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

3.* The structures of dianions and disodium salts of echinochrome A and their reactions with the hydroperoxyl radical

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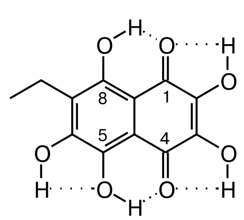
Quantum chemical calculations and the conformational analysis of dianions, radical dianions, disodium salts, and radicals of disodium salts of 7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A) were carried out at the (U)B3LYP/6-31G(d) and (U)B3LYP/6-311G(d) levels of theory. The heats of reactions of the hydroperoxyl radical ($HOO^{\bullet}$) with the isomers of dianions and disodium salts of echinochrome A with the lowest Gibbs free energies were estimated. All reactions of these isomers of dianions and disodium salts of echinochrome A with $HOO^{\bullet}$ in the gas phase are exothermic. The isomer of the dianion of echinochrome A with the lowest Gibbs free energy, which is formed by the heterolysis of the 2β- and 6β-OH groups, is the more effective antioxidant than the isomer of the 2,6-disodium salt with the lowest Gibbs free energy.

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

Previously,[1,2] we have investigated the antioxidant properties of 7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone (echinochrome A, (Et)NZ(β-OH)$_3$ (**1**), where NZ is naphthazarin (5,8-dihydroxy-1,4-naphthoquinone)), its monoanions, and undissociated monosodium salts, in the reactions with the hydroperoxyl radical ($HOO^{\bullet}$) by the (U)B3LYP/6-311G(d) method. Echinochrome A is the active principle of the pharmacopoeial drug Histochrome®, which is used in the cardiac therapy and ophthalmology. It was shown that echinochrome A is most effective as a radical scavenger when it exists either as the monoanion formed by the heterolysis of the O—H bond in one of β-OH groups or as the undissociated monosodium salt.



Echinochrome A

* For Part 2, see Ref. 1.

The determination of p*K* of the β-OH groups in echinochrome A by thin-layer voltammetry (polarography)[3] and the potentiometric titration[4] showed that at pH = 7.4 (normal pH of the human blood), compound **1** can exist in the form of both mono- and disodium salts and, in the case of their dissociation, as mono- and dianions. The structures and the antioxidant properties of the dianions and undissociated disodium salts of echinochrome A remained unknown.

The aim of the present work is to study the structures and the antioxidant properties of dianions **2—11** and undissociated disodium salts **22—31** of echinochrome A in the reactions with the hydroperoxyl radical, as well as the structures of the corresponding products, *viz.*, radical dianions **12—21** and radicals of disodium salts **32—61**, by the density functional theory and to compare the antioxidant properties of neutral echinochrome A, its mono- and dianions, and mono- and disodium salts.

Experimental

Quantum chemical calculations were carried out by the B3LYP density functional method[5] in the 6-31G(d) and 6-311G(d) basis sets with the use of the GAUSSIAN 03 program.[6] The electronic energies *E* of all major isomers of dianions, radi-

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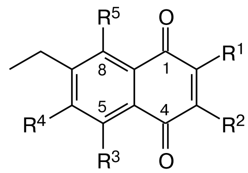


1—61

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

Compound	R[1]	R[2]	R[3]	R[4]	R[5]
32	ONa	ONa	O$^\bullet$	OH	OH
33	ONa	ONa	OH	O$^\bullet$	OH
34	ONa	ONa	OH	OH	O$^\bullet$
35	ONa	O$^\bullet$	ONa	OH	OH
36	ONa	OH	ONa	O$^\bullet$	OH
37	ONa	OH	ONa	OH	O$^\bullet$
38	ONa	O$^\bullet$	OH	ONa	OH
39	ONa	OH	O$^\bullet$	ONa	OH
40	ONa	OH	OH	ONa	O$^\bullet$
41	ONa	O$^\bullet$	OH	OH	ONa
42	ONa	OH	O$^\bullet$	OH	ONa
43	ONa	OH	OH	O$^\bullet$	ONa
44	O$^\bullet$	ONa	ONa	OH	OH
45	OH	ONa	ONa	O$^\bullet$	OH
46	OH	ONa	ONa	OH	O$^\bullet$
47	O$^\bullet$	ONa	OH	ONa	OH
48	OH	ONa	O$^\bullet$	ONa	OH
49	OH	ONa	OH	ONa	O$^\bullet$
50	O$^\bullet$	ONa	OH	OH	ONa
51	OH	ONa	O$^\bullet$	OH	ONa
52	OH	ONa	OH	O$^\bullet$	ONa
53	O$^\bullet$	OH	ONa	ONa	OH
54	OH	O$^\bullet$	ONa	ONa	OH
55	OH	OH	ONa	ONa	O$^\bullet$
56	O$^\bullet$	OH	ONa	OH	ONa
57	OH	O$^\bullet$	ONa	OH	ONa
58	OH	OH	ONa	O$^\bullet$	ONa
59	O$^\bullet$	OH	OH	ONa	ONa
60	OH	O$^\bullet$	OH	ONa	ONa
61	OH	OH	O$^\bullet$	ONa	ONa

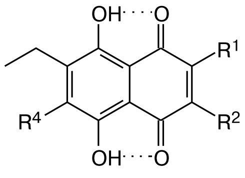
cal dianions, disodium salts, and their radicals derived from the echinochrome A molecule were evaluated by full geometry optimization with the 6-311G(d) basis set in the ground electronic state. The zero-point energy (ZPE) corrections and the temperature corrections G_T and H_T were calculated in the same basis set. The Gibbs free energies G and the enthalpies H were calculated taking into account all electronic, translational, rotational, and vibrational degrees of freedom at $T = 298.15$ K. The ground-state wave functions were calculated in the single-determinant approximation by the spin-restricted B3LYP method for compounds **1—7**, **11—13**, **16—18**, and HOOH and by the spin-unrestricted UB3LYP method for compounds **8—10**, **14**, **15**, **19**, and $HOO^\bullet$ and the $H^\bullet$ atom.

Geometry optimization of isomers of compounds **2—61** was conducted until |**grad**| $\le 10^{-6}$ au Å^{-1}. The assignment of the stationary points on the potential energy surface to minima and saddle points was made based on the calculated normal vibrational frequencies. If the theoretical spectrum includes one imaginary frequency, the stationary point is a saddle point; if there are no imaginary frequencies, the stationary point corresponds to a minimum.

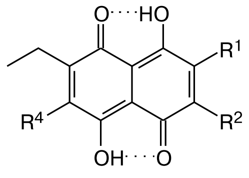
Results and Discussion

Dianions and radical dianions of echinochrome A. Dianions **2**, **4**, and **7**, which are formed by heterolysis of the O—H bond only in the β-OH groups of compound **1**, and radical dianion **13** generated by homolytic cleavage (homolysis) of the O—H bond in the β-OH group of these dianions can exist in four tautomeric forms (**A**, **B**, **C**, and **D**).

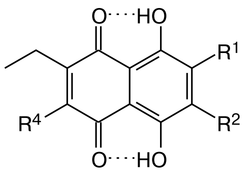
At the same time, the number of possible tautomeric forms of dianions and their radicals formed by heterolysis of the O—H bond or by homolysis of one α-OH group reduces to two. In particular, one minimum on the ground-state potential energy surface corresponds to each pair of tautomers **A**, **D** and **B**, **C** of dianions **3**, **6**, and **9** and radical dianions **12**, **15**, and **18** (Fig. 1). Analogously, only one minimum on the ground-state potential energy surface corresponds to each pair of tautomers **A**, **B** and **C**, **D** of dianions **5**, **8**, and **11** and radical dianions **14**, **17**, and **21** (Fig. 2).



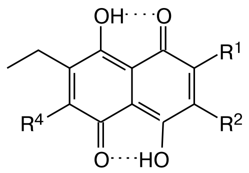
A
(**2**, **4**, **7**, **13**)



B
(**2**, **4**, **7**, **13**)



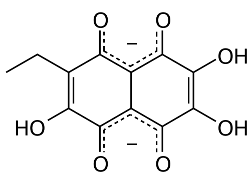
C
(**2**, **4**, **7**, **13**)



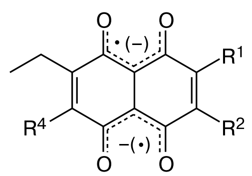
D
(**2**, **4**, **7**, **13**)

R^1, R^2, R^4 = OH, O^-, or $O^\bullet$

Dianion **10** and radical dianions **16**, **19**, and **20** generated by heterolysis or by heterolysis and homolysis of both α-OH groups of compound **1** have no tautomeric forms.



10



16, **19**, **20**

R^1, R^2, R^4 = OH, O^-

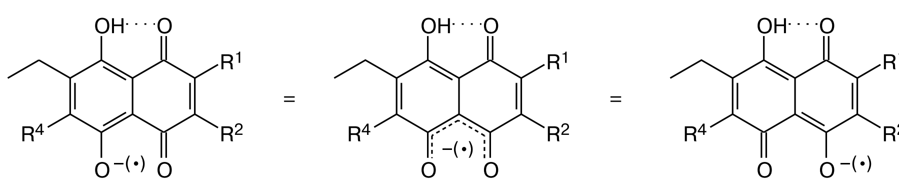
Each tautomeric form of compounds **2—9** and **11** can exist as a mixture of rotamers with respect to internal rotation of β- and/or α-OH groups around the corresponding C—O bonds.

The initial screening of the Gibbs free energies for 47 isomers of dianions **2—11** was performed at the B3LYP/6-31G(d) level of theory. The screening of dianions **2—11** revealed one major isomer with the lowest Gibbs free energy for each of them (Table 1). The geometric and energy characteristics of the major isomers were refined by the B3LYP/6-311G(d) method.* Of all the major isomers of dianions **2—11**, isomer $\mathbf{4C}_{(3)}$ is energetically most favorable.** This isomer is formed by heterolysis of the 2β- and 6β-OH groups. The Gibbs free energy of isomer $\mathbf{4C}_{(3)}$ is 3.7—28.6 kcal mol^{-1} lower than those of the major isomers of dianions **2**, **3**, and **5—11**. The geometry of isomer $\mathbf{4C}_{(3)}$ is shown in Fig. 3.

As can be seen from Fig. 3, the C(2)—O^- and C(6)—O^- bonds lengths (1.274 and 1.249 Å, respectively) in dianion $\mathbf{4C}_{(3)}$ generated by heterolysis of the 2β- and 6β-OH groups are comparable to the bond lengths of the carbonyl groups C(8)=O and C(5)=O (1.283 and 1.263 Å, respectively). In the dianion, the distances between the oxygen atoms *R*(O(1)...O(8)) and *R*(O(4)...O(5)) in the chelate rings stabilized by strong intramolecular hydrogen bonds involving α-OH groups are 0.10 and 0.07 Å shorter than the corresponding distances in molecule **1**. The close separation between the oxygen atoms in the chelate rings is responsible for a decrease in the barriers to α-OH proton

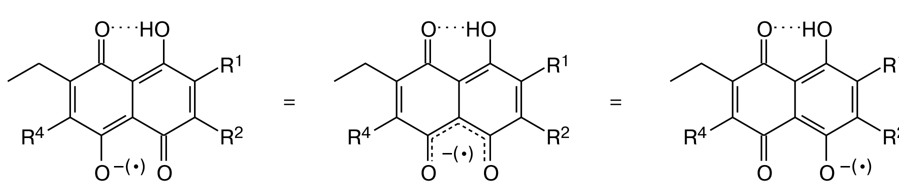
* A similar procedure was used to study radical dianions, disodium salts, and radicals of disodium salts of echinochrome A.

** For the rotamers of the β- and α-OH groups of the echinochrome A molecule, the subscript in parentheses denotes the number of the carbon atom at which the OH group is rotated by 180°.[1,2] For all OH groups, their positions in the main isomer **1A** of compound **1** is taken as the initial position.



