26C	—[d]	—
27A	26.1	$2.42 \cdot 10^{-18}$
27C	24.2	$5.92 \cdot 10^{-17}$
28A$_{(3)}$	11.3	$1.65 \cdot 10^{-7}$
28C$_{(3)}$	6.7	$3.94 \cdot 10^{-4}$
28C$_{(1,3)}$	11.3	$1.53 \cdot 10^{-7}$
29A$_{(3)}$	11.4	$1.31 \cdot 10^{-7}$
29C$_{(3)}$	10.6	$5.52 \cdot 10^{-7}$
30	10.0	$1.57 \cdot 10^{-6}$
31A	15.3	$1.83 \cdot 10^{-10}$
31C	16.2	$4.21 \cdot 10^{-11}$

[a] **7B** → **7A**.

[b] **12B** → **12A**.

[c] **14B** → **14A**.

[d] **26C** → **26A**.

1, the gain in the Gibbs free energy for rotamer **7A**$_{(3)}$ formed by rotating the 3β-OH group about the C(3)—O bond by about 180° relative to isomer **7A** is small (merely 0.1 kcal mol^{-1}, see Table 2).

A B3LYP/6-31G(d) estimation of the Gibbs free energies of all isomers of monosodium salts **7**—**11** showed that the major isomer is tautomeric form **9A** formed upon neutralization of the 5α-OH group. The *G* value for isomer **9C** is 2.6 kcal mol^{-1} higher than for isomer **9A** (see Table 2). The Gibbs free energies of isomers **11A** and **11C** of monosodium salt **11**, which is formed upon neutralization of the 8α-OH group, are, respectively, 4.8 and 3.9 kcal mol^{-1} higher than that of the major isomer **9A**.

The *G* values of the major isomers **7A**, **8A**, and **10A** (neutralization of the 2β-OH, 3β-OH, and 6β-OH groups, respectively) are 5.6, 7.6, and 6.4 kcal mol^{-1} higher than that of isomer **9A** (see Table 2). The structures of the major isomers of monosodium salts **7**—**11** are shown in Fig. 2. It should be noted that both B3LYP/6-311G(d) and B3LYP/6-311G(d,p) calculations predict virtually identical geometric parameters of these salts, namely, the bond lengths differ by less than 0.01 Å while the bond angles differ by a few tenth of a degree.

Conformational analysis of monosodium salts **7**—**11** made it possible to estimate the statistical weights and percentages of all isomers of these salts (see Experimental). Unexpectedly, it was found that the percentages of only two isomers, **9A** and **9C**, of salt **9**, which is formed upon neutralization of the 5α-OH group of compound **1**, is about 99.8%. The content of isomers **11A** and **11C** of salt **11** formed upon neutralization of the 8α-OH group is nearly 0.17%, while the total percentages of isomers of monosodium salts **7** and **10** formed upon neutralization of the 2β-OH and 6β-OH groups, are ~0.016 and 0.007%, respectively. The smallest content was found for all isomers of salt **8** ($\sim 3 \cdot 10^{-4}$%), which is formed upon neutralization of the 3β-OH group.

Estimation of statistical weigths of monosodium salts showed that the reaction of molecule **1** with NaOH should result in salts involving the α-OH groups as major products (up to 99.97%), while the amount of all other salts involving the β-OH groups is predicted to be at most 0.03%. This gave us an impetus to estimate the heats ($-\Delta H_r$) of neutralization reactions of the α-OH and β-OH groups of compound **1** with sodium hydroxide and to elucidate their interrelations with the estimated heterolytic dissociation energies of the O—H bonds, because both parameters characterize the acidity of the OH groups involved in acid-base reactions.

$$\mathbf{1A} + \mathrm{NaOH} \longrightarrow \mathbf{7A} + \mathrm{H_2O} \qquad (4)$$

$$\mathbf{1A} + \mathrm{NaOH} \longrightarrow \mathbf{8A} + \mathrm{H_2O} \qquad (5)$$

$$\mathbf{1A} + \mathrm{NaOH} \longrightarrow \mathbf{9A} + \mathrm{H_2O} \qquad (6)$$

$$\mathbf{1A} + \mathrm{NaOH} \longrightarrow \mathbf{10D} + \mathrm{H_2O} \qquad (7)$$

$$\mathbf{1A} + \mathrm{NaOH} \longrightarrow \mathbf{11C} + \mathrm{H_2O} \qquad (8)$$

