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Antioxidant Properties of Conjugates of 20-Hydroxyecdysone Derivatives with a Polysubstituted Chromanylaldehyde

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Abstract—The antioxidant properties of 20-hydroxyecdysone and its conjugates with (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)acetaldehyde are reported for initiated 1,4-dioxane oxidation as a model reaction. The antioxidant activity of these compounds is characterized in terms of the effective rate constant of inhibition, fk_{In}.

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Ecdysteroids constitute the widest and most frequently encountered class of steroids and execute a wide variety of biological functions [1–9]. Due to their availability and low toxicity, they are used in the synthesis of medicines for treatment of cardiovascular diseases, hepatitis, and disorders caused by the intensification of cell membrane lipid peroxidation [10–15]. 20-Hydroxyecdysone exerts a pronounced therapeutic effect at concentrations as low as 10^{-8} mol/l, thus demonstrating its high biological activity [7, 8].

20-Hydroxyecdysone and its derivatives possess obvious antiradical and antioxidant properties. Their antioxidant effect on blood serum lipid oxidation is comparable with the effects of hydroquinone and butylhydroxytoluene [11–17]. However, the data characterizing the antioxidant activity (AOA) of 20-hydroxyecdysone and its structural analogues are semiquantitative, so it is impossible to predict the effectiveness of these antioxidants under variable conditions. The parameters used to characterize the AOA of ecdysteroids include the yield of propanedial (final oxidation product), the amount of diene conjugates, the concentration that reduces the rate of oxidation of the model substrate by 50%, and the 2,2-diphenyl-1-picrylhydrazyl radical (**DPPH•**) accepting efficiency [13–19]. However, the most objective measure of AOA is the inhibition rate constant determined by kinetic methods.

The AOA of 20-hydroxyecdysone (**I**) can be enhanced by using it in the form of conjugates (**II**, **III**) with the α-tocopherol analogue (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)acetaldehyde (**IV**). Here, we report a quantitative study of the antioxidant properties of compounds **I**–**III**.

EXPERIMENTAL

The structural formulas of the compounds examined are presented in Scheme 1.

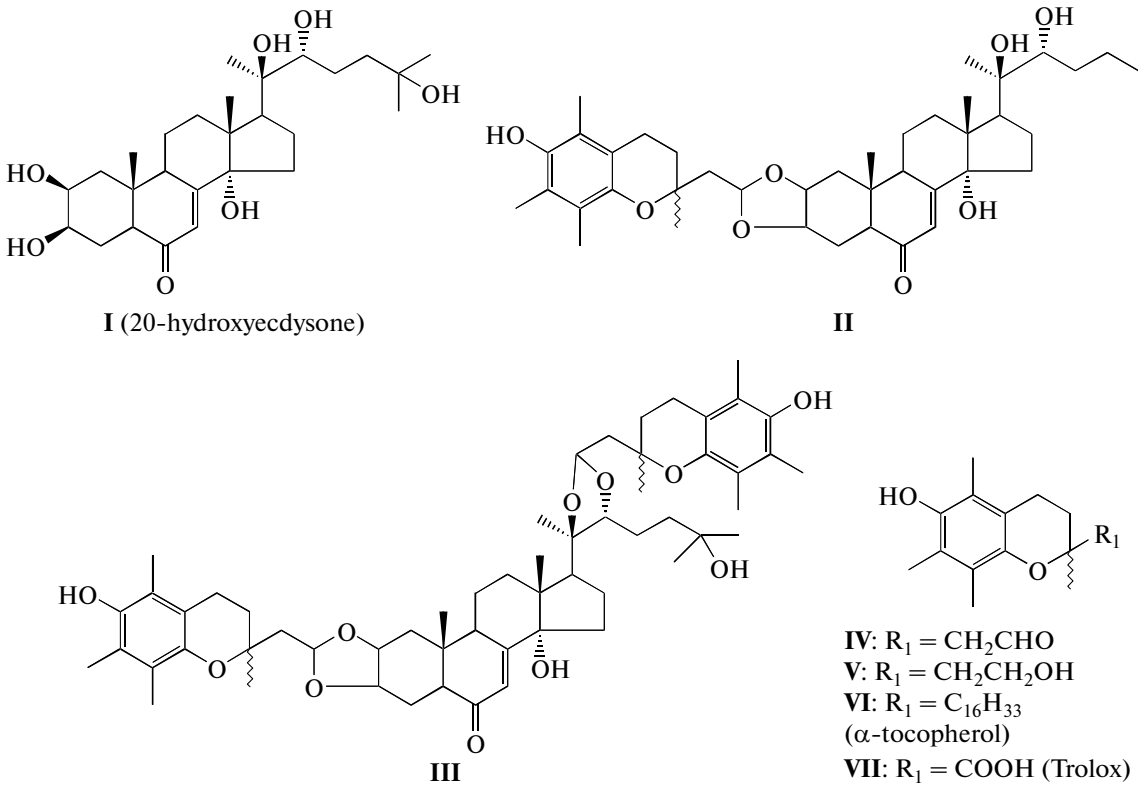
Conjugates **II**–**III** were characterized by melting point and optical rotation angle measurements and ^{1}H and ^{13}C NMR spectroscopy.