A
(**3**, **6**, **9**, **12**, **15**, **18**)

D
(**3**, **6**, **9**, **12**, **15**, **18**)

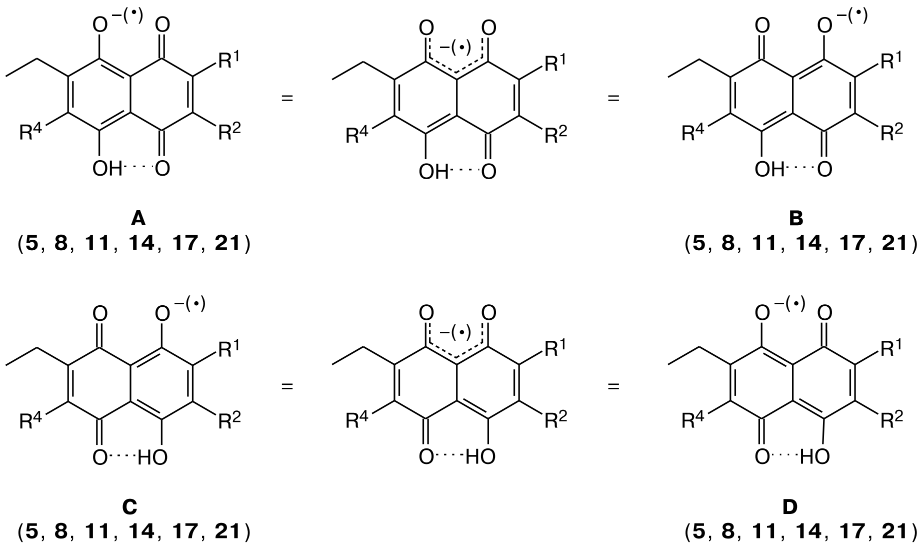


B
(**3**, **6**, **9**, **12**, **15**, **18**)

C
(**3**, **6**, **9**, **12**, **15**, **18**)

R^1, R^2, R^4 = OH, O^-, or $O^\bullet$

Fig. 1. Pairs of equivalent tautomers **A** = **D** and **B** = **C** of dianions **3**, **6**, and **9** and radical dianions **12**, **15**, and **18**.



A (**5**, **8**, **11**, **14**, **17**, **21**)

B (**5**, **8**, **11**, **14**, **17**, **21**)

C (**5**, **8**, **11**, **14**, **17**, **21**)

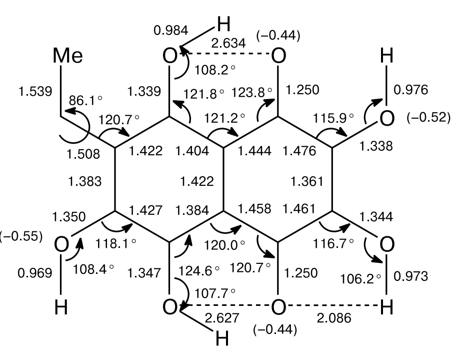
D (**5**, **8**, **11**, **14**, **17**, **21**)

R^1, R^2, R^4 = OH, O^- or $O^{\bullet}$

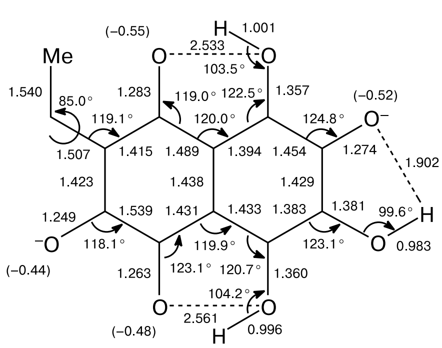
Fig. 2. Pairs of equivalent tautomers **A** = **B** and **C** = **D** of dianions **5**, **8**, and **11** and radical dianions **14**, **17**, and **21**.

transfer between tautomeric forms and facilitates it, thus enhancing the mobility of the dianion.

The heterolysis of two β-OH groups in dianion **4C** leads to a decrease in the homolysis energy (D_{OH}) of the 3β-OH group that remains intact. For instance, the 3β-OH group in isomer $\mathbf{4C}_{(3)}$ forms a stronger intramolecular hydrogen bond with $O^-(2)$ (R(O(3)H...O^-(2) = 1.902 Å) compared to the corresponding bond in neutral molecule **1** (R(O(3)H...O(4) = 2.086 Å). As a result, the O—H bond in the 3β-OH group is longer (0.983 Å) than that in neutral molecule **1** (0.973 Å) and, as shown below in the consideration of the reaction of dianion $\mathbf{4C}_{(3)}$ with the hydroperoxyl radical, D_{OH} for this reaction (56.9 kcal mol^{-1}) is much lower than that for molecule **1** (69.3 kcal mol^{-1}).[1]



1A



$\mathbf{4C}_{(3)}$

Fig. 3. Geometry of the neutral molecule of compound **1A** and the main isomer $\mathbf{4C}_{(3)}$ of dianion **4**. Atomic charges are given in parentheses.

In all schemes and figures, the total charges expressed as a minus sign on the oxygen atoms of β- and α-OH groups of dianions and radical dianions are arbitrary and indicate only which OH groups underwent heterolysis. For example, the charge on the O(2) atom* in dianion $\mathbf{4C}_{(3)}$ generated by heterolysis of the 2β- and 6β-OH groups of compound **1** is –0.52 e,** whereas the charge on the O(6) atom is –0.44 e (see Fig. 3). The calculated charges on the oxygen atoms of the OH groups that underwent heterolysis vary from the lowest value (–0.37 e on O(5) of dianion **9A**) to the highest value (–0.56 e on O(3) of dianion $\mathbf{7C}_{(2)}$).

The statistical weights (g) of the isomers of dianions **2—11** were estimated by the equation

$$g_{\mathbf{X}i} = [\exp(-\Delta G_{\mathbf{X}_i}/RT)]/[\Sigma_{\mathbf{X}_i}\exp(-\Delta G_{\mathbf{X}_i}/RT)], \quad (1)$$

where summation was carried out over all 47 isomeric (tautomeric-rotameric) forms of dianions **X** = **2—11**; $\Delta G_{\mathbf{X}_i} = G_{\mathbf{X}_i} - G(\mathbf{4C}_{(3)})$, where $G(\mathbf{4C}_{(3)})$ is the Gibbs free

* The subscript in parentheses denotes the number of the carbon atom to which this oxygen atom is bound.

** The atomic charges were calculated according to Mulliken.

Table 1. Relative Gibbs free energies (ΔG) and the percentages (g) of dianions **2—11** and radical dianions **12—21** of echinochrome A calculated by the (U)B3LYP/6-31G(d) method

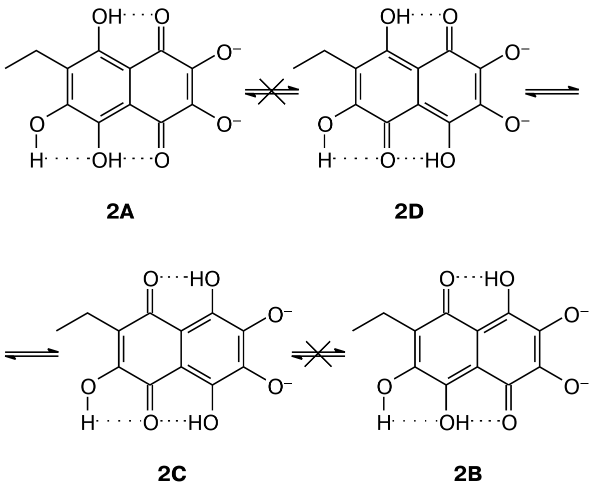
Compound	ΔG /kcal mol^{-1}	g (%)
2A	—[a]	—
2B	—[b]	—
2C	28.1	$2.40 \cdot 10^{-19}$
2C$_{(1)}$	28.7	$8.71 \cdot 10^{-20}$
2C$_{(4)}$	24.1	$1.95 \cdot 10^{-16}$
2C$_{(1,4)}$	25.0	$4.80 \cdot 10^{-17}$
2D	27.6	$5.87 \cdot 10^{-19}$
2D$_{(4)}$	25.5	$1.80 \cdot 10^{-17}$
3A(D)	16.4	$9.34 \cdot 10^{-11}$
3A$_{(3)}$ (**3D**$_{(3)}$)	16.8	$4.45 \cdot 10^{-11}$
3C(B)	16.6	$6.78 \cdot 10^{-11}$
3C$_{(3)}$ (**3B**$_{(3)}$)	17.1	$2.77 \cdot 10^{-11}$
3C$_{(1)}$	18.1	$5.58 \cdot 10^{-12}$
4A	3.6	0.24
4A$_{(3)}$	2.2	2.31
4A$_{(5)}$	8.7	$4.46 \cdot 10^{-5}$
4A$_{(3,5)}$	9.1	$2.13 \cdot 10^{-5}$
4B$_{(3)}$	3.0	0.65
4C	5.6	$7.57 \cdot 10^{-3}$
4C$_{(3)}$	0.0	94.74
4C$_{(1)}$	13.1	$2.53 \cdot 10^{-8}$
4D$_{(3)}$	2.3	1.86
5A	22.5	$3.00 \cdot 10^{-15}$
5A$_{(3)}$ (**5B**$_{(3)}$)	19.4	$6.01 \cdot 10^{-13}$
5C	22.8	$1.97 \cdot 10^{-15}$
5C$_{(3)}$ (**5D**$_{(3)}$)	17.6	$1.21 \cdot 10^{-11}$
6A	14.2	$3.83 \cdot 10^{-9}$
6A$_{(2)}$ (**6D**$_{(2)}$)	12.5	$6.98 \cdot 10^{-8}$
6C$_{(2)}$ (**6B**$_{(2)}$)	12.6	$5.80 \cdot 10^{-8}$
7A	8.5	$5.30 \cdot 10^{-5}$
7A$_{(2)}$	6.6	$1.38 \cdot 10^{-3}$
7A$_{(5)}$	10.4	$2.30 \cdot 10^{-6}$
7A$_{(2,5)}$	9.1	$2.06 \cdot 10^{-5}$
7B$_{(2)}$	7.6	$2.50 \cdot 10^{-4}$
7C$_{(2)}$	3.7	0.19
7C$_{(4)}$	11.0	$8.77 \cdot 10^{-7}$
7C$_{(2,4)}$	8.6	$5.12 \cdot 10^{-5}$
7D$_{(2)}$	6.4	$2.03 \cdot 10^{-3}$
8A	28.4	$1.57 \cdot 10^{-19}$
8A$_{(2)}$ (**8B**$_{(2)}$)	28.6	$1.05 \cdot 10^{-19}$
8C	25.5	$2.00 \cdot 10^{-17}$
8C$_{(2)}$ (**8D**$_{(2)}$)	26.0	$8.03 \cdot 10^{-18}$
8C$_{(4)}$	22.6	$2.71 \cdot 10^{-15}$
9A (9D)	10.0	$4.36 \cdot 10^{-6}$
9C (9B)	11.5	$3.77 \cdot 10^{-7}$
10	16.7	$5.44 \cdot 10^{-11}$
11A (11B)	17.8	$8.22 \cdot 10^{-12}$
11A$_{(5)}$ (**11B**$_{(5)}$)	17.4	$1.54 \cdot 10^{-11}$
11C (11D)	19.4	$5.81 \cdot 10^{-13}$
12A (12D)	10.6	$8.00 \cdot 10^{-7}$
12C (12B)	10.6	$9.01 \cdot 10^{-7}$
12C$_{(1)}$ (**12B**$_{(1)}$)	16.6	$3.54 \cdot 10^{-11}$
13A	0.2	36.54
13A$_{(5)}$	5.1	$9.36 \cdot 10^{-3}$
13B	0.9	11.02
13B$_{(1)}$	9.7	$4.11 \cdot 10^{-6}$
13B$_{(1,4)}$	14.0	$2.62 \cdot 10^{-9}$
13C	0.0	49.51
13C$_{(4)}$	5.1	$9.36 \cdot 10^{-3}$
13C$_{(1,4)}$	15.1	$4.61 \cdot 10^{-10}$
13D	1.7	2.91
14A (14B)	19.1	$4.63 \cdot 10^{-13}$
14C (14D)	17.3	$1.11 \cdot 10^{-11}$
14C$_{(4)}$ (**14D**$_{(4)}$)	21.8	$5.29 \cdot 10^{-15}$
15A	13.0	$1.39 \cdot 10^{-8}$
15A$_{(3)}$	14.9	$6.35 \cdot 10^{-10}$
15B (15C)	12.8	$2.20 \cdot 10^{-8}$
15B$_{(1)}$ (**15C**$_{(1)}$)	22.0	$3.65 \cdot 10^{-15}$
15C$_{(3)}$ (**15B**$_{(3)}$)	14.6	$1.00 \cdot 10^{-9}$
16	21.8	$5.29 \cdot 10^{-15}$
16$_{(3)}$	22.8	$9.95 \cdot 10^{-16}$
17A	18.7	$9.57 \cdot 10^{-13}$
17A$_{(3)}$ (**17B**$_{(3)}$)	16.8	$2.57 \cdot 10^{-11}$
17A$_{(5)}$	24.3	$7.47 \cdot 10^{-17}$
17A$_{(3,5)}$	24.1	$1.11 \cdot 10^{-16}$
17C(D)	18.9	$1.74 \cdot 10^{-12}$
17C$_{(3)}$ (**17D**$_{(3)}$)	14.3	$1.53 \cdot 10^{-9}$
18A	10.6	$8.28 \cdot 10^{-7}$
18A$_{(2)}$	8.7	$2.22 \cdot 10^{-5}$
18B	11.6	$1.67 \cdot 10^{-7}$
18B$_{(2)}$	8.2	$4.84 \cdot 10^{-5}$
19	20.0	$1.03 \cdot 10^{-13}$
19$_{(2)}$	23.7	$2.00 \cdot 10^{-16}$
20	16.4	$4.88 \cdot 10^{-11}$
21A	18.1	$2.82 \cdot 10^{-12}$
21A$_{(2)}$	22.1	$2.93 \cdot 10^{-15}$
21C	18.2	$2.30 \cdot 10^{-12}$
21C$_{(2)}$	22.2	$2.78 \cdot 10^{-15}$
21C$_{(4)}$	22.0	$3.53 \cdot 10^{-15}$