The heats of gas-phase reactions (4)—(8) were estimated as the differences between the sums of the formation enthalpies of the major isomers of isolated reactant molecules and products. B3LYP/6-311G(d,p) calculations of reaction (6), which involves the 5α-OH group of compound **1**, gave $-\Delta H_r = 42.3$ kcal mol^{-1}. This is 4.6 kcal mol^{-1} higher than the heat of reaction (8) involving the 8α-OH group. The heats of reactions (4), (7), and (5) involving the 2β-OH, 6β-OH, and 3β-OH groups are 36.4, 36.0, and 34.3 kcal mol^{-1}, respectively (obtained from B3LYP/6-311G(d,p) calculations).

For all OH groups in compound **1**, correlations between the heterolytic dissociation energies (ΔG_H) of the O—H bonds and the heats of their reactions with NaOH (Fig. 4) clearly demonstrate that these groups cannot be attributed to the same type of OH-acids. At the same time, separate consideration of the α-OH and β-OH groups of echinochrome A leads to interrelations where lower ΔG_H values correspond to higher $-\Delta H_r$ values. At the same ΔG_H values of the α-OH and β-OH groups, the $-\Delta H_r$ of the α-OH groups is about 7 kcal mol^{-1} higher than that of the β-OH groups. A possible reason for this difference is that the major isomers of undissociated monosodium salts **7A**—**10A** and **11C** are not identical to the major isomers of monoanions **2C**$_{(3)}$, **3C**$_{(2)}$, **4A**, **5A**$_{(5)}$, and **6C**, which are formed upon heterolysis of those OH groups that form the corresponding monosodium salts.

Echinochrome A undergoes the neutralization with NaOH in the tautomeric form **1A**. Monosodium salts (pri-

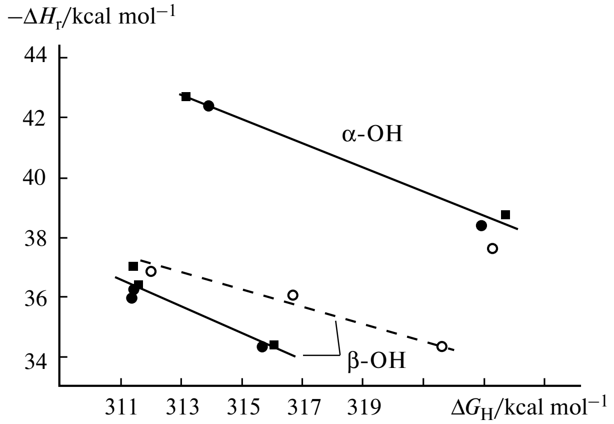
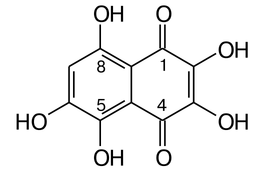


Fig. 4. Correlations between heats of reactions (4)—(8) ($-\Delta H_r$) and heterolytic dissociation energies (ΔG_H) of OH groups of compound **1** obtained from B3LYP/6-311G(d,p) calculations. Solid circles correspond to the major isomers of monoanions and undissociated monosodium salts of compound **1**, squares correspond to spinochrome D, and open circles correspond to monoanions and monosodium salts of compound **1** in the tautomeric form **A**.

mary products) also exist in the same form **A**, which is the major one for all of them, except for salt **11**. At the same time, in the case of heterolytic dissociation of OH groups of compound **1** the corresponding monoanions that are initially formed in the tautomeric form **A** undergo transformations to the major isomeric structures listed above as a result of conformational transitions.

Correlations between ΔG_{H} and $-\Delta H_{\mathrm{r}}$ for monoanions and undissociated monosodium salts of compound **1** existing in the same isomeric form **A** (the major isomer of this compound) are shown in Fig. 4. As can be seen, these correlations for the β-OH groups, established for both the major isomers and the tautomeric form **A**, differ insignificantly and even match each other for the α-OH groups.