Compound II. Mp 138–140°C; $[\alpha]_D^{20} = +19.3°$ (c = 1.77, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.80 s, 0.97 s, 1.18 s, 1.24 s, 1.34 s, 1.36 s, 1.43 s, 2.11 s, 2.17 s, 2.25 s, 2.61 s, 3.65 m, 4.13 m, 4.17 m, 4.69 s, 5.22 br s ($w_{1/2}$ = 16.4 Hz), 5.82 s, 7.35–7.50 m.

Compound III. Mp 128–130°C; $[\alpha]_D^{20} = +20.7°$ (c = 2.03, $CHCl_3$)); ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.90 s, 1.00 s, 1.17 s, 1.27 s, 1.28 s, 1.37 s, 2.11 s, 2.18 s, 2.24 s, 2.63 m, 3.62 m, 4.10 m, 4.14 m, 4.71 s, 5.18 br s ($w_{1/2}$ = 13.0 Hz), 5.24 br s ($w_{1/2}$ = 13.0 Hz), 5.85 s, 7.35–7.50 m.

The AOA of 20-hydroxyecdysone (**I**) and its derivatives **II** and **III** was studied for the initiated oxidation of 1,4-dioxane as a model reaction at 348 K. 1,4-Dioxane was purified by a standard procedure [19]. The initiator was azobisisobutyronitrile (AIBN). 1,4-Dioxane solutions of the initiator and the compounds examined were placed in a temperature-controlled glass reactor. Oxygen uptake kinetics was recorded using a multipurpose differential manometric setup described in detail by Emanuel and Gal [18]. The inhibiting effect of 20-hydroxyecdysone and its derivatives was estimated from oxygen uptake rate data at the initial stage of the process.

The initiation rate was calculated as $w_i = k_i$[AIBN], where k_i is the initiation rate constant. We assumed that $k_i = 2ek_d$, where k_d is the AIBN decomposition



Scheme 1. Structural formulas of compounds **I**–**VII**.

rate constant and e is the probability of a radical escaping into the bulk. In these calculations, we used the k_d value measured in cyclohexanol,

$$\log k_d = 17.70 - 35/(4.575T \times 10^{-3}), \quad (1)$$

and $e = 0.5$ [20–23].

The AOA of the compounds was characterized by the effective inhibition rate constant fk_{In}, where f is the radical capacity of the antioxidant (AO), defined as the number of radicals decaying on one AO molecule in the chain termination events [13]. Induction periods were calculated as was suggested by Tsepalov et al. [23].

For comparison, the antioxidant properties of (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)ethanol (**V**), α-tocopherol (**VI**), and the water-soluble α-tocopherol derivative Trolox (**VII**) were studied under the same conditions.

RESULTS AND DISCUSSION

All of the compounds exert a pronounced inhibiting effect on the initiated oxidation of 1,4-dioxane. Typical oxygen uptake curves obtained in the presence of compounds **I**, **II**, **V**, **VI**, and **VII** are plotted in Fig. 1.

From the initial portions of the oxygen uptake curves, we derived the oxidation rate of the model substrate (w_0) in the presence of each particular antioxidant. The dependences of w_0 on the concentrations of compounds **II**, **III**, and **VII** introduced into 1,4-dioxane being oxidized are shown in Fig. 2.