[a] **2A** → **2D**.
[b] **2B** → **2C**.

energy of the main isomer **4C**$_{(3)}$ of dianion **4**. The percentage (g (%)) of the ith isomer was calculated as $g_{\mathbf{X}_i} \cdot 100\%$.

The estimation of the percentage of all dianions of compound **1** showed that of ten dianions **2—11**, dianion **4** formed by heterolysis of the 2β- and 6β-OH groups is predominant in the gas phase (without taking into account the effect of the medium). The percentage of all its isomers is ~99.8%. The percentage of all isomers of di-

Scheme 1



anion **7** generated by heterolysis of the 3β- and 6β-OH groups is ~0.2%. The percentage of the other dianions (**2**, **3**, **5**, **6**, and **8—11**) is very low ($10^{-19}\% \le g \le 10^{-6}\%$).

Optimization of the geometric and energy parameters of dianion **2** generated by heterolysis of the 2β- and 3β-OH groups of compound **1** showed that of four possible tautomeric forms, only the tautomers **C** and **D** exist (Scheme 1, see Table 1).

Isomer $\mathbf{2C}_{(4)}$ is the major isomer of dianion **2**.

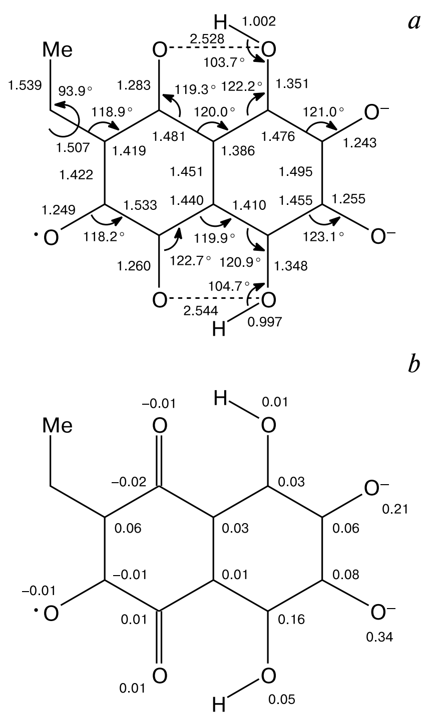


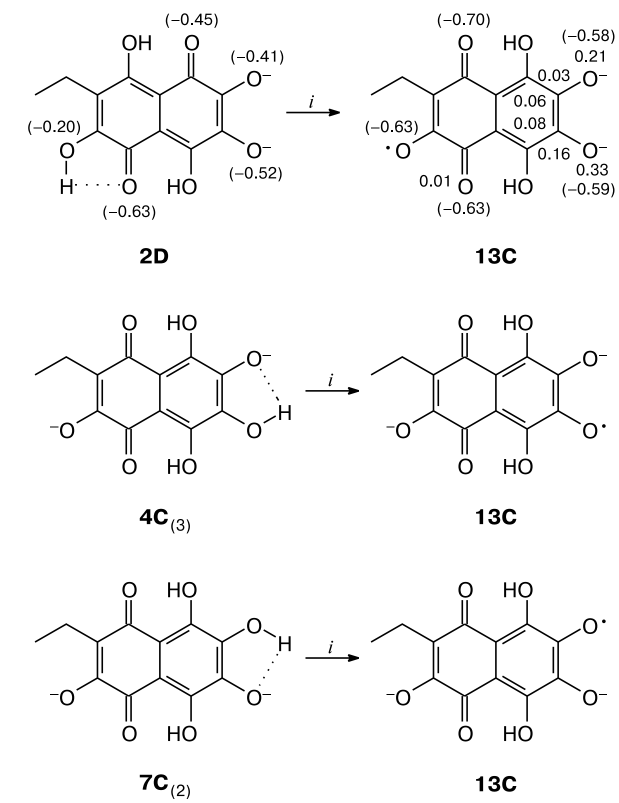
Fig. 4. Geometry (*a*) and the spin populations on the atoms (*b*) of the main isomer **13C** of radical dianion **13**.

When analyzing the reactants of dianions **2—11** with the HOO$^•$ radical, only the initial and final states of the major isomeric forms of the reactants were considered assuming that the reaction system is in thermodynamic equilibrium in the initial and final steps.

Quantum chemical calculations of radical dianions in the ground state showed that the spin densities are not mainly located on the oxygen atoms of the OH groups which underwent the O—H bond homolysis (Fig. 4, structure **13C**). As mentioned above, this also holds for the electron densities (charges). The results of calculations are unexpected from the viewpoint of classical organic chemistry. Assuming that the charges and spin densities are localized only on the oxygen atoms of those hydroxy groups which underwent heterolysis and homolysis, the reactions of ten dianions **2—11** with the HOO$^•$ radical should produce 30 radical dianions. However, according to our quantum chemical calculations, the number of structurally different radical dianions is ten (see below). In turn, they give 40 possible isomers (tautomeric-rotameric forms).

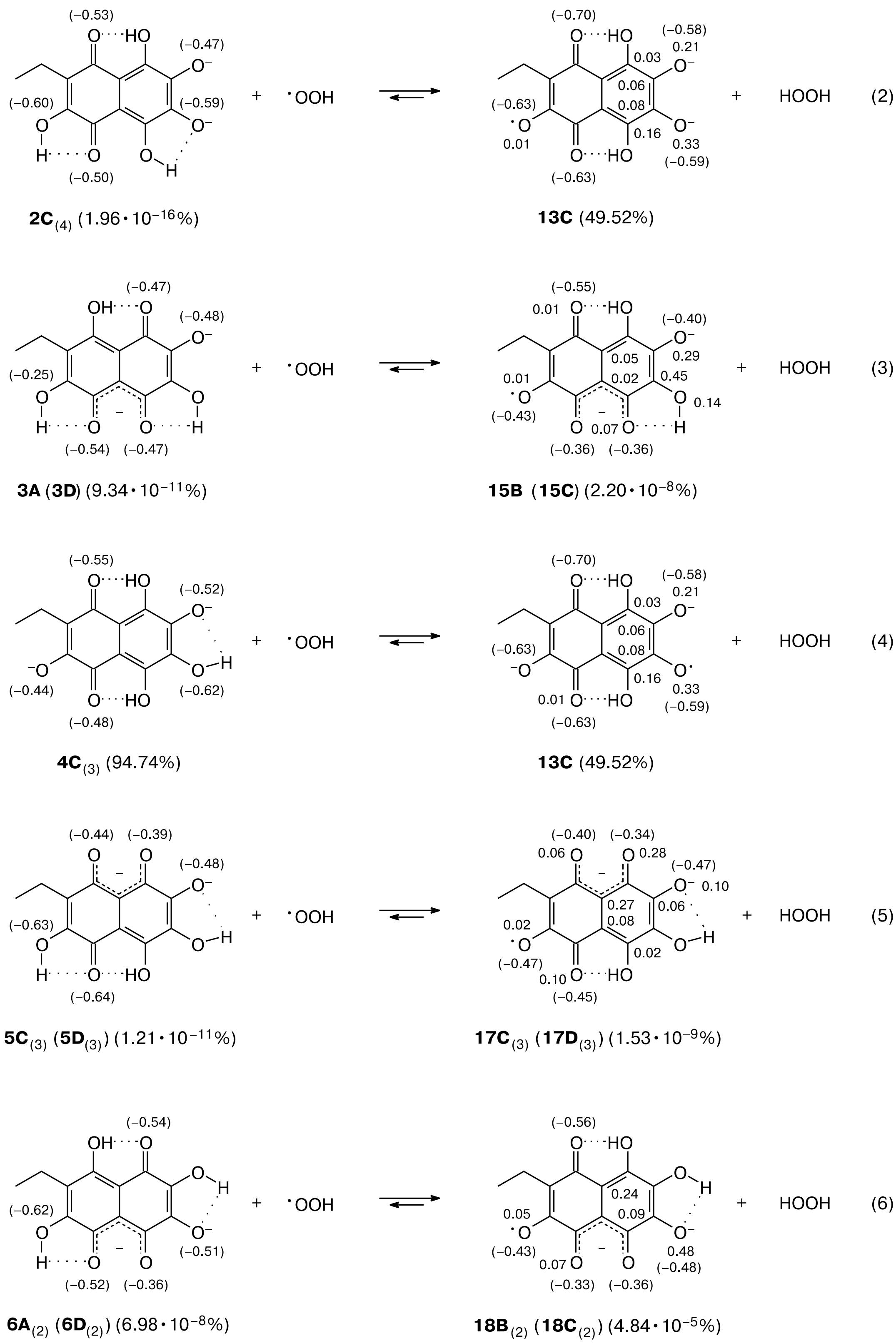
This can be exemplified by radical dianion **13** formed in the reactions of the HOO$^•$ radical with dianions **2**, **4**, and **7** (Scheme 2). Full geometry optimization of radical **13** showed no expected positive spin density on the O(6)

