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# Chromato–Mass Spectrometric Identification of Unusual Products of 4-Isopropylphenol Oxidation in Aqueous Solutions

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Received August 14, 2017

Abstract—4-Isopropylphenol has been chosen as the simplest object to model the processes of oxidation of organic compounds with air oxygen in aqueous media, since it contains a hydrogen atom at the tertiary carbon atom in the α-position with benzene ring and a hydroxyl group enabling mass-spectrometric detection of the products in the negative ions mode. It has been stated that oxidation of 4-isopropylphenol with air oxygen in aqueous media becomes noticeable as the solution pH approaches the p$K_a$ value of the substrate (10.25). The major product [4-isopropyl-2-(4-isopropylphenoxy)phenol] is formed via nucleophilic addition of the starting 4-isopropylphenol at the intermediate product of its oxidation, quinone methide. Intensity of electrochemical oxidation can be tubed by changing the electrode potential. The highest conversion of 4-isopropylphenol has been observed at potential 1.5–3.0 V, the formed compounds being the products of transformation of the same quinone methide intermediate. The obtained data have explained the formation and diversity of dimeric and oligomeric products of oxidation of natural flavonoids.

**Keywords:** 4-isopropylphenol, aqueous media, dissolved air oxygen, free-radical oxidation, electrochemical oxidation, quinone methides

**DOI:** 10.1134/S1070363218010024

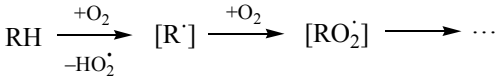
Oxidation of natural and technogenic organic compounds with dissolved air oxygen in aqueous media is among the principal processes of their transformation [1–4]. Therefore, elucidation of the regularities of such processes is important for the characterization of decompositions of toxic compounds in different environmental objects. Moreover, this is essential for the understanding of many *in vivo* oxidation processes. For example, identification of products of oxidation of biologically active compounds is a prerequisite of elucidation of their antioxidant activity mechanisms. The structure of the oxidation products of many organic flavonoids including the most widely spread quercetin [5, 6] and, hence, the nature of its antioxidant activity have been scarcely studied so far.

Oxidation of organic compounds with dissolved oxygen (ground state: triplet $^3S_g^-$) generally obeys the regulations of free-radical reactions, their first stage being elimination of the most reactive hydrogen atoms (mainly these at tertiary carbon atoms or in benzyl and α-position with respect to heteroatoms) yielding peroxide radicals $RO_2^{\cdot}$ and products of their further transformations (Scheme 1).

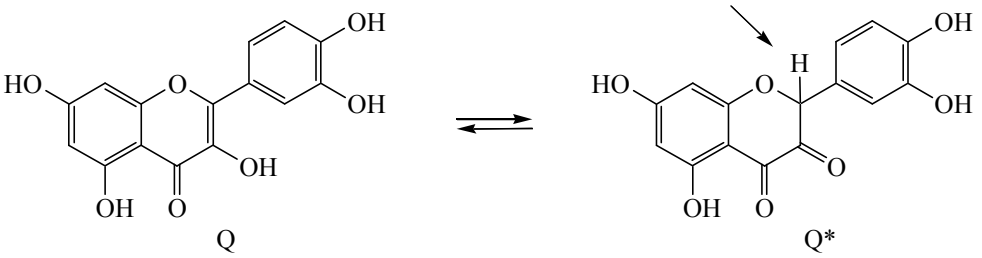
The suggested schemes of quercetin (Q) oxidation [5, 6] include such process involving the hydrogen atom in position 2 of the pyran fragment of its tautomer (Q*) as the first stage (Scheme 2).

Systematization (as an independent stage of identification) of the earlier known products of quercetin oxidation aiming to assign their structures with the chromatographic peaks [6] has shown that half of the detected products (28 of 57) have not been identified, and only 8 of the products have been identified unambiguously. The identified oxidation products include poorly stable peroxides and hydroperoxides, unstable

**Scheme 1.**



**Scheme 2.**




hemiketals, hydrate and enol forms; their preparative isolation from aqueous solutions is practically impossible. Moreover, the mixtures of the products of flavonoids oxidation often contain dimers and trimers of debatable origin, even at low concentration [6]. In this regard, characterization of products of oxidation of simpler model compounds under the same conditions seems reasonable.