For determining the effective inhibition rate constant fk_{In}, experimental data were fitted to Eqs. (2) and (3). Equation (2) was used in the processing of only those experimental data which were obtained under conditions of the chain oxidation of 1,4-dioxane [20, 23]:

$$F = \frac{w_0^0}{w_0} - \frac{w_0}{w_0^0} = fk_{In}[AO]/\sqrt{2k_6 w_i}, \quad (2)$$

$$\Delta O_2 = \frac{k_2}{fk_{In}}[RH]\ln\left(1 - \frac{t}{\tau}\right), \quad (3)$$

where w_0^0 and w_0 are the initial oxygen uptake rates in 1,4-dioxane oxidation in the absence and presence of AO, respectively; [AO] is the AO concentration; k_{In} and $2k_6$ are the rate constant of chain termination on AO and the rate constant of quadratic-law chain termination on the peroxyl radicals of the substrate, respectively [20–23]; ΔO_2 is the amount of oxygen absorbed in time t (see Fig. 1); τ is the induction period

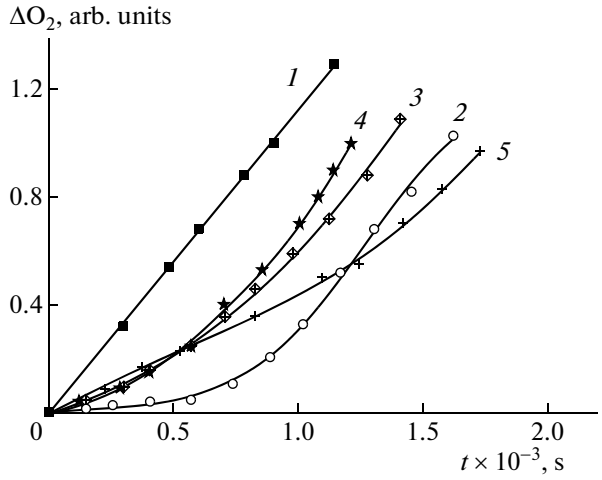


Fig. 1. Oxygen uptake kinetics for 1,4-dioxane oxidation (*1*) in the absence and (*2–5*) in the presence of α-tocopherol analogues: (*2*) [**VII**] = 4.4×10^{-5} mol/l, (*3*) [**V**] = 4.0×10^{-5} mol/l, (*4*) [**II**] = 4.0×10^{-5} mol/l, and (*5*) [**I**] = 1.2×10^{-3} mol/l. The initiation rate is $w_i = 1 \times 10^{-7}$ mol l^{-1} s^{-1}; $T = 348$ K.

in oxygen absorption; [RH] is the 1,4-dioxane concentration ([RH] = 11.75 mol/l); and k_2 is the rate constant of chain propagation in the oxidation of the model substrate ($k_2 = 7.9$ l mol^{-1} s^{-1} at 348 K [20]).

By way of example, we show the fit between Eq. (3) and the experimental data for compounds **II**, **III**, **V**, and **VI** (Fig. 3).

Equations (2) and (3) are obeyed satisfactorily for all of the compounds examined (with a correlation factor of >0.96). The dependences of the inhibition parameter F calculated via Eq. (2) on the concentrations of the antioxidants are plotted in Fig. 3. From the slopes of these lines, we derived fk_{In} values. In these calculations, we used the value of the quadratic-law chain termination rate constant known from the literature, $2k_6 = 6.67 \times 10^7$ l mol^{-1} s^{-1} [20], which is practically independent of temperature. The values of the effective rate constants of inhibition are listed in the table. For comparison, we present, in the same table, tocopherol equivalents (TE) calculated as

$$TE = fk_{In}/fk_{\alpha\text{-tocopherol}}. \quad (4)$$

As is clear from the table, the fk_{In} values calculated using formulas (2) and (3) are fairly similar.

The inhibition rate curves for 1,4-dioxane oxidation flatten out as the oxidant concentration is increased (Fig. 2). For compound **I**, this takes place at [AO] > 9.0×10^{-4} mol/l; for compounds **II**, **III**, and **VI**, at [AO] = 1.0×10^{-4} mol/l; for **V**, at [AO] = 2.9×10^{-4} mol/l; for **VII**, at [AO] = 4.5×10^{-5} mol/l. The linear-law disappearance of 1,4-dioxane peroxyl radicals on inhibitor molecules is likely dominant under these conditions.

The experimental rates of inhibited substrate oxidation under these limiting conditions in the presence of compounds **I**–**III** and **V**–**VII** were fitted to the following equation [20–22]:

$$fw_i = w_0^{limit} \quad (5)$$

from which the radical capacity of the inhibitors was estimated at $f \approx 2$.