Scheme 2



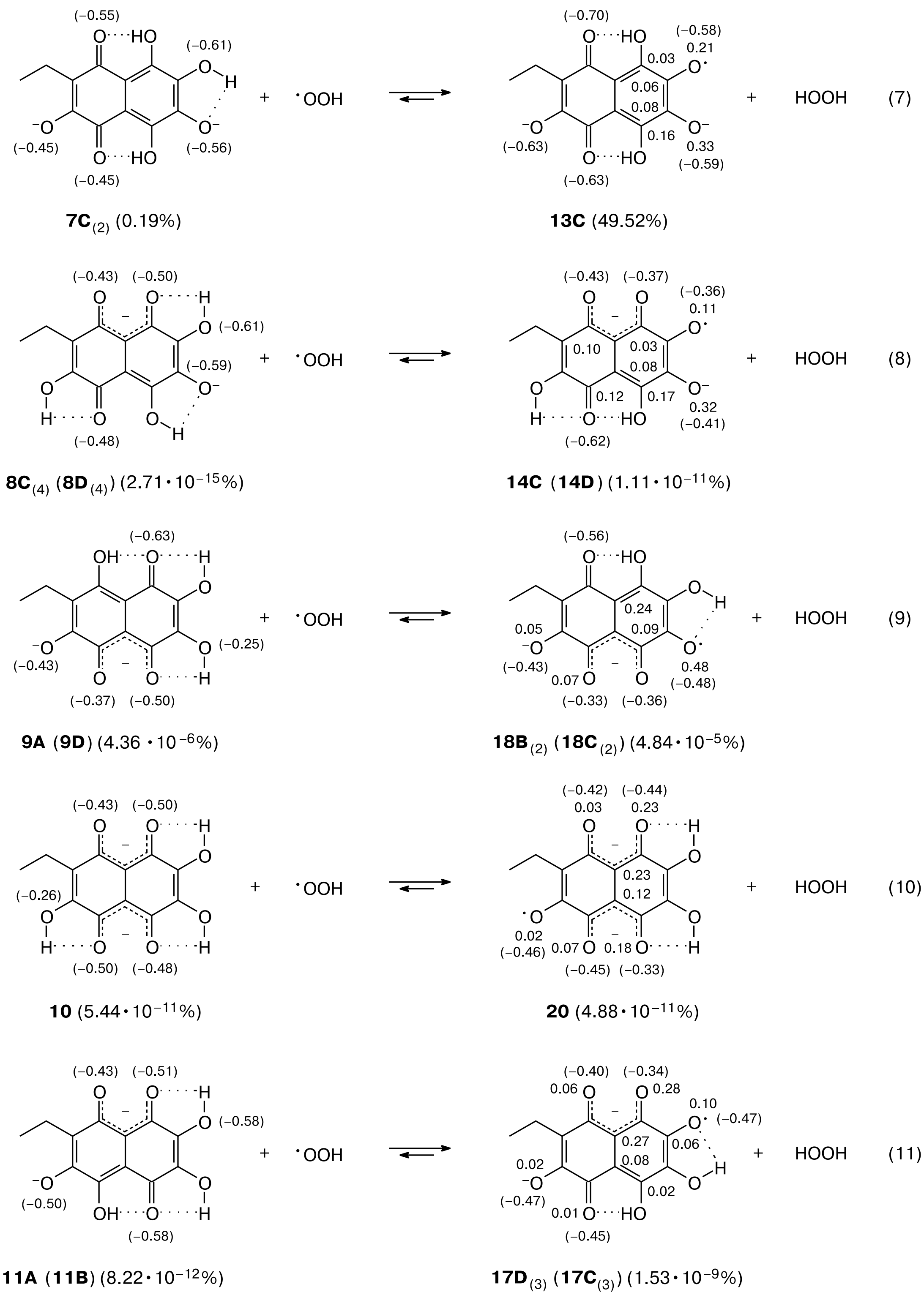
i. Homolysis.

Scheme 3



+ ·OOH
+ HOOH
(2)
$\mathbf{2C}_{(4)}$ ($1.96 \cdot 10^{-16}$%)
13C (49.52%)
(3)
3A (**3D**) ($9.34 \cdot 10^{-11}$%)
15B (**15C**) ($2.20 \cdot 10^{-8}$%)
(4)
$\mathbf{4C}_{(3)}$ (94.74%)
13C (49.52%)
(5)
$\mathbf{5C}_{(3)}$ ($\mathbf{5D}_{(3)}$) ($1.21 \cdot 10^{-11}$%)
$\mathbf{17C}_{(3)}$ ($\mathbf{17D}_{(3)}$) ($1.53 \cdot 10^{-9}$%)
(6)
$\mathbf{6A}_{(2)}$ ($\mathbf{6D}_{(2)}$) ($6.98 \cdot 10^{-8}$%)
$\mathbf{18B}_{(2)}$ ($\mathbf{18C}_{(2)}$) ($4.84 \cdot 10^{-5}$%)

Scheme 4



atom, whereas the highest positive spin populations were found on the O(2) and O(3) atoms (see Fig. 4). Hence, compound **13** should be considered as the "biradical" with the radical centers O(2) and O(3).

According to calculations, radical dianion **13** is formed when the homolysis of β-OH groups occurs in dianions **4** or **7**, as shown in Scheme 2 (in Schemes 2 and 3, the charges on the oxygen atoms of the carbonyl groups and

OH groups, which underwent the O—H bond heterolysis or homolysis, are given in parentheses). However, from a formal point of view, these reactions should give different radicals.

Radical dianions **12** and **14—21** are formed in the reactions of dianions with the HOO$^•$ radical. Hereinafter, the spin populations in radical dianions in all schemes and figures are given only for the oxygen atoms of the α- or β-OH groups that underwent the O—H bond homolysis. If the same radical dianion is generated from different dianions of compound **1**, the same number was assigned to it. For example, dianions **2**, **4**, and **7** give the same radical **13** (see Scheme 2).

For the major isomers of dianions **2—11**, only the reaction with the HOO$^•$ radical giving the major radical dianion was considered. Delocalization of the spin and electron densities in radical dianions (see above) reflects the fact that reactions (2)—(11) give only six structurally different major radical dianions, radical dianion **13C** being the main species. For the radical dianions (Schemes 3 and 4), shown are the spin populations equal to or higher than 0.01. These schemes also give the percentage of the isomers of dianions and radical dianions (in parentheses) near their symbols of the isomers.

The calculations of the structures of radical dianions showed that the positive spin density is mainly localized in the moiety containing no ethyl groups. A small portion of the positive spin density is localized in the moiety containing the ethyl substituent only in the case when one or both α-OH groups undergo the O—H bond homolysis.

Estimation of the statistical weights of all 40 isomers of radical dianions **12—21** by Eq. (1)* using the Gibbs free energies showed that in the gas phase the total percentage of all isomers of radical dianion **13** is higher than 99.99%. Two isomers, **13C** (49.52%) and **13A** (36.55%), are the energetically most favorable tautomeric forms of this radical. The geometry and the spin populations on the atoms of the main isomer **13C** are given in Fig. 4. The total percentage of all other radical dianions **12** and **14—21** is at most ~10^{-7}%.

An analysis of the geometry of radical dianion **13C** shows that the C(2)—O$^-$, C(3)—O$^-$, and C(6)—O$^•$ bond lengths are equal within ±0.006 Å and are 0.034 Å shorter than those for the carbonyl groups at the C(5) and C(8) atoms. It should be noted that the distances between the oxygen atoms in the hydrogen-bonded chelate rings in radical dianion **13C** are shorter than in dianion $\mathbf{4C}_{(3)}$ (see Fig. 3).

The homolysis energies (D_{OH}) of the α- and β-OH groups of dianions **12—21** were evaluated as the differences

$$\Delta G = G[(^-O)_2(EtNZOH)] - G[(^-O)_2(EtNZO^•)] - G(H^•),$$

according to the equation:

$$(^-O)_2(EtNZOH) \rightarrow (^-O)_2(EtNZO^•) + H^•, \quad (12)$$

where $(^-O)_2$(EtNZOH) is the echinochrome A dianion involved in the reaction, $(^-O)_2$(EtNZO$^•$) is the radical dianion formed in the reaction with the HOO$^•$ radical, and H$^•$ is the hydrogen atom.

The enthalpies of reactions (2)—(11) (ΔH_r) and their energy balances (ΔG_r) were evaluated using to the general scheme

$$\Delta F_r = F((^-O)_2(EtNZO^•)) + F(HOOH) - F((^-O)_2(EtNZOH)) - F(HOO^•),$$

$F = H$ or G,

using the reaction equation

$$(^-O)_2(EtNZOH) + HOO^• \rightarrow (^-O)_2(EtNZO^•) + HOOH. \quad (13)$$

The equilibrium constants K of reactions (2)—(11) were calculated by the equation

$$K = \exp(-\Delta G_r)/RT). \quad (14)$$

The relative efficiencies of the antioxidant properties of the dianions were estimated by comparing the D_{OH} values for the O—H bonds in the β- and α-OH groups and the ΔH_r values for the reactions with the HOO$^•$ radical. The estimation of D_{OH} for the O—H bonds in the β- and α-OH groups of dianions of compound **1** in reactions (2)—(11) (Table 2) showed that the dianions are more effective antioxidants than the corresponding monoanions. The D_{OH} values for the O—H bonds in the β-OH groups of monoanions[2] of compound **1** are in the range of 57.9—62.5 kcal mol^{-1} (*cf.* 31.9—56.9 kcal mol^{-1} for dianions). Reactions (2)—(11) are exothermic and characterized by the ΔH_r values from −11.2 to −36.1 kcal mol^{-1} (see Table 2). The ΔH_r values for the corresponding reactions of monoanions of compound **1** vary from −5.2 to −9.7 kcal mol^{-1}.

A comparison of the ΔH_r and K values for the reactions of the HOO$^•$ radical with the main isomers of the monoanion of echinochrome A** and dianion $\mathbf{4C}_{(3)}$ shows that reaction (4) is characterized by ΔH_r = −11.2 kcal mol^{-1} and $K = 6.8 \cdot 10^8$, which are two and six times larger, respectively, than the corresponding values for the reaction of the monoanion with this radical.[2]

Disodium salts of echinochrome A and their radicals. Disodium salts **22**, **24**, and **27** and their radicals **33**, **38**, and **47**, which are formed by replacement of protons of only β-OH groups in compound **1** by Na^+ cations fol-

* For radical dianions, the statistical weights were estimated by calculating the relative Gibbs free energies as $\Delta G_{\mathbf{X}_i} = G_{\mathbf{X}_i} - G(\mathbf{13C})$, where G(**13C**) is the Gibbs free energy of the main isomer **13C** of radical dianion **13**.

** The main isomeric form of the monoanion of compound **1** is formed by heterolysis of the O—H bond in the 6β-OH group of the tautomeric form **A**.[1]

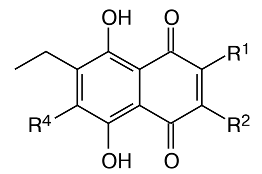
Table 2. Homolytic dissociation energies of the O—H bonds (D_{OH}) in dianions of echinochrome A, the heats of reactions (ΔH_r), the Gibbs free energies (ΔG_r), and the equilibrium constants (K) of the reactions of the hydroperoxyl radical with the major isomers of dianions of echinochrome A calculated by the (U)B3LYP/6-31G(d) method

Reaction	D_{OH}	$-\Delta H_r$	$-\Delta G_r$	K
	kcal mol^{-1}			
2C$_{(4)}$ → **13C** (2)	31.9	36.1	37.0	$1.32 \cdot 10^{27}$
3A → **15B** (3)	52.7	14.9	16.2	$7.62 \cdot 10^{11}$
4C$_{(3)}$ → **13C** (4)	56.9	11.2	12.1	$6.80 \cdot 10^{8}$
5C$_{(3)}$ → **17C**$_{(3)}$ (5)	53.3	13.4	15.7	$3.11 \cdot 10^{11}$
6A$_{(2)}$ → **18B**$_{(2)}$ (6)	51.2	15.5	17.8	$1.04 \cdot 10^{13}$
7C$_{(2)}$ → **13C** (7)	53.2	14.8	15.2	$1.38 \cdot 10^{11}$
8C$_{(4)}$ → **14C** (8)	50.2	20.9	22.4	$2.54 \cdot 10^{16}$
9A → **18B**$_{(2)}$ (9)	54.4	12.5	14.6	$4.78 \cdot 10^{10}$
10 → **20** (10)	54.9	11.3	14.0	$1.89 \cdot 10^{10}$
11A → **17D**$_{(3)}$ (11)	53.0	13.9	16.0	$4.91 \cdot 10^{11}$

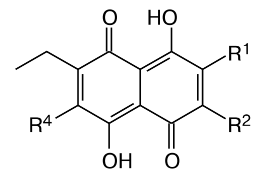
Note. Calculated by the (U)B3LYP/6-311G(d) method: $\Delta G = G(\mathrm{HOOH}) - G(\mathrm{HOO^{\bullet}}) = 390.7$ kcal mol^{-1}, $\Delta H = H(\mathrm{HOOH}) - H(\mathrm{HOO^{\bullet}}) = 390.4$ kcal mol^{-1}; $G(\mathrm{H^{\bullet}}) = 321.8$ kcal mol^{-1}.

lowed by the hydrogen atom abstraction from OH groups of these salts, can exist in the ground state as four tautomeric forms (**A**, **B**, **C**, and **D**).