Similar differences between ΔG_{H} and $-\Delta H_{\mathrm{r}}$ values for the α-OH and β-OH groups were also found for yet another pentahydroxy-1,4-naphthoquinone, spinochrome D (**32**)* (analog of compound **1**). The ΔG_{H} and $-\Delta H_{\mathrm{r}}$ values obtained for the α-OH and β-OH groups of molecule **1** and spinochrome D are in good agreement (see Fig. 4).



32

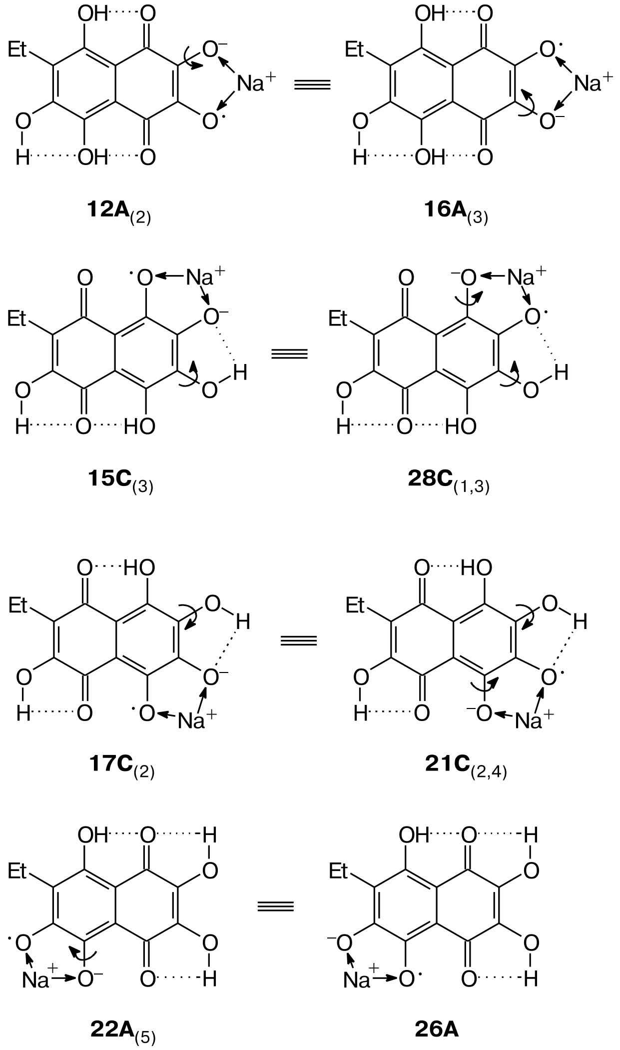
Since the correlations between the heterolytic dissociation energies of the O—H bonds and the heats of reactions of the α-OH and β-OH groups of compound **1** with NaOH are different, we believe that the α-OH and β-OH groups of molecule **1** should be treated as two different types of OH-acids. Therefore, the acidity series (see above) for all OH groups of molecule **1** should be divided into two sequences, namely, one for the β-OH groups (2β-OH ≥ ≥ 6β-OH > 3β-OH) and the other for the α-OH groups (5α-OH > 8α-OH) based on the estimated ΔG_{H} values.

Probably, correlations between $-\Delta H_{\mathrm{r}}$ and ΔG_{H} for the reactions of α-OH and β-OH groups of molecule **1** with NaOH are to a great extent determined by the nature of the rings involving the sodium cation of the ONa group and O atoms of neighboring functional groups (C=O or OH). For instance, in salts **9** and **11** the sodium cation forms a rather strong six-membered ring (dissociation energies of O—Na—O bonds are 123.1—130.8 kcal mol^{-1}). In salts **7**, **8**, and **10**, it forms a weaker five-membered ring (dissociation energies of the O—Na—O bonds are 117.0—120.2 kcal mol^{-1}, obtained from B3LYP/6-311G(d) calculations). At vibrational energies $E_{\mathrm{vibr}} \le$ $\le$ 10 kcal mol^{-1}, the displacement of Na$^+$ from the equilibrium position in salt **9** is about twice as small as in salt **7** (see Fig. 3).

Radicals of monosodium salts of echinochrome A. To access the antioxidant activity of undissociated salts **7—11** in reactions with HOO$^\bullet$, we have preliminarily performed conformational analysis of all isomers of radicals **12—31**. Isomers **12A**$_{(2)}$ and **16A**$_{(3)}$ correspond to the same minimum on the ground-state PES; this also holds for pairs of isomers **15C**$_{(3)}$ and **28C**$_{(1,3)}$, **17C**$_{(2)}$ and **21C**$_{(2,4)}$, and **22A**$_{(5)}$ and **26A** (Fig. 5).