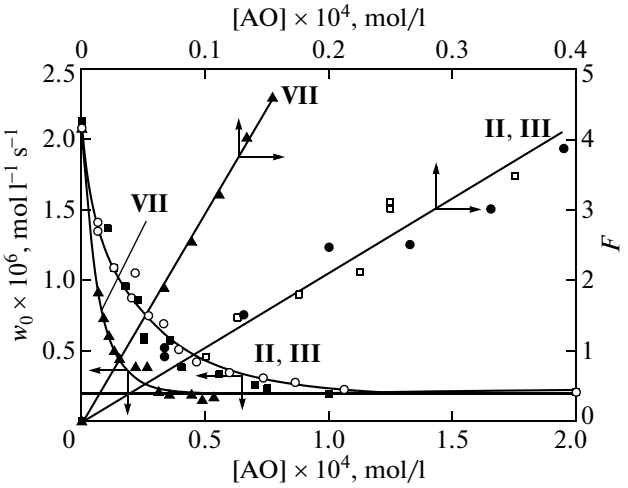


Fig. 2. Initial 1,4-dioxane oxidation rate and the inhibition parameter F as a function of the concentrations of **II**, **III**, and **VII** at 348 K. The initiation rate is $w_i = 1 \times 10^{-7}$ mol l^{-1} s^{-1}.

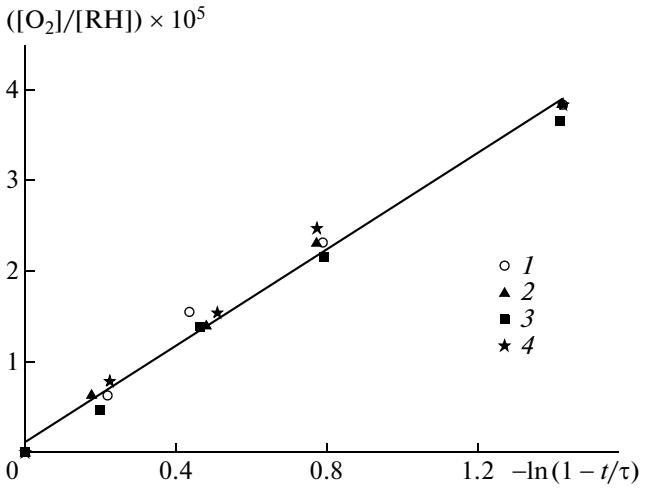


Fig. 3. Anamorphoses of oxygen uptake curves in the coordinates of Eq. (3) for inhibited 1,4-dioxane oxidation in the presence of **II**, **III**, **V**, and **VI**: (*1*) [**VI**] = 5.1×10^{-5} mol/l, (*2*) [**II**] = 4.0×10^{-5} mol/l, (*3*) [**III**] = 4.65×10^{-5} mol/l, and (*4*) [**V**] = 4.0×10^{-5} mol/l.

AOA data for α-tocopherol and its analogues at 348 K

Compound	$fk_{In} \times 10^{-5}$, l mol^{-1} s^{-1}		f	$k_{In} \times 10^{-5}$, l mol^{-1} s^{-1}	TE
	calculated using formula (2) [20]	calculated using formula (3) [23]			
I	0.08 ± 0.01	–	–	0.04 ± 0.01	0.03
II	3.2 ± 0.3	3.5 ± 0.4	2	1.60 ± 0.15	1.30
III	2.2 ± 0.3	3.6 ± 0.4	2	1.10 ± 0.15	0.90
V	0.8 ± 0.1	3.0 ± 0.3	2	0.40 ± 0.05	0.32
VI (α-tocopherol)	2.5 ± 0.4	2.6 ± 0.3	2	1.3 ± 0.2	1.00
VII	8.0 ± 1.0	10.0 ± 1.0	2	4.0 ± 0.5	3.20