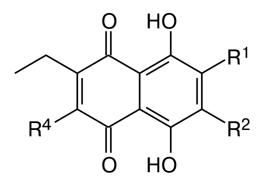
For disodium salts of compound **1** formed with the involvement of one α-OH group and their radicals, there are two possible tautomeric forms. Thus each pair of the tautomers **A**, **D** and **B**, **C** of disodium salts **23**, **26**, and **29** and radicals of disodium salts **35—37**, **44—46**, and **53—58**



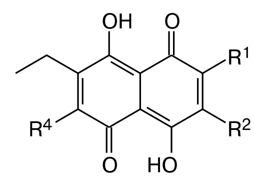
A
(**22**, **24**, **27**, **33**, **38**, **47**)



B
(**22**, **24**, **27**, **33**, **38**, **47**)



C
(**22**, **24**, **27**, **33**, **38**, **47**)



D
(**22**, **24**, **27**, **33**, **38**, **47**)

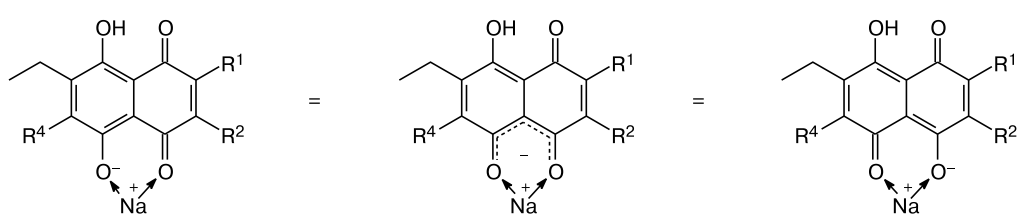
22, **24**, **27**: R^1, R^2, R^4 = ONa, OH;
33, **38**, **47**: R^1, R^2, R^4 = ONa, $O^{\bullet}$

is characterzied by one minimum on the ground-state potential energy surface of these compounds (Fig. 5).*

Analogously, only one minimum on the potential energy surface corresponds to each pair of the tautomers **A**, **B** and **C**, **D** of disodium salts **25**, **28**, and **31** and radicals of disodium salts **41—43**, **50—52**, and **56—61** (Fig. 6).

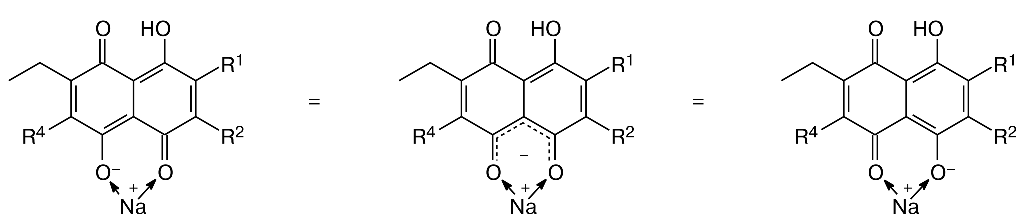
Disodium salt **30** formed involving both α-OH groups of compound **1**, as well as radicals **56—58** generated from

* In the sodium salts of compound **1**, the coordination number of the Na^+ cation is 2.[1] In the schemes and figures, both coordination bonds of the Na^+ cation are indicated by identical arrows.



A
(**23**, **26**, **29**, **35—37**, **44—46**, **53—58**)

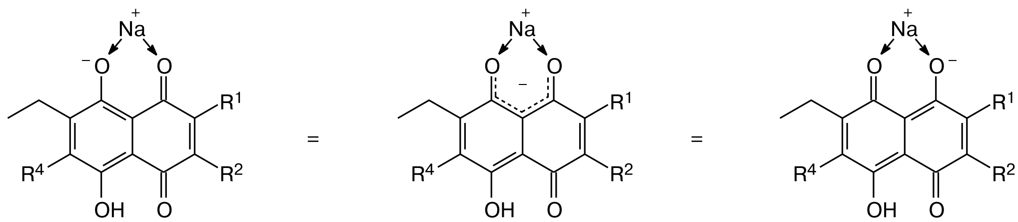
D
(**23**, **26**, **29**, **35—37**, **44—46**, **53—58**)



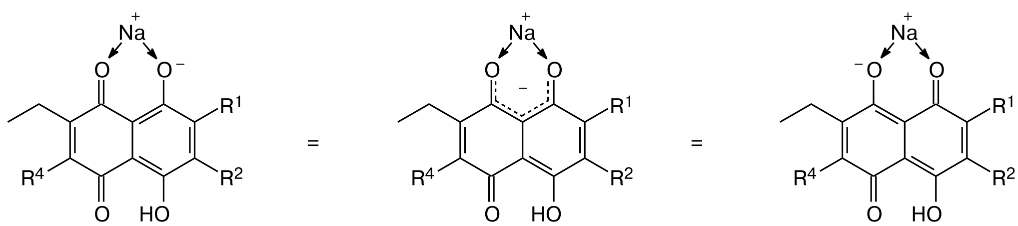
B
(**23**, **26**, **29**, **35—37**, **44—46**, **53—58**)

C
(**23**, **26**, **29**, **35—37**, **44—46**, **53—58**)

Fig. 5. Pairs of equivalent tautomers **A** = **D** and **B** = **C** of disodium salts **23**, **26**, and **29** and radicals of disodium salts **35—37**, **44—46**, and **53—58**.



A (**25**, **28**, **31**, **41—43**, **50—52**, **56—61**) **B** (**25**, **28**, **31**, **41—43**, **50—52**, **56—61**)

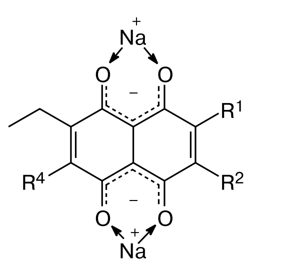


C (**25**, **28**, **31**, **41—43**, **50—52**, **56—61**) **D** (**25**, **28**, **31**, **41—43**, **50—52**, **56—61**)

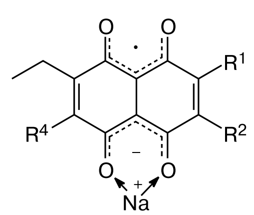
25, **28**, **31**: R^1, R^2, R^4 = ONa, OH; **41—43**, **50—52**, **56—61**: R^1, R^2, R^4 = ONa, OH, $O^•$

Fig. 6. Pairs of equivalent tautomers **A** = **B** and **C** = **D** of disodium salts **25**, **28**, and **31** and radicals of disodium salts **41—43**, **50—52**, and **56—61**.

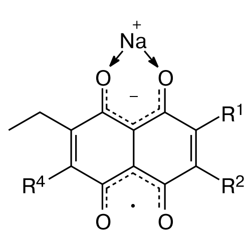
this salt, have no tautomeric forms. Radicals of disodium salts **37**, **42**, **46**, **51**, **55**, and **61** formed by replacement of the proton in one of α-OH groups by the Na^+ cation and by abstraction of the hydrogen atom from the other α-OH group have no tautomeric forms as well.



30, **56—58**



37, **46**, **55**



42, **51**, **61**

30, **56—58**: R^1, R^2, R^4 = OH, $O^•$;
37, **42**, **46**, **51**, **55**, **61**: R^1, R^2, R^4 = ONa, OH

Like dianions of compound **1**, disodium salts **22—31** can exist in different isomeric states as rotamers with respect to internal rotation of β-OH and ONa groups around the corresponding C—O bonds and tautomers resulting from the bridging proton transfer between α-OH groups. Previously, geometry optimization of monosodium salts of compound **1** has shown that the neutralization of the α-OH or β-OH group with sodium hydroxide affords a salt containing the Na^+ cation with the coordination number 2. The structure of molecule **1** is such that the formation of the monosodium salt at each of five OH groups can lead to the formation of the second coordination bond between the Na^+ cation and the oxygen atom of the adjacent carbonyl group. The rotamers with respect to internal rotation of the ONa group in monosodium salts of compound **1**, in which the second coordination bond is formed between the Na^+ cation and the oxygen atom of the adjacent β- or α-OH group, are energetically less favorable.[2]

The screening of 34 isomers of 10 disodium salts **22—31** revealed ten major isomers, *viz.*, **22A**, **23A**$_{(3)}$, **24D**$_{(3)}$, **25A**$_{(2)}$, **26A**$_{(3)}$, **27A**, **28A**, **29D**, **30**, and **31C** (Table 3). Among them, the isomer of disodium salt **24D**$_{(3)}$ is the main one. It is formed by replacement of protons in the 2β- and 6β-OH groups by Na^+ cations. The geometry of this isomer is shown in Fig. 7.

As in monosodium salts of compound **1**,[1] disodium salt **24D**$_{(3)}$ contains the sodium cations with the coordination number 2. The Na—O(2) and Na—O(3) bond lengths differ by only 0.014 Å; the Na—O(5) and Na—O(6) bond lengths differ by 0.022 Å.

Unlike monosodium salts of compound **1**, for two isomers (**25A**$_{(2)}$ and **26A**$_{(3)}$) of the ten major isomers of disodium salts, the energetically more favorable rotamers

Table 3. Relative Gibbs free energies (ΔG) and the percentages (g) of disodium salts **22—31** and radicals of disodium salts **32—61** of echinochrome A calculated by the (U)B3LYP/6-31G(d) method

