QUANTITATIVE STUDY OF ANTIOXIDANT PROPERTIES
OF PHENOLCARBOXYLIC ACIDS FROM *Larix sibirica* BARK

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Antioxidant properties of caffeic, ferulic, vanillic, and 4-hydroxybenzoic acids isolated from Larix sibirica *Ledeb. bark were studied using model radical-chain reactions for oxidation of propan-2-ol and 1,4-dioxane under kinetic control. Quantitative characteristics of the antioxidant activities were determined as effective rate constants fk_{In} for oxidation chain termination of the phenolcarboxylic acids. It was found that caffeic acid had the highest antioxidant activity among the investigated natural compounds.*
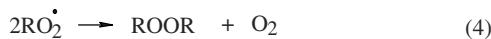
Key words: phenolcarboxylic acids, antioxidants, radical-chain reactions, initiated oxidation, inhibition rate constant.

Phenolcarboxylic acids possess a broad spectrum of biological activity and are promising sources for fabricating phytopreparations and parapharmaceuticals [1]. Adding these compounds to technical and food products and medical preparations can increase their shelf-life [2-5]. It has been proved that representatives of this class of compounds must be applied for nonspecific therapy of many diseases connected with advanced peroxide lipid oxidation [1, 6]. Thus, cinnamic and ferulic acids are found in agents intended to decrease the toxic effects of chemotherapy [5]. *p*-Coumaric, ferulic, and caffeic acids exhhibit antibacterial activity. Derivatives of cinnamic acid are widely used to fabricate photo-protective additives for cosmetics [2, 7]. Addition of ferulic acid to food products protects an organism from the toxic action of nitroso compounds in them [2]. Caffeic and ferulic acids decrease the oxidation rate of lard, isopropylbenzene, and ethylbenzene [8]. However, primarily semi-quantitative data on the antioxidant action of phenolcarboxylic acids have been reported. This does not enable an objective conclusion about their antioxidant properties to be made. Therefore, the goal of our work was to study quantitatively the antioxidant properties of caffeic (CA), ferulic (FA), vanillic (VA), and 4-hydroxybenzoic (4-HA) acids using manometry and atmospheric oxygen absorption.

The model reaction for studying the antioxidant activity (AOA) of the studied compounds was radical-chain oxidation of propan-2-ol and 1,4-dioxane (348 K, azodiisobutyronitrile initiator, $V_i = 1\cdot10^{-7}$ mol/L·s initiation rate). The inhibition effectiveness of compounds was estimated from the decrease in the initial rate of oxygen absorption upon oxidation of the model substrates. The experimental methods of the kinetics studies have been described in detail [9]. The rate constant of oxidation chain termination fk_{In}, where f is the radical capacity of the antioxidant (AO) that indicates the number of radical intermediates consumed per single AO molecule during chain termination, was used as the quantitative characteristic of the AOA [10]. The ionol equivalent (IE), a quantity estimated by comparing experimental fk_{In} values for the studied compounds with that for ionol, was used as a characteristic for the AOA of the studied compounds.

We studied quantitatively the AOA of the four phenolcarboxylic acids CA, FA, VA, and 4-HA using initiated oxidation of propan-2-ol and 1,4-dioxane as model reactions. Initiated oxidation of the model substrates under the standard experimental conditions followed a kinetic regime for a radical-chain mechanism with quadratic chain termination according to Reaction (4) and included several basic steps that are common for most organic compounds [11]:
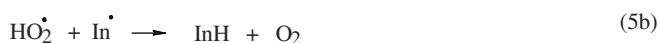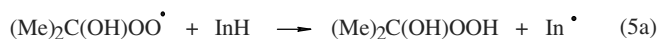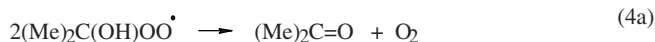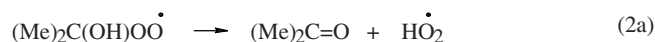

$$I \longrightarrow 2r^\bullet \ , \quad r^\bullet + RH \longrightarrow rH + R^\bullet \qquad (i)$$

$$R^\bullet + O_2 \longrightarrow R\overset{\bullet}{O}_2 \qquad (1)$$

$$R\overset{\bullet}{O}_2 + RH \longrightarrow ROOH + R^\bullet \qquad (2)$$

$$ROOH \longrightarrow R\overset{\bullet}{O} + HO^\bullet \qquad (3)$$

$$2R\overset{\bullet}{O}_2 \longrightarrow ROOR + O_2 \qquad (4)$$

$$R\overset{\bullet}{O}_2 + InH \longrightarrow ROOH + In^\bullet \qquad (5)$$

where $R^\bullet$ and $R\overset{\bullet}{O}_2$ are alkyl and peroxide radicals of 1,4-dioxane or hydroxyalkyl and hydroperoxyl radicals of propan-2-ol $(Me)_2C(OH)OO^\bullet$, respectively.