To select the major isomers of radicals **12—31** (hereafter, major radicals), we estimated the Gibbs free energies (*G*) of their possible isomeric forms by the UB3LYP/6-31G(d) method. Based on the *G* values thus determined, the percentages of isomeric forms of radicals were calculated (see Table 2). The energy characteristics of five types of major radicals of monosodium salts were refined by the UB3LYP/6-311G(d) method and then used for estimation of the equilibrium constants and heats of quenching reactions of the HOO$^\bullet$ radical (Scheme 1, Table 3).

Like salts **7—11**, radicals **12—31** can exist as mixtures of tautomeric forms **A—D**; in turn, each of them can be



12A$_{(2)}$ **16A**$_{(3)}$

15C$_{(3)}$ **28C**$_{(1,3)}$

17C$_{(2)}$ **21C**$_{(2,4)}$

22A$_{(5)}$ **26A**

Fig. 5. Pairwise equivalent isomers of radicals **12A**$_{(2)}$ and **16A**$_{(3)}$, **15C**$_{(3)}$ and **28C**$_{(1,3)}$, **17C**$_{(2)}$ and **21C**$_{(2,4)}$, **22A**$_{(5)}$ and **26A**.

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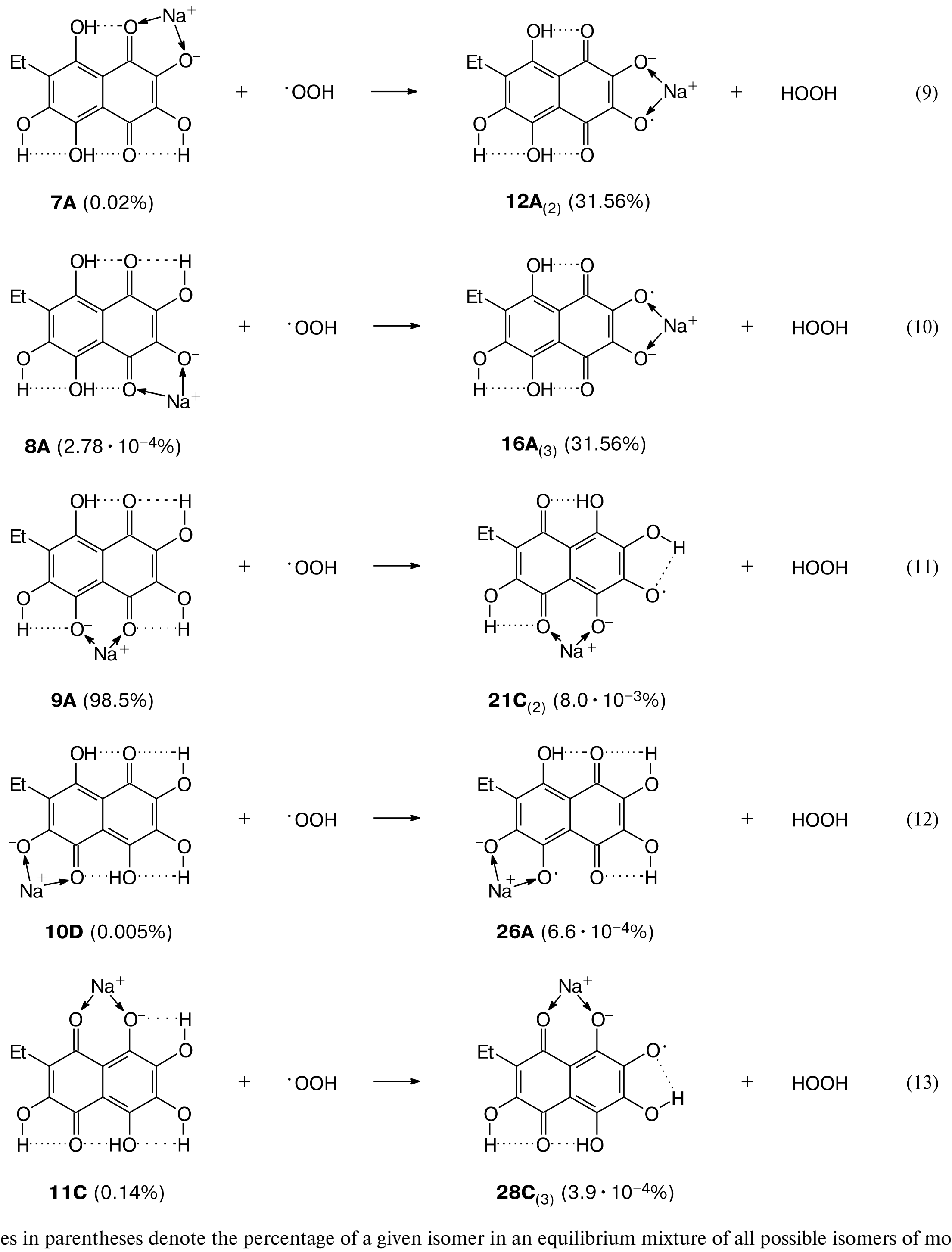
* The *G* and *H* values obtained from B3LYP/6-311G(d,p) calculations of spinochrome D and all major isomers of its monosodium salts are not presented here.