Compound	ΔG /kcal mol^{-1}	g (%)
22A	13.3	$1.75 \cdot 10^{-8}$
22B	—[a]	—
22C	18.4	$2.79 \cdot 10^{-12}$
22D	15.3	$5.45 \cdot 10^{-10}$
23A (**23D**)	2.4	1.65
23A$_{(3)}$ (**23D**$_{(3)}$)	1.9	3.70
23C (**23B**)	—[b]	—
24A	5.1	0.02
24B	—[c]	—
24C	—[d]	—
24D	2.1	2.62
24D$_{(2)}$	12.5	$6.57 \cdot 10^{-8}$
24D$_{(3)}$	0.0	90.64
25A (**25B**)	16.0	$1.63 \cdot 10^{-10}$
25A$_{(2)}$	11.8	$2.01 \cdot 10^{-7}$
25C (**25D**)	13.5	$1.22 \cdot 10^{-8}$
25C$_{(2)}$	13.5	$1.22 \cdot 10^{-8}$
26A (**26D**)	13.2	$2.10 \cdot 10^{-8}$
26A$_{(3)}$	7.1	$5.62 \cdot 10^{-4}$
26C (**26B**)	14.7	$1.46 \cdot 10^{-9}$
27A	12.9	$3.34 \cdot 10^{-8}$
27B	18.4	$2.79 \cdot 10^{-12}$
27C	15.4	$4.98 \cdot 10^{-10}$
27D	14.1	$3.98 \cdot 10^{-9}$
28A (**28B**)	9.9	$5.03 \cdot 10^{-6}$
28C (**28D**)	11.1	$6.79 \cdot 10^{-7}$
29D	10.1	$3.90 \cdot 10^{-6}$
29D$_{(3)}$	13.2	$4.35 \cdot 10^{-8}$
29D$_{(3,5)}$	18.9	$2.65 \cdot 10^{-12}$
29C (**29B**)	13.6	$9.63 \cdot 10^{-9}$
29C$_{(3,5)}$	21.1	$2.59 \cdot 10^{-14}$
30	2.5	1.37
30$_{(3)}$	20.4	$1.09 \cdot 10^{-13}$
31A (**31B**)	9.0	$2.16 \cdot 10^{-5}$
31C (**31D**)	6.4	$1.98 \cdot 10^{-3}$
32A (**32D**)	5.4	$2.72 \cdot 10^{-3}$
32A$_{(2,4)}$ (**32D**$_{(2,4)}$) = **35A**$_{(2)}$	0.0	25.12
32A$_{(4)}$	1.9	1.09
32C (**32B**)	7.6	$6.75 \cdot 10^{-5}$
32C$_{(2,4)}$	0.8	6.66
33A	6.3	$6.27 \cdot 10^{-4}$
33A$_{(5)}$	4.0	0.03
33B	—[e]	—
33C	—[f]	—
33D	11.1	$1.84 \cdot 10^{-7}$
34A (**34B**)	16.1	$4.04 \cdot 10^{-11}$
34C (**34D**)	16.7	$1.35 \cdot 10^{-11}$
35A (**35D**)	1.9	1.09
35A$_{(2)}$ (**35D**$_{(2)}$)	0.0	25.12
35A$_{(5)}$ (**35D**$_{(5)}$)	5.4	$2.70 \cdot 10^{-3}$
35A$_{(2,5)}$ (**35D**$_{(2,5)}$)	12.9	$8.3 \cdot 10^{-8}$
35C (**35B**)	—[g]	—
35C$_{(2)}$ = **44C**$_{(3)}$	0.8	6.64
36A (**36D**)	9.7	$1.91 \cdot 10^{-6}$
40A (**40B**)	—[l]	—
40C (**40D**)	18.2	$1.18 \cdot 10^{-12}$
41A (**41B**)	17.8	$2.11 \cdot 10^{-12}$
41A$_{(2)}$ (**41B**$_{(2)}$)	4.6	0.01
41A$_{(2),(3)}$	5.1	$4.35 \cdot 10^{-3}$
41D (**41C**)	15.5	$1.09 \cdot 10^{-10}$
41D$_{(2)}$ (**41C**$_{(2)}$) = **50C**$_{(3)}$	3.5	0.07
41D$_{(2),(3)}$	9.7	$1.97 \cdot 10^{-6}$
42	25.4	$7.30 \cdot 10^{-17}$
43A	25.0	$1.15 \cdot 10^{-17}$
43C	26.7	$6.42 \cdot 10^{-19}$
44A	13.1	$6.02 \cdot 10^{-9}$
44A$_{(3)}$ = **35A**$_{(2)}$	0.0	25.12
44A$_{(3),(2)}$	1.9	1.09
44C	13.9	$1.56 \cdot 10^{-9}$
44C$_{(3)}$	0.8	6.64
45A	17.3	$5.09 \cdot 10^{-12}$
45A$_{(5)}$ = **48A** = **54D**$_{(4)}$	7.3	$1.18 \cdot 10^{-4}$
45C (**45B**)	19.5	$1.26 \cdot 10^{-13}$
45B$_{(5)}$ = **48C** = **54C**$_{(4)}$	8.5	$1.40 \cdot 10^{-5}$
46	30.1	$2.31 \cdot 10^{-21}$
47A	9.7	$1.85 \cdot 10^{-6}$
47A$_{(3)}$	3.6	0.06
47B	13.8	$1.91 \cdot 10^{-9}$
47B$_{(3)}$	7.0	$1.60 \cdot 10^{-4}$
47C	13.9	$1.60 \cdot 10^{-9}$
47C$_{(3)}$	2.7	0.21
47D	13.0	$8.09 \cdot 10^{-9}$
47D$_{(3)}$ = **38D**$_{(2)}$	2.6	0.30
48A (**48D**)	7.3	$1.18 \cdot 10^{-4}$
48C (**48B**)	8.5	$1.40 \cdot 10^{-5}$
49A	33.7	$4.94 \cdot 10^{-24}$
49C	33.2	$1.26 \cdot 10^{-23}$
50A	5.1	$4.35 \cdot 10^{-3}$
50A$_{(3)}$	4.6	0.01
50C	9.7	$1.97 \cdot 10^{-6}$
50D$_{(3)}$ = **41C**$_{(2)}$	3.5	0.07
51	10.0	$1.11 \cdot 10^{-6}$
52A	15.5	$1.11 \cdot 10^{-10}$
52C	18.5	$6.49 \cdot 10^{-13}$
53A	19.7	$8.53 \cdot 10^{-14}$
53A$_{(3)}$	18.3	$9.76 \cdot 10^{-13}$
53C	21.3	$6.41 \cdot 10^{-15}$
53C$_{(3)}$	19.7	$8.74 \cdot 10^{-14}$
54A (**54D**)	15.3	$1.47 \cdot 10^{-10}$
54A$_{(2)}$	17.0	$8.77 \cdot 10^{-12}$
54D$_{(4)}$ (**54A**$_{(4)}$)	7.3	$1.18 \cdot 10^{-4}$
54C	18.0	$1.69 \cdot 10^{-12}$
54C$_{(2)}$	16.7	$1.55 \cdot 10^{-11}$
55	29.6	$4.81 \cdot 10^{-21}$
56	10.9	$2.45 \cdot 10^{-7}$
56$_{(3)}$	10.5	$5.11 \cdot 10^{-7}$
57	8.8	$9.22 \cdot 10^{-6}$
57$_{(2)}$	9.8	$1.58 \cdot 10^{-6}$

(*to be continued*)

Table 3 (*continued*)

Compound	ΔG /kcal mol^{-1}	g (%)	Compound	ΔG /kcal mol^{-1}	g (%)
36C (36B)	—^{h}	—	**57**$_{(2,5)}$	10.4	$5.69 \cdot 10^{-7}$
37	14.2	$1.00 \cdot 10^{-9}$	**57**$_{(5)}$	10.0	$1.18 \cdot 10^{-6}$
38A	6.7	$3.02 \cdot 10^{-4}$	**58**	13.1	$5.86 \cdot 10^{-9}$
38A$_{(2)}$ = **47A**$_{(3)}$	3.6	0.06	**59A**	17.6	$3.27 \cdot 10^{-12}$
38B	—^{i}	—	**59A**$_{(3)}$	15.9	$5.48 \cdot 10^{-11}$
38C	—^{j}	—	**59C**	13.5	$3.48 \cdot 10^{-9}$
38C$_{(2)}$	2.7	0.25	**59C**$_{(2)}$	10.3	$7.59 \cdot 10^{-7}$
38D	4.1	0.03	**60A**	14.8	$3.51 \cdot 10^{-10}$
38D$_{(2)}$	2.6	0.30	**60A**$_{(2)}$	16.0	$4.52 \cdot 10^{-11}$
38D$_{(5)}$	5.0	$5.96 \cdot 10^{-3}$	**60C**	12.0	$4.05 \cdot 10^{-8}$
39A (39D)	9.4	$3.09 \cdot 10^{-6}$	**60C**$_{(2)}$	13.7	$2.47 \cdot 10^{-9}$
39C (39B)	—^{k}	—	**61**	9.7	$2.10 \cdot 10^{-6}$

^{a} **22B** → **22A**. ^{b} **23C(B)** → **23A(D)**. ^{c} **24B** → **24D**. ^{d} **24C** → **24D**. ^{e} **32B** → **32A**. ^{f} **32C** → **32D**. ^{g} **35C(B)** → **35A(D)**. ^{h} **36C(B)** → **36A(D)**. ^{i} **38B** → **38A**. ^{j} **38C** → **38D**. ^{k} **39C(B)** → **39A(D)**. ^{l} **40A(B)** → **40C(D)**.

with respect to internal rotation of the ONa group are those in which the second coordination bond is formed between the Na^+ cation and the oxygen atom of the adjacent 3β-OH or 2β-OH group, respectively. This is associated with the fact that disodium salts **25** and **26**, which are formed by replacement of the protons in the 1α- (in the tautomeric form **C**) and 2β-OH groups and in the 4α- (in the tautomeric form **C**) and 3β-OH groups, respectively, in the *ortho* positions with respect to each other by Na^+ cations, are nonplanar due to electrostatic interactions between the Na^+ cations. In these salts, the Na^+ cations deviate from the plane of the naphthazarin moiety by 16—24°, and the deformation of the naphthazarin moiety is characterized by angles of 0.5—4°.

Rotamers **25A**$_{(2)}$ and **26A**$_{(3)}$ of disodium salts **25** and **26** obtained by rotation of the ONa groups around the corresponding C(2)—O and C(3)—O bonds by ~180° have planar geometries and are the major isomers of these salts. The Gibbs free energies of these rotamers are higher than those of tautomers **25A** and **26A** by 4.2 and 6.0 kcal mol^{-1}, respectively (see Table 3). The barrier to the transition **25A** → **25A**$_{(2)}$ estimated by the B3LYP/6-31(d) method is $E^{\#}$ = 13.6 kcal mol^{-1}.

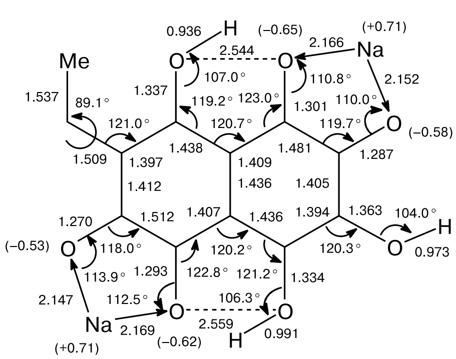


Fig. 7. Geometry of the main isomer **24D**$_{(3)}$ of disodium salt **24**. Atomic charges are given in parentheses.

Only one of the major isomers (**29D**) of disodium salt **29**, which is formed by replacement of the protons of the 5α- and 6β-OH groups in the *ortho* positions with respect to each other by Na^+ cations, is nonplanar due to electrostatic interactions between the Na^+ cations. Isomer **29D**$_{(3,5)}$ obtained by rotation of the ONa and 3β-OH groups around the corresponding C(5)—O and C(3)—O bonds by ~180° is planar. However, the Gibbs free energy of this isomer is ~8.8 kcal mol^{-1} lower than that of tautomer **29D** (see Table 3).

The percentages of the disodium salts were estimated using their statistical weights* based on the results of the screening of all 34 isomers according to Eq. (1). Under thermodynamic equilibrium, only the following three of the ten disodium salts under consideration are present in substantial amounts: **23** (2,5-$(ONa)_2$), 5.34%; **24** (2,6-$(ONa)_2$), 93.28%; and **30** (5,8-$(ONa)_2$), 1.37%. The total percentage of all other types of salts is at most 0.01%.

In the reactions of disodium salts **22—31** with the HOO$^{\bullet}$ radical, only the major isomers of both the salts involved in these reactions and the radicals generated from these salts were considered. The major isomers of radicals of disodium salts were found by screening 97 isomers of 30 radicals **32—61** (see Table 3). The screening revealed six structurally different main radicals: **32A**$_{(2,4)}$, **38D**$_{(2)}$, **41D**$_{(2)}$, **54D**$_{(4)}$, **57**, and **61**.

* For disodium salts of compound **1**, the statistical weights were estimated by calculating the relative Gibbs free energies of the isomers as $\Delta G_{\mathbf{X}i} = G_{\mathbf{X}i} - G(\mathbf{24D}_{(3)})$, where $G(\mathbf{24D}_{(3)})$ is the Gibbs free energy of the main isomer **24D**$_{(3)}$ of disodium salt **24**.

This is attributed to the fact that radicals $\mathbf{35A}_{(2)}$ and $\mathbf{44A}_{(3)}$ generated in the reactions of salts **23** and **26**, respectively, with the HOO$^{\bullet}$ radical are identical to isomeric form $\mathbf{32A}_{(2,4)}$ of radical **32**, whereas radicals $\mathbf{47D}_{(3)}$ and $\mathbf{50D}_{(3)}$ generated in the reactions of salts **24** and **25** with the HOO$^{\bullet}$ radical are identical to radicals $\mathbf{38D}_{(2)}$ and $\mathbf{41D}_{(2)}$, respectively. The factors responsible for this structural identity were considered above by an example of the reactions of dianions of compound **1** with the HOO$^{\bullet}$ radical.

Radical $\mathbf{32A}_{(2,4)}$ is the main species among the major radicals of disodium salts. Its Gibbs free energy is 2.6—9.7 kcal mol^{-1} higher than those of the other major radicals of disodium salts. The geometry and the positive spin populations on the atoms of the main isomer $\mathbf{32A}_{(2,4)}$ are given in Fig. 8.

In radical $\mathbf{32A}_{(2,4)}$, the Na—O(4) and Na—O(5) bond lengths in the six-membered ring involving the Na$^+$ cation are equal within ±0.002 Å, whereas the Na—O(2) and Na—O(3) bond lengths in the five-membered ring (as in molecule $\mathbf{24D}_{(3)}$, see Fig. 7) differ by ~0.01 Å.

The statistical weights* of radicals of disodium salts were evaluated by Eq. (1) taking into account all 97 isomers. The estimation showed that under thermodynamic equilibrium only five of all possible radicals of disodium salts are formed in substantial amounts: **32** (2,3-$(ONa)_2$-5-O$^{\bullet}$), 32.87%; **35** (2,5-$(ONa)_2$-3-O$^{\bullet}$), 32.87%; **44** (3,5-$(ONa)_2$-2-O$^{\bullet}$), 32.87%; **38** (2,6-$(ONa)_2$-3-O$^{\bullet}$), 0.55%; **47** (3,6-$(ONa)_2$-2-O$^{\bullet}$), 0.55%. The above-mentioned percentage of each of the five types of radicals includes the total percentage of all possible tautomeric-rotameric forms of the particular radical.