Adding an AO to the reaction mixture decreased the oxidation rate of the model substrate because of chain termination at the inhibitor [Reaction (5)], which has also been previously reported [10].

Furthermore, it should be considered that oxidation of the used model substrates occurred by different mechanisms. According to the literature [12], oxidation of propan-2-ol under our experimental conditions is propagated by hydroperoxyl radicals formed by decomposition of primary hydroxyperoxyl radicals $(Me)_2C(OH)OO^\bullet$ (Reaction 2a):

$$(Me)_2C(OH)O\overset{\bullet}{O} \longrightarrow (Me)_2C=O + H\overset{\bullet}{O}_2 \qquad (2a)$$

$$H\overset{\bullet}{O}_2 + RH \longrightarrow H_2O_2 + R^\bullet \qquad (2b)$$

$$2(Me)_2C(OH)O\overset{\bullet}{O} \longrightarrow (Me)_2C=O + O_2 \qquad (4a)$$

$$(Me)_2C(OH)O\overset{\bullet}{O} + InH \longrightarrow (Me)_2C(OH)OOH + In^\bullet \qquad (5a)$$

$$H\overset{\bullet}{O}_2 + In^\bullet \longrightarrow InH + O_2 \qquad (5b)$$

We took this into account in determining fk_{In} for the natural compounds.

Additions of the studied compounds decreased the initial oxidation rate of propan-2-ol and 1,4-dioxane, which confirms their AO activity. The AOA of the compounds varied over a rather wide range (Figs. 1 and 2). Thus, adding FA and CA to propan-2-ol in the concentration range $(0.16\text{-}1.16) \cdot 10^{-4}$ M for FA and $(1.0\text{-}8.4) \cdot 10^{-5}$ M for CA decreased its oxidation rate by 1.2-6.7 and 1.5-7.3 times, respectively, whereas an analogous effect was not observed for 4-HA upon addition of it to propan-2-ol in the concentration range $(1.6\text{-}6.5) \cdot 10^{-3}$ M. Figure 1a shows as an example the initial rate of oxygen absorption upon oxidation of propan-2-ol as a function of the concentration of added phenolcarboxylic acid.

Figures 1a and 1b show that the decreased oxidation rates of propan-2-ol and 1,4-dioxane in the studied concentration range of phenolcarboxylic acids still followed a chain regime for oxidation of the model substrates. In this instance, Eq. (1) can be used to determine the inhibition rate constants fk_{In} [10]:

$$F = (v_0^0 / v_0) - (v_0 / v_0^0) = fk_{In}[InH](2k_6 v_i)^{1/2}, \qquad (1)$$

where v_0^0 and v_0 are the initial rates of oxygen absorption upon oxidation of the model substrates in the absence and presence, respectively, of inhibitors (mol/L·s); k_{In}, the inhibition rate constant (L/mol·s); f, the stoichiometric inhibition coefficient; $[InH]$, the inhibitor concentration (M); and k_6, the rate constant of recombination of radical intermediates involved in oxidation chain termination of propan-2-ol and 1,4-dioxane (L/mol·s) [10, 11].

The experimental results are linear in coordinates of Eq. (1) (Fig. 2) with a high correlation coefficient (r = 0.991).

Effective inhibition rate constants fk_{In}, the values of which are given in Table 1, were found from the slopes of these functions for each compound (Fig. 2). The IE values are given for comparison of the AOA effectiveness of these compounds.

Comparison of the data indicates that the effective inhibition rate constants depend on the nature of the AO and on the nature of the oxidized model substrate. Thus, the AOA effectiveness of the phenolcarboxylic acids is about 5 times greater in propan-2-ol than in 1,4-dioxane. This result has been reported for secondary alcohols and is explained by two factors. First, hydroperoxyl radicals formed through Reaction (2a) are more reactive in chain propagation and termination reactions than peroxyl radicals from 1,4-dioxane. Second, phenoxyl radicals formed upon oxidation of phenolic compounds are regenerated by chain termination according to Reaction (5b) [12].