Scheme 1



Note. Values in parentheses denote the percentage of a given isomer in an equilibrium mixture of all possible isomers of monosodium salts of compound **1** or radicals of these salts.

a mixture of rotamers with respect to internal rotations of the OH and ONa groups about the corresponding C—O bonds. Rotamers with respect to internal rotation of the β-OH groups linked by the intramolecular hydrogen bond (IMHB) with O atoms bearing conditionally the unpaired electron have a small energy gain ($\Delta G = G(\mathbf{17C}) - G(\mathbf{17C}_{(2)}) = 0.2$ kcal mol^{-1}).

For radicals **12—31**, the energetically more favorable isomers are those in which Na^+ forms a coordination bond with the radical center $O^\bullet$. For instance, in isomer **12A**

Table 3. Homolytic dissociation energies of the O—H bonds (D_{OH}) in monosodium salts of compound **1**, heats of reactions ($-\Delta H_r$), the Gibbs free energies ($-\Delta G_r$), and the equilibrium constants (*K*) for quenching reactions of the hydroperoxyl radical by monosodium salts of compound **1** (calculated by the B3LYP/6-311G(d) method)

Reaction	D_{OH}	$-\Delta H_r$	$-\Delta G_r$	K
	kcal mol^{-1}			
7A → **12A**$_{(2)}$ (9)	57.0	11.3	12.0	$5.81 \cdot 10^8$
8A → **16A**$_{(3)}$ (10)	55.1	13.3	13.9	$1.54 \cdot 10^{10}$
9A → **21C**$_{(2)}$ (11)	64.3	1.0	2.1	34.6
10D → **26A** (12)	62.1	5.6	6.8	$1.03 \cdot 10^5$
11C → **28C**$_{(3)}$ (13)	64.7	3.2	4.3	$1.30 \cdot 10^3$

rotation of the ONa group about the corresponding C—O bond leads to a more stable isomer **12A**$_{(2)}$ (Fig. 6; see also Table 2). Usually, among radical isomers with respect to internal rotation of the ONa group, the energetically more favorable are those isomers in which Na$^+$ forms a coordination bond with the radical center O$^•$, which is not involved in the formation of an IMHB with the β-OH groups (see Fig. 6).

According to UB3LYP/6-31G(d) calculations, isomeric transitions **12A** → **12A**$_{(2)}$ and **22A** → **22A**$_{(5)}$ are characterized by almost equal barriers to internal rotation of the ONa group ($E^{\neq} \approx 16.1$ kcal mol^{-1}), which is about 2 kcal mol^{-1} higher than for the transition **21C**$_{(2)}$ → **21C**$_{(2,4)}$ ($E^{\neq} \approx 14.5$ kcal mol^{-1}).

The quenching reactions of the HOO$^•$ radical by monosodium salts of echinochrome A studied in this work obey the following common rule: if a salt exists as the major isomer, the energy expenditure is the smallest for homolysis of the O—H bond of the α-OH or β-OH group in *ortho*-position with respect to the OH group that forms the salt. For salts **7**, **8**, and **10**, the major (most stable) isomers with respect to the β-OH groups are the isomers of radicals **12A**$_{(2)}$, **16A**$_{(3)}$, and **26A**, whereas for salts **9** and **11** these are the isomers **21C**$_{(2)}$ and **28C**$_{(3)}$ with respect to the α-OH groups.