Salts **25**, **26**, and **29**, in which the ONa groups are in the *ortho* positions with respect to each other, as well as radicals **41**, **44**, and **54** generated from these salts by O—H bond homolysis are nonplanar. In these radicals, the Na$^+$ cations deviate from the plane of the naphthazarin moiety by 14—26°. Rotational isomers $\mathbf{41D}_{(2)}$, $\mathbf{44A}_{(3)}$, and $\mathbf{54D}_{(5)}$ are planar and are the major isomers of radicals **41**, **44**, and **54** because their Gibbs free energies are 12.0, 13.1, and 8.1 kcal mol^{-1} higher than those of isomers **41D**, **44A**, and **54D**, respectively.

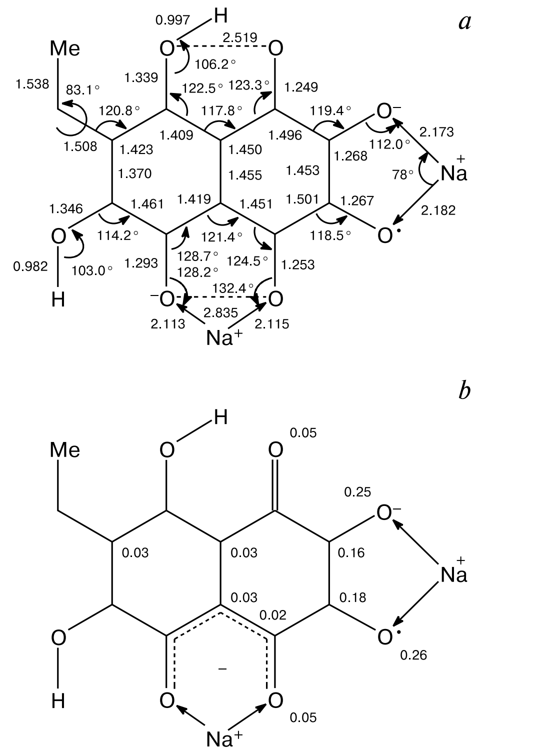
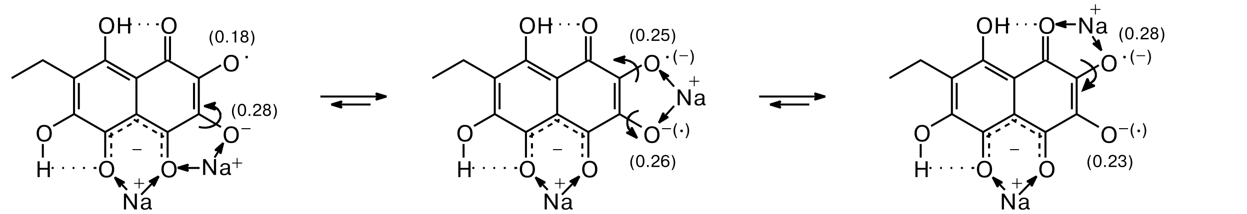


Fig. 8. Geometry (*a*) and the spin populations on the atoms (*b*) of the main isomer $\mathbf{32A}_{(2,4)}$ of radical of disodium salt **22**.

Only isomers of radicals of disodium salts of compound **1**, which are formed as a result of the single rotation of the ONa group around the corresponding C—O bond, are considered above. At the same time, successive rotations of the ONa group around the corresponding C—O bonds can occur in radicals **35**, **41**, and **44** without steric hindrance. Scheme 5 exemplifies rotation of the ONa group around the C(3)—O bond followed by rotation

* For radicals of disodium salts of compound **1**, the statistical weights were estimated by calculating the relative Gibbs free energies of the isomers as $\Delta G_{\mathbf{X}_i} = G_{\mathbf{X}_i} - G(\mathbf{32A}_{(2,4)})$, where $G(\mathbf{32A}_{(2,4)})$ is the Gibbs free energy of the main isomer $\mathbf{32A}_{(2,4)}$ ($\mathbf{35A}_{(2)} = \mathbf{44A}_{(3)}$) of the radical of disodium salt **32**.

Scheme 5



$\mathbf{44A}(\mathbf{44D}) = \mathbf{32A}_{(2,4),(3)}(\mathbf{32D}_{(2,4),(3)}) = \mathbf{35A}_{(2),(3)}(\mathbf{35D}_{(2),(3)})$ (ΔG = 13.1 kcal mol^{-1})

$\mathbf{44A}_{(3)}(\mathbf{44D}_{(3)}) = \mathbf{32A}_{(2,4)}(\mathbf{32D}_{(2,4)}) = \mathbf{35A}_{(2)}(\mathbf{35D}_{(2)})$ (ΔG = 0.0 kcal mol^{-1})

$\mathbf{44A}_{(3),(2)}(\mathbf{44D}_{(3),(2)}) = \mathbf{32A}_{(4)}(\mathbf{32D}_{(4)}) = \mathbf{35A}(\mathbf{35D})$ (ΔG = 1.9 kcal mol^{-1})

around the C(2)—O bond in radical **44** resulting in the migration of the Na^+ cation from the O(4) and O(3) atoms in isomer **44A** (**44D**) to the O(2) and O(1) atoms in isomer $\mathbf{44A}_{(3),(2)}(\mathbf{D}_{(3),(2)})$.* The barrier $E^{\#}$ to the first isomeric transition **44A** → $\mathbf{44A}_{(3)}$ evaluated at the UB3LYP/6-31G(d) level of theory is ~5.2 kcal mol^{-1}, and the barrier to the second isomeric transition $\mathbf{44A}_{(3)}$ → $\mathbf{44A}_{(3),(2)}$ is ~18.5 kcal mol^{-1}.

Reactions (15)—(24) afford only six of ten structurally different major isomeric forms of radicals of disodium salts of compound **1** (Schemes 6 and 7). The D_{OH} values for the O—H bonds in α- and β-OH groups of disodium salts, the enthalpies of the reactions (15)—(24) (ΔH_r), the Gibbs free energies (ΔG_r), and the equilibrium constants (K) were estimated as described above for dianions of compound **1** (see the reactions (12) and (13) and Eq. (14)).

The calculated values are given in Table 4. In the structural formulas (see Schemes 6 and 7), the charges on the oxygen atoms of the carbonyl groups and the OH groups, which underwent the O—H bond heterolysis or homolysis, are given in parentheses. For radicals, shown are only the spin populations on the atoms, which are equal to or higher than 0.01. The percentages of isomers of disodium salts and their radicals are given in parentheses near their symbols.

A distinctive feature of the reactions of the $HOO^{\bullet}$ radical with disodium salts of echinochrome A compared to the reactions of dianions is that the reaction (17) of isomer $\mathbf{24D}_{(3)}$, which is the thermodynamically most favorable species of the ten major isomers of disodium salts, gives radical $\mathbf{38D}_{(2)}$ ($\mathbf{47D}_{(3)}$) thermodynamically less favorable than the main radical $\mathbf{32A}_{(2,4)}$ ($\mathbf{35A}_{(2)}$ = $\mathbf{44A}_{(3)}$) (see Schemes 6 and 7).

In the reactions with the $HOO^{\bullet}$ radical, disodium salts of compound **1** are less active antioxidants than dianions. The D_{OH} values for the O—H bonds in α- and β-OH groups of dianions of compound **1** are in the range 31.9—56.9 kcal mol^{-1}, whereas the corresponding values for disodium salts are, as a rule, larger (46.6—66.6 kcal mol^{-1}). The enthalpies of the reactions (15)—(24) vary from −1.3 to −20.6 kcal mol^{-1}, whereas the enthalpies of reactions (2)—(11) vary from −11.2 to −36.1 kcal mol^{-1} (see Tables 2 and 4). The ΔH_r value for reaction (17) (−4.84 kcal mol^{-1}) is 2.3 times smaller than that for reaction (4).

A comparison of the ΔH_r values for the reactions of the $HOO^{\bullet}$ radical with the main isomer $\mathbf{24D}_{(3)}$ and the main isomer of the monosodium salt (the monosodium salt of compound **1** at position 5)[1] shows that the disodium salt is the more effective antioxidant in the reaction with the $HOO^{\bullet}$ radical than the monosodium salt, because ΔH_r is almost five times larger.

Isomer $\mathbf{4C}_{(3)}$ of dianion **4** generated by heterolysis of the 2β- and 6β-OH groups is the main isomer of the possible isomeric forms of dianions of echinochrome A. The percentage of $\mathbf{4C}_{(3)}$ is 94.7%; the total percentage of all other isomers of dianion **4** is 5.1%.

The reactions of the $HOO^{\bullet}$ radical with dianions **2—11** are exothermic. Isomer $\mathbf{2C}_{(4)}$ should exhibit the strongest antioxidant properties of all the dianions, although it percentage is very low. The enthalpy of the reaction of isomer $\mathbf{2C}_{(4)}$ with the $HOO^{\bullet}$ radical is −36.6 kcal mol^{-1} and the equilibrium constant is $1.32 \cdot 10^{27}$.

Under thermodynamic equilibrium, salt **24** generated by replacement of the protons of the β-OH groups at the C(2) and C(6) atoms by Na^+ cations is the energetically most favorable of all possible disodium salts of echinochrome A in the gas phase. The percentage of salt **24** in the mixture of all disodium salts of echinochrome A is ~93.3%. Isomer $\mathbf{24D}_{(3)}$ is the main isomer of salt **24**. The tautomeric form of this isomer is retained for radical **38**.

The reactions of salts **22—31** with the $HOO^{\bullet}$ radical are exothermic. Reactions (15), (16), and (18)—(22) are characterized by $\Delta H_r \geq 10$ kcal mol^{-1}.

The main isomer $\mathbf{4C}_{(3)}$ is the more effective antioxidant in the reaction with the $HOO^{\bullet}$ radical than the main isomer $\mathbf{24D}_{(3)}$. The values $-\Delta H_r$ = 11.2 kcal mol^{-1} and $-\Delta G_r$ = 12.1 kcal mol^{-1} for reaction (4) are more than twice as large as the corresponding values (4.8 and 5.6 kcal mol^{-1}) for reaction (17). This also holds for monoanions and, all the more, for monosodium salts of compound **1**.

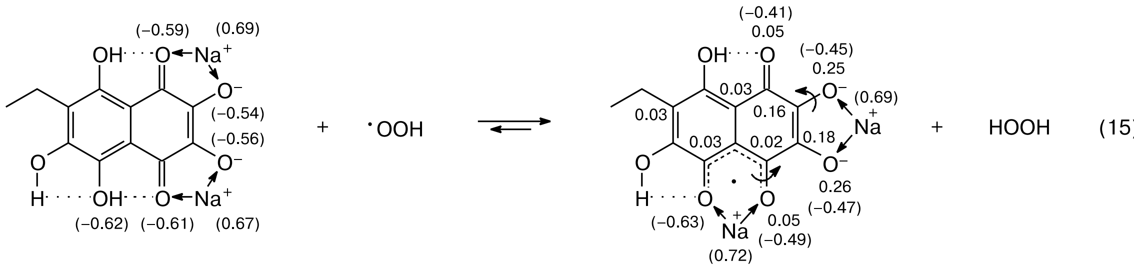
The present study shows that the Na^+ cation in radicals **35**, **41**, and **44** can migrate from one pair of oxygen

Table 4. Homolytic dissociation energies of the O—H bonds (D_{OH}) in the main isomers of disodium salts of echinochrome A, the heats of reactions (ΔH_r), the Gibbs free energies (ΔG_r), and the equilibrium constants (K) of the reactions of the hydroperoxyl radical with the major isomers of disodium salts of echinochrome A calculated by the (U)B3LYP/6-31G(d) method