TABLE 1. Kinetics of Antioxidant Activity of Phenolcarboxylic Acids

| Compound	$fk_{In} \cdot 10^{-4}$, L/mol·s	IE
Propan-2-ol		
Caffeic acid	13.60±2.00	3.20
Ferulic acid	5.60±0.40	1.30
4-Hydroxybenzoic acid	0.06±0.01	0.015
Vanillic acid	0.60±0.05	0.16
Ionol	4.20±0.40	1.00
1,4-Dioxane		
Caffeic acid	2.00±0.40	1.700
Ferulic acid	1.30±0.20	1.100
Vanillic acid	0.10±0.01	0.09
Ionol	1.20±0.20	1.00
Cumene		
Caffeic acid	9.70	5.90
Ferulic acid	0.90	0.54
4-Hydroxybenzoic acid	0.27	0.16
Vanillic acid	1.05	0.64
Ionol	1.65	1.00
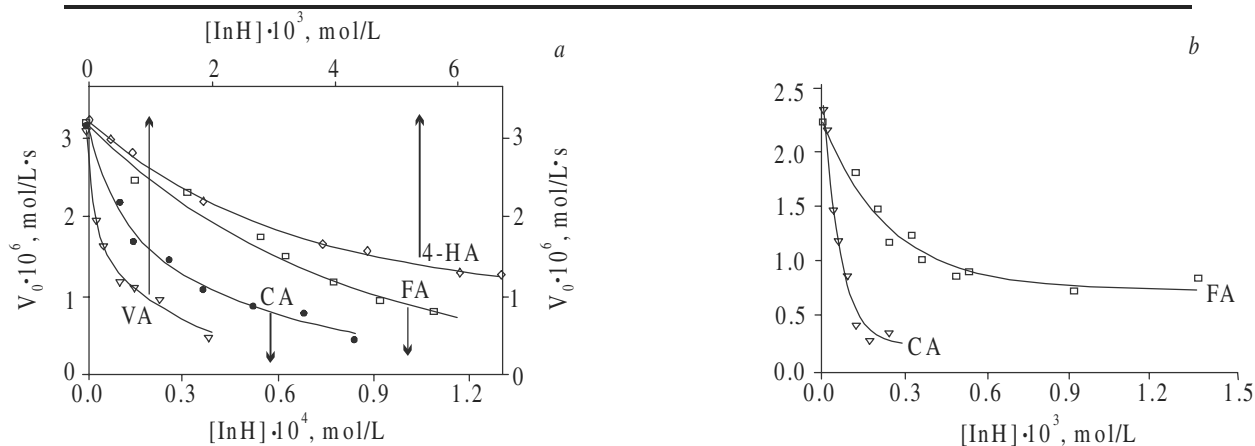
Fig. 1. Rate of oxygen absorption upon oxidation of propan-2-ol as a function of initial concentration of FA (*a*) and of 1,4-dioxane as a function of initial concentrations of CA and FA (*b*); T = 75°C, $V_i = 1 \cdot 10^{-7}$ mol/L·s.

FA and CA, in which the electron-accepting carboxylic group is separated from the aromatic ring by a vinyl bridge, typically have higher fk_{In} values. This is typical of hydroxycinnamic acid derivatives in which the conjugation of the vinyl group with the aromatic system causes high delocalization of electron density in the phenoxyl radicals formed through Reactions (5) and (5a). This conclusion is supported by results of quantum-chemical investigations of phenolcarboxylic acids [13]. A comparison of the AOA of VA and 4-HA suggests that compounds containing aromatic groups with electron-donating substituents are more effective AO.

Table 1 shows that CA is twice as reactive as FA. The high AOA of CA relative to FA is due to formation of an intramolecular H-bond between the two hydroxyls situated on neighboring C atoms. As a result, the H atom not involved in H-bond formation becomes more labile (because the H-bond forms at the expense of electron density of the O atom bonded to it) and, therefore, is more reactive toward radicals.
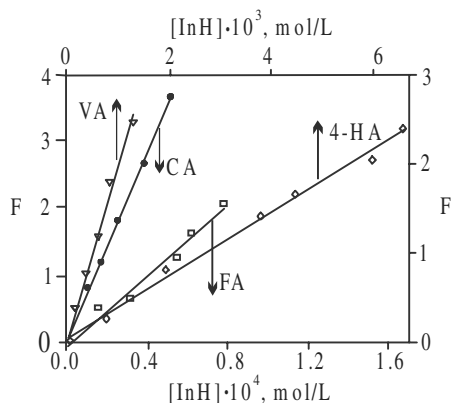
Fig. 2. Effective inhibition parameter of propan-2-ol oxidation as a function of FA concentration according to Eq. (1); $V_i = 1 \cdot 10^{-7}$ mol/L·s; T = 348 K.