The same result is obtained by fitting experimental data to the equation

$$\tau = \frac{f[AO]^a}{w_i}. \quad (6)$$

In the AO concentration range examined, the exponent a is 0.5 and the τ versus [AO] curves tend to have a limit (Fig. 4). For compounds **I** and **VII**, this curve passes through a maximum. At the same time, in the [AO] = (0.1–0.6) × 10^{-5} mol/l range this function is linear ($a = 1$) and the dependence of τ on the antioxidant concentration is described by the classical equation [20]. At AO concentrations above (0.6–1.0) × 10^{-4} mol/l, a significant role is likely played by the transfer of the oxidation chain to the inhibitor [20–23]:

$$In^{\bullet} + RH \xrightarrow{k_{10}} InH + R^{\bullet}.$$

The results of this study suggest that the 2,3-monoconjugate (**II**) and 2,3:20,22-bisconjugate (**III**) between 20-hydroxyecdysone (**I**) and (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)acetaldehyde (**IV**), which a have a chromanyl moiety in their structure, are similar in AOA to α-tocopherol (**VI**) (table). The AOA of 20-hydroxyecdysone (**I**) itself is 30 times lower than the AOA of **VI**. Thus, with the structures of inhibitors **II** and **III** taken into account, it can be deduced that the mechanism of their antioxidant action is similar to that of α-tocopherol. It can also be assumed

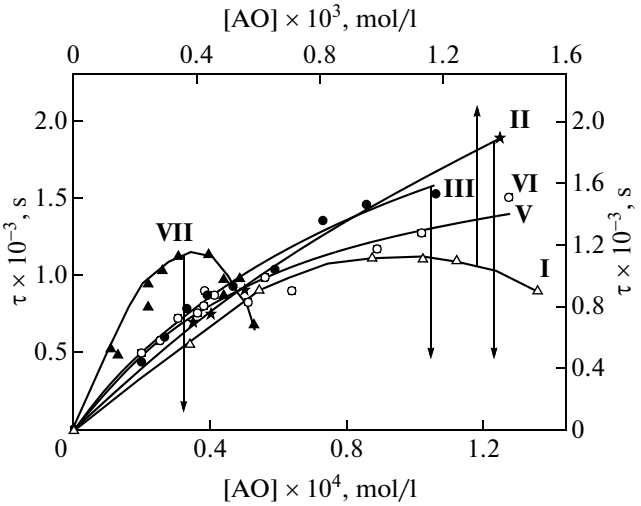
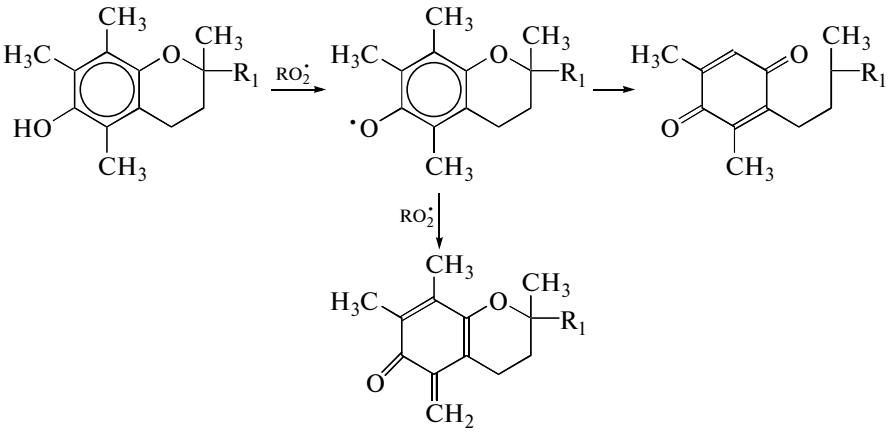


Fig. 4. Induction period as a function of the AO concentration.



Scheme 2. Intercation of 1,4-dioxane peroxyl radicals with α-tocopherol and its analogues.

that the two chromanyl fragments in **III** work independently. The likely mechanism of the interaction of 1,4-dioxane peroxyl radicals with antioxidant molecules is presented in Scheme 2.

CONCLUSIONS

The antioxidant properties of conjugates of 20-hydroxyecdysone with a polysubstituted chromanylaldehyde were studied for initiated 1,4-dioxane oxidation as a model reaction and were compared with those of α-tocopherol.

The effective rate constants of the inhibition of initiated 1,4-dioxane oxidation at 348 K for 20-hydroxyecdysone (**I**), its conjugates **II** and **III**, α-tocopherol (**VI**), and its analogues **V** and **VII** are $k_{In} \times 10^{-5} = 0.04$, 1.6, 1.1, 0.4, 1.3, and 4.0 l mol^{-1} s^{-1}, respectively.

The AOA of the tocopherol derivatives is mainly due to the inhibiting effect of the chromanyl moiety.

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