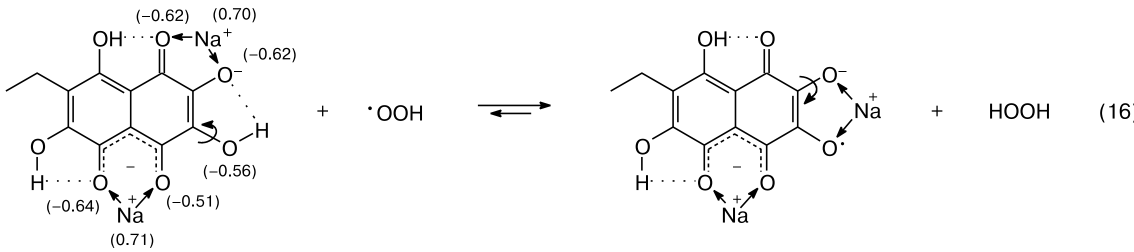
Reaction	D_{OH}	$-\Delta H_r$	$-\Delta G_r$	K
	kcal mol^{-1}			
22A → $\mathbf{32A}_{(2,4)}$ (15)	46.6	20.6	22.3	$1.32 \cdot 10^{15}$
$\mathbf{23A}_{(3)}$ → $\mathbf{35A}_{(2)}$ (16)	58.6	9.5	10.4	$3.93 \cdot 10^{7}$
$\mathbf{24D}_{(3)}$ → $\mathbf{38D}_{(2)}$ (17)	63.4	4.8	5.6	$11.70 \cdot 10^{3}$
$\mathbf{25A}_{(2)}$ → $\mathbf{41D}_{(2)}$ (18)	53.5	14.9	15.5	$2.22 \cdot 10^{11}$
$\mathbf{26A}_{(3)}$ → $\mathbf{44A}_{(3)}$ (19)	54.2	14.0	14.8	$6.48 \cdot 10^{10}$
27A → $\mathbf{47D}_{(3)}$ (20)	51.2	17.6	17.7	$9.91 \cdot 10^{12}$
28A → $\mathbf{50D}_{(3)}$ (21)	54.3	13.9	14.7	$5.48 \cdot 10^{10}$
29D → $\mathbf{54D}_{(5)}$ (22)	57.5	11.1	11.5	$2.47 \cdot 10^{8}$
30 → **57** (23)	66.6	1.3	2.4	$5.65 \cdot 10^{3}$
31C → **61** (24)	63.1	3.8	5.9	$2.01 \cdot 10^{4}$

* The double rotation of a ONa group around two different C—O bonds in radicals of disodium salts is denoted by subscripts, each being enclo sed in individual parentheses. In rotamers of radicals of disodium salts, the rotations of different ONa groups around different C—O bonds are denoted by subscripts enclosed in common parentheses.

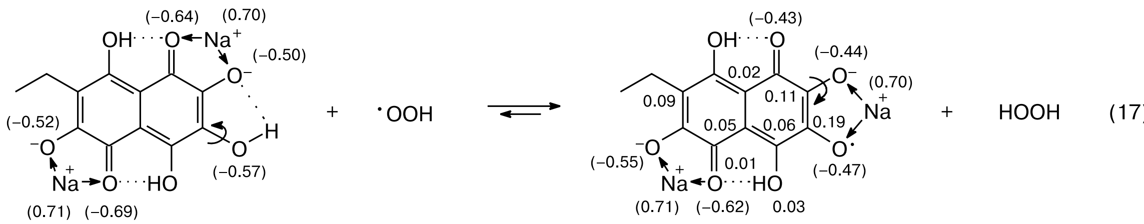
Scheme 6



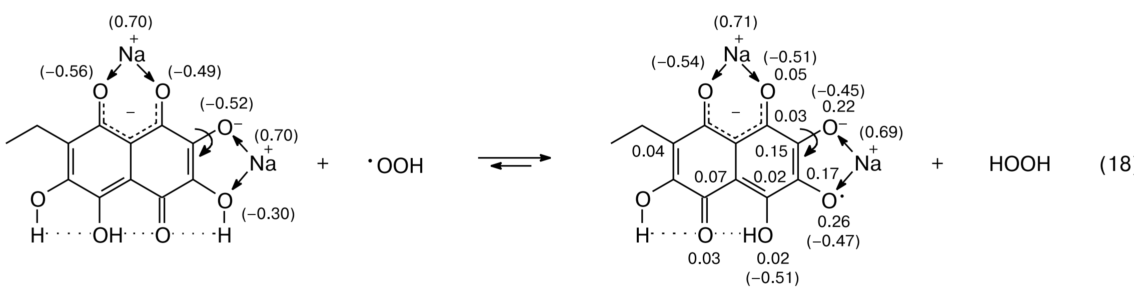
22A ($1.75 \cdot 10^{-8}$%) **32A**$_{(2,4)}$ (**32D**$_{(2,4)}$) = **35A**$_{(2)}$ (**D**$_{(2)}$) = **44A**$_{(3)}$ (**44D**$_{(3)}$) (25.12%)



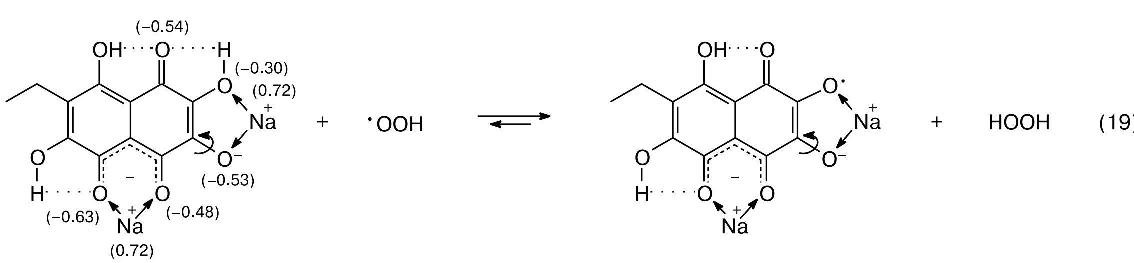
23A$_{(3)}$ (**23D**$_{(3)}$) (3.70%) **35A**$_{(2)}$ (**35D**$_{(2)}$) (25.12%)



24D$_{(3)}$ (90.64%) **38D**$_{(2)}$ = **47D**$_{(3)}$ (0.30%)

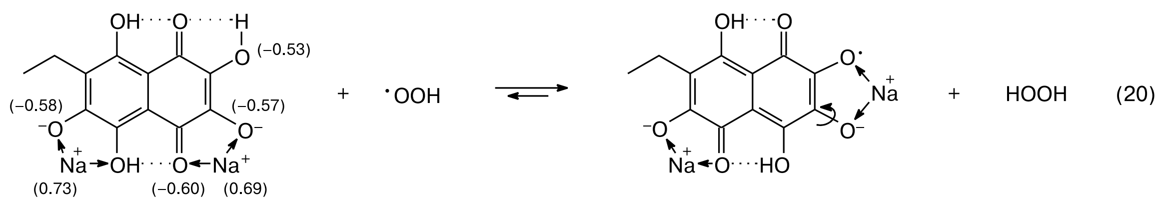


25A$_{(2)}$ (**25B**$_{(2)}$) ($2.01 \cdot 10^{-7}$%) **41D**$_{(2)}$ (**41C**$_{(2)}$) = **50D**$_{(3)}$ (**50C**$_{(3)}$) (0.07%)

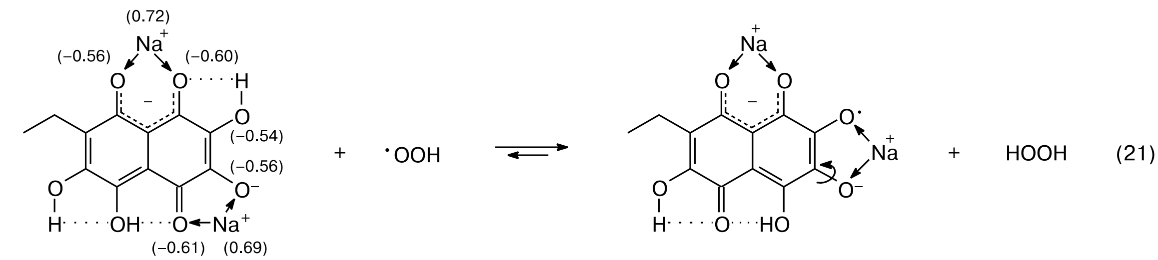


26A$_{(3)}$ (**26D**$_{(3)}$) ($5.62 \cdot 10^{-4}$%) **44A**$_{(3)}$ (**44D**$_{(3)}$) (25.12%)

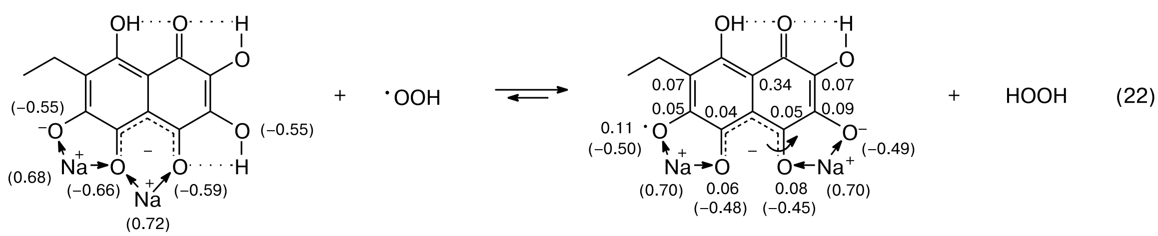
Scheme 7



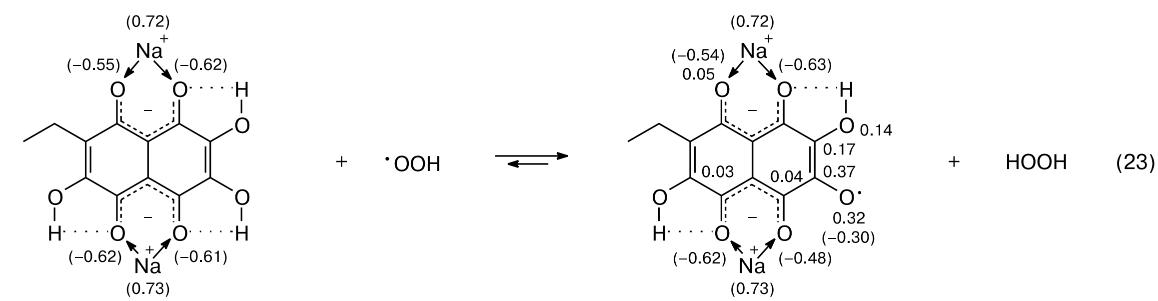
27A ($3.34 \cdot 10^{-8}$%) **47D**$_{(3)}$ (0.30%)



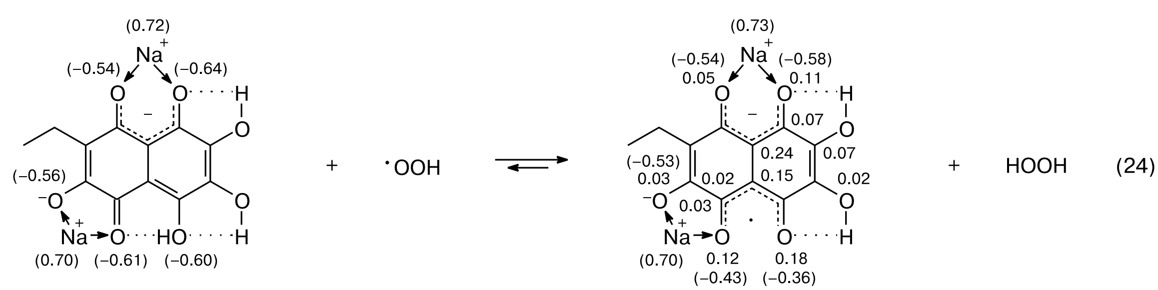
28A (**28B**) ($5.03 \cdot 10^{-6}$%) **50D**$_{(3)}$ (**50C**$_{(3)}$) (0.07%)



29D (**29A**) ($3.90 \cdot 10^{-6}$%) **45A**$_{(5)}$ = **48A** = **54D**$_{(4)}$ (**54C**$_{(4)}$) ($1.18 \cdot 10^{-4}$%)



30 (1.37%) **57** ($1.58 \cdot 10^{-6}$%)



31C (**31D**) ($1.98 \cdot 10^{-3}$%) **61** ($2.10 \cdot 10^{-6}$%)

atoms (for example, from the O(4) and O(3) atoms in isomer **44A**) to another pair (to the O(2) and O(1) atoms in isomer $\mathbf{44A}_{(3),(2)}$) through successive rotations of the ONa group around the corresponding C—O bonds.

The estimations of the antioxidant properties of disodium salts and dianions and the investigation of the antioxidant properties of monosodium salts[1] and monoanions[2] of echinochrome A, as well as of neutral echinochrome A,[2] indicate that isomer $\mathbf{4C}_{(3)}$ is the most effective antioxidant with respect to the HOO$^\bullet$ radical.

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