Our results agree well with the literature for the AOA of phenolcarboxylic acids. For example, it was demonstrated [14-17] that the AO properties of CA for lard, triglycerides, and methyl esters of sunflower and olive oils last longer than those of FA, VA, and 4-HA. These same compounds have very high rate constants for inhibition of initiated oxidation of cumene (Table 1).

EXPERIMENTAL

Phenolcarboxylic acids [caffeic (CA), ferulic (FA), 4-hydroxybenzoic (4-HA), and vanillic (VA) acids] were isolated from the outer bark of *Larix sibirica* Ledeb. by the literature method [18]. The purity of the compounds was >90% according to HPLC.

Propan-2-ol and 1,4-dioxane were purified beforehand by standard methods [19, 20].

ACKNOWLEDGMENT

REFERENCES

1. T. K. Chumbalov, L. T. Pashinina, and Z. A. Leiman, *Khim. Prir. Soedin.*, 284 (1973).
2. M. A. Kasumov, *Pishch. Promst.*, No. 12, 21 (1991).
3. L. Nystrom, T. Achrenius, A.-M. Lampi, R. A. Moreau, and V. Piironen, *Food Chem.*, **101**, No. 3, 947 (2007).
4. J. H. Chen and C.-T. Ho, *J. Agric. Food Chem.*, **45**, 2374 (1997).
5. N. A. Zakharova, G. N. Bogdanov, M. N. Zaprometov, N. A. Tyukavkina, K. E. Kruglyakova, and N. M. Emmanuel′, *Zh. Obshch. Khim.*, **42**, No. 6, 1414 (1972).
6. A. V. Simonyan, *Khim.-farm. Zh.*, No. 2, 21 (1993).
7. F. A. M. Silva, F. Borges, C. Guimaraes, L. F. C. Jose, M. C. Lima, and S. Reis, *J. Agric. Food Chem.*, **48**, 2122 (2000).
8. O. T. Kasaikina, V. D. Kortenska, E. M. Marinova, I. F. Rusina, and N. V. Yanishlieva, *Izv. Akad. Nauk, Ser. Khim.*, No. 6, 1119 (1997).

9. V. R. Khairullina, G. G. Garifullina, A. Ya. Gerchikov, L. A. Ostroukhova, and V. A. Babkin, *Khim. Prir. Soedin.*, 131 (2006).

10. E. T. Denisov and V. V. Azatyan, *Inhibition of Chain Reactions* [in Russian], IFKhCh RAN, Chernogolovka (1997).

11. E. T. Denisov, N. I. Mitskevich, and V. E. Agabekov, *Mechanism of Liquid-Phase Oxidation of Acid-Containing Compounds* [in Russian], Nauka i Tekhnika, Minsk (1975).

12. E. T. Denisov and T. G. Denisova, *Neftekhimiya*, **46**, No. 5, 305 (2006).

13. E. G. Bakalbassis, V. S. Melissas, M. Tsimidou, M. Tsolaki, and A. Vafiadis, *Lipids*, **36**, No. 2, 181 (2001).

14. E. M. Marinova, N. V. Yanishlieva, and A. G. Toneva, *Riv. Ital. Sostanze Grasse*, **83**, No. 1, 6 (2006).

15. V. L. Yanishlieva and E. M. Marinova, *Fat Science Technol.*, **94**, No. 10, 374 (1992).

16. E. M. Marinova and N. V. Yanishlieva, *Fat Science Technol.*, **94**, No. 11, 428 (1992).

17. V. D. Kortenska, M. P. Velikova, N. V. Yanishlieva, I. R. Totzeva, V. S. Bankova, and M. C. Marcucci, *Eur. J. Lipid Sci. Technol.*, **104**, No. 1, 19 (2002).

18. N. V. Ivanova, L. A. Ostroukhova, V. A. Babkin, S. Z. Ivanova, and O. V. Popova, *Khim. Rastit. Syr'ya*, No. 4, 5 (1999).

19. E. T. Denisov and V. M. Solyanikov, *Neftekhimiya*, **9**, No. 3, 116 (1969).

20. A. Weissberger and E. S. Proskauer, *Organic Solvents*, 2nd Ed., rev. by J. A. Riddick and E. E. Toops, Jr., Interscience Pubs., Inc., New York (1955).