The structures of, and spin density distributions in, the major radicals of monosodium salts for all five OH groups of compound **1** are shown in Fig. 7. It should be noted that, similarly to salts **7**—**11** themselves, both UB3LYP/6-311G(d) and UB3LYP/6-311G(d,p) calculations predict the same values of the geometric parameters of their radicals. Only in the identical isomers **12A**$_{(2)}$ and **16A**$_{(3)}$ the spin density is mainly localized on C atoms and on the O atoms bound to them in those β-OH groups that were ionized and homolyzed.

As mentioned above, reactions (9)—(13) (see Scheme 1) were considered only taking the major isomers of salts **7**—**11**

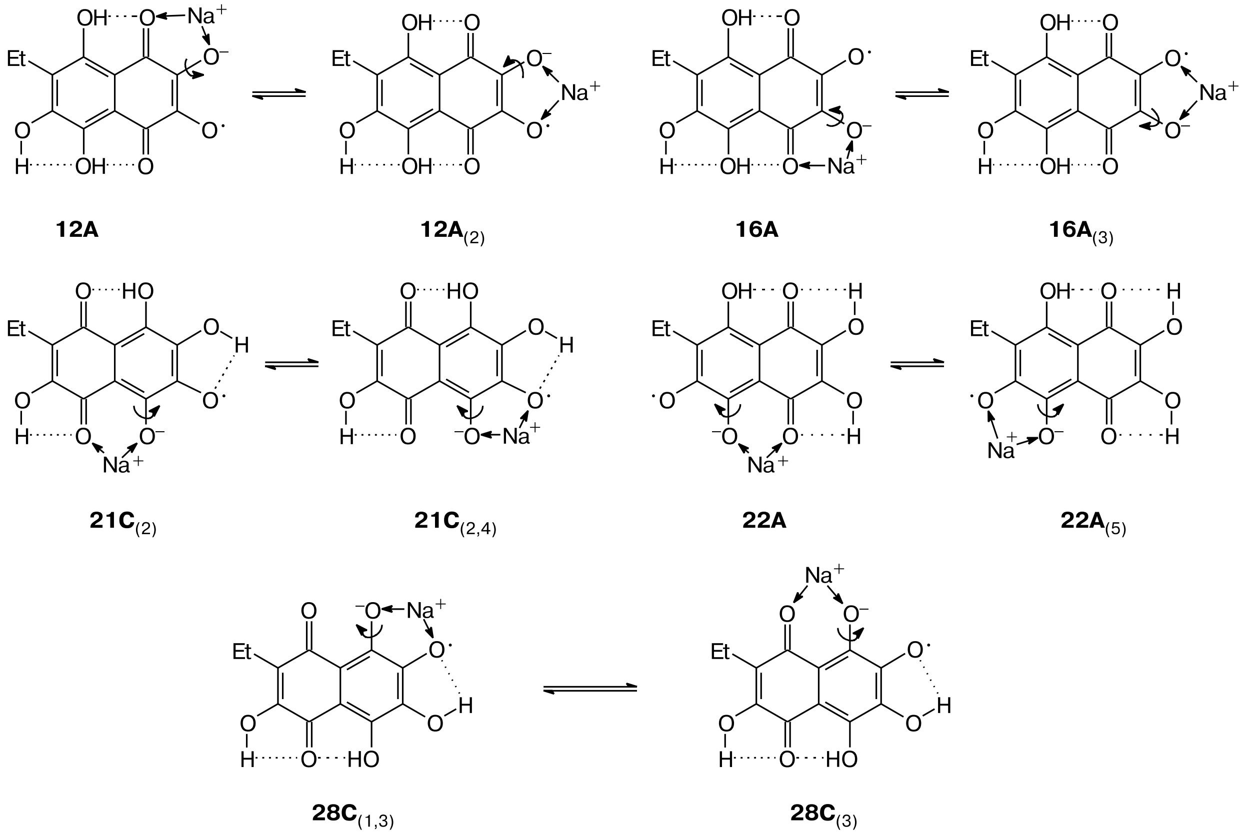


Fig. 6. Rotamers of radicals **12**, **16**, **21**, **22**, and **28** with respect to internal rotation of the ONa groups.

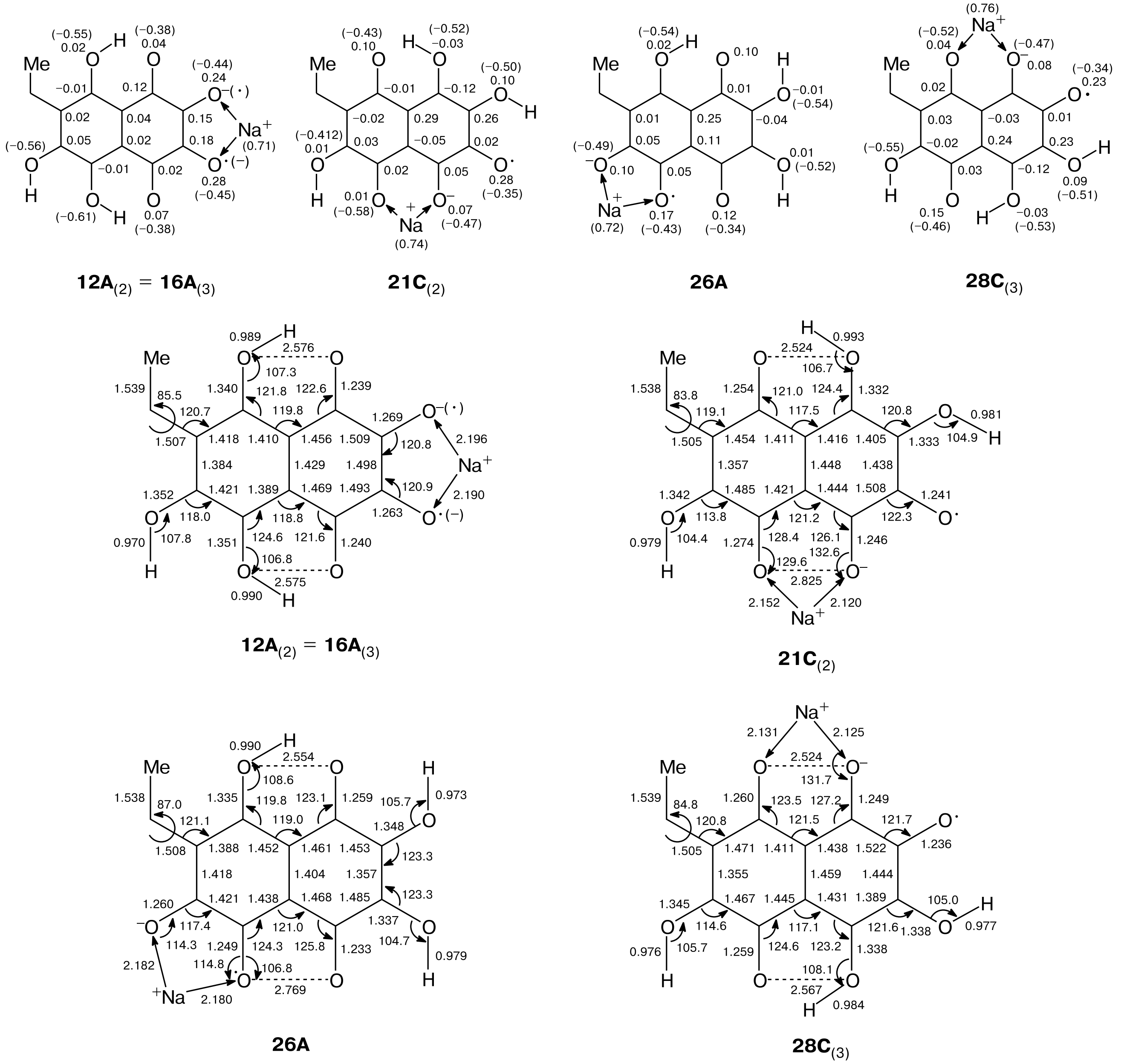


Fig. 7. Spin density distribution in (upper row) and geometric parameters of (middle and bottom rows) the major isomers **12A**$_{(2)}$ = **16A**$_{(3)}$, **21C**$_{(2)}$, **26A** and **28C**$_{(3)}$ of radicals of the corresponding monosodium salts (obtained from UB3LYP/6-311G(d) calculations). Figures in parentheses denote selected atomic charges.

and radicals formed from them as examples. The Gibbs free energies (ΔG_r) and the enthalpies (ΔH_r) of these reactions were estimated as the differences between the total Gibbs free energies or enthalpies of products and reactants obtained from (U)B3LYP/6-311G(d) calculations. For instance, for reaction (9) one has

$$\Delta G_r = [G(\mathbf{12A}_{(2)}) + G(\mathrm{HOOH})] - [G(\mathbf{7A}) + G(\mathrm{HOO}^\bullet)],$$

$$\Delta H_r = [H(\mathbf{12A}_{(2)}) + H(\mathrm{HOOH})] - [H(\mathbf{7A}) + H(\mathrm{HOO}^\bullet)].$$

The equilibrium constant K_i for the ith reaction was determined from the equation

$$K_i = \exp(-\Delta G_{r,i}/RT).$$

The parameters ΔH_r, ΔG_r, and K for reactions (9)—(13) are listed in Table 3.

The heats of quenching reactions of hydroperoxyl radical with monosodium salts of echinochrome A were used as measures for comparative estimation of their antioxidant activity. The results obtained show that reactions (9)—(13) are exothermic. The maximum heat ($-\Delta H_r$ = 13.3 kcal mol^{-1}) is evolved in the reaction of $HOO^{\bullet}$ quenching by isomer **8A** (see Table 3, reaction (10)).

Low percentage (equilibrium concentration) of some monosodium salts of compound **1** in the mixture does not significantly limit their action as quenchers of the hydroperoxyl radical, because the equilibrium constant for the quenching reaction is inversely proportional to the concentration of the initial substrate. For instance, if the concentration ratio of the major isomers **9A** and **7A** is $1.23 \cdot 10^4$, the ratio of the equilibrium constants for reactions (9) and (11) that involve these isomers is $1.68 \cdot 10^7$, which is about 10^3 times larger than the concentration ratio.

The homolytic dissociation energies of the O—H bonds (D_{OH}) in the α-OH and β-OH groups of monosodium salts **7**—**11** were estimated from the relation

$$(OH)_4(Et)NZ(O^-Na^+) \longrightarrow {}^{\bullet}O(OH)_3(Et)NZ(O^-Na^+) + H^{\bullet} \tag{14}$$

using the Gibbs free energies ((U)B3LYP/6-311G(d) calculations). They are listed in Table 3. As in the case of monoanions of compound **1**,[1] the minimum value of D_{OH} (55.1 kcal mol^{-1}) was obtained for undissociated monosodium salts of echinochrome A (homolysis of the 2β-OH group in isomer **8A**).

Thus, according to our quantum chemical calculations, the β-OH and α-OH groups of compound **1** belong to different types of acids. The acidities of the β-OH and α-OH groups of echinochrome A change as follows: 2β-OH ≥ 6β-OH > 3β-OH and 5α-OH > 8α-OH.

The heat effects of the reactions of compound **1** with NaOH are larger for the α-OH groups than for the β-OH groups. The ΔH_r values of these OH groups correlate with the corresponding heterolytic dissociation energies.

Of all possible products of the gas-phase reaction of molecule **1** with NaOH under thermodynamic equilibrium, monosodium salt **9** is energetically most favorable. Its percentage in the mixture of all monosodium salts of compound **1** is about 99.5%.

The single-well shape of the potential for the motion of Na^+ between two O atoms in molecules **7** and **9** indicates that this cation forms strong bonds with both oxygen atoms. Almost symmetrical potential for isomer **9A** formally matches single-well proton potentials in molecules containing very strong centrosymmetric hydrogen bonds O...H...O.

For all monosodium salts **7**—**11**, quenching of the hydroperoxyl radical is an exothermic process, the most active antioxidants being isomers **7A** and **8A**. A comparison of the heats of reactions of undissociated monosodium salts and monoanions of compound **1** with $HOO^{\bullet}$ showed that these salts are at least competitive with monoanions in efficiency of quenching of $HOO^{\bullet}$.

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