The chosen objects should contain hydrogen atoms at the tertiary carbon atoms. The simplest compound of this type is isopropylbenzene [solubility in water $S$ (mol/L) at 20°C, p$S$ = –log$S$ = 3.39±0.01, i. e. about 0.1 g/L]. However, this hydrophobic hydrocarbon (log $P$ 3.66±0.20 [7]) is noticeably volatile (vapor pressure at 20°C is 3.3 mmHg), and this leads to significant losses of the substrate from its aqueous solution in contact with air, especially during bubbling. The second issue is the detection of the formed products (including phenol, dimethylphenylmethanol, benzoic acid, and cumene hydroperoxide [4]): UV detection is advantageous for these species, since mass-spectrometric (HPLC-MS) detection of the signals of positive as well as negative ions of the compounds containing no phenolic hydroxyls is inefficient.

In view of the above reasons, more hydrophilic and less volatile 4-isopropylphenol (log $P$ 2.90±0.02, $S$ ~ 1.1 g/L) seems more suitable as the model compounds for the study of free-radical oxidation with air oxygen in aqueous solutions. Moreover, this compound is interesting as such, since it models the behavior of 4-substituted alkylphenols (4-octyl- and 4-nonyl-), products of hydrolysis of their polyethoxy derivatives used as nonionic surfactants [8] and exhibiting endocrine toxicity.

Herein, we discuss the results of chromato–mass spectrometric (HPLC-ES-MS) identification of products of oxidation of 4-isopropylphenol as a model compound to elucidate the oxidation of more complex compounds with air oxygen.

**Oxidation of 4-isopropylphenol with air oxygen in alkaline medium.** To model oxidation of 4-isopropylphenol with air oxygen, we prepared its solutions in a mixture of aqueous solution of $NH_4HCO_3$ (25 mM, pH ≈ 8.5) and acetonitrile (80 : 20 v/v) with concentration ranging from 0.77 to 1.0 mg/mL and bubbled them with air (~2 L/min) during 4 h. Since no oxidation products were detected under those conditions, the solution pH was adjusted to ~10 with aqueous ammonia, and bubbling with air was continued during 1 h.

Properties of the oxidation products were unknown before the experiment, therefore to minimize the information loss, both of negative and positive ions detection modes were used for recording the electrospray mass spectra. In the positive ions mode, the collected chromatograms contained numerous peaks most of which corresponded to background signals of admixtures; those could be eliminated by parallel analysis of the specimens without the characterized component or containing different concentrations of it. As a result, a small number of oxidation products was left for further consideration, much less than in the case of quercetin [6], showing better selectivity of 4-isopropylphenol oxidation.

Table 1 lists retention times ($t_R$) of major products of oxidation of 4-isopropylphenol in two regimes of separation (I and II), values of linear-logarithmic retention indices in regime II, and mass numbers of the $[M + H]^+$ ions in the positive ions detection mode (designated as "+") and of the $[M - H]^-$ ions in the negative ions detection mode (symbol "–"). If the mass spectrum contained strong signals of fragmentation ions, their mass numbers are indicated and assigned as well.

Even the small number of the listed components clearly revealed the prominently different informative content of the negative and positive detection modes. For example, the compounds with retention times

**Table 1.** Components detected in the products of 4-isopropylphenol oxidation with air oxygen in an aqueous solution at pH ~10[a]

| $t_R$, min (I) | $t_R$, min (II) | RI (II) | *m*/*z* $[M + H]^+$ or $[M - H]^-$ (*m*/*z* of fragmentation ions) | Compound |
|---|---|---|---|---|
| 1.5 | – | | (+) 151 (110 [$M + H - C_3H_5$]) | $C_9H_{12}O - 2H + [O]$ (structure not determined) |
| 1.7 | – | | (+) 151 (110 [$M + H - C_3H_5$]) | $C_9H_{12}O - 2H + [O]$ (structure not determined) |
| *16.6* | *12.1* | 929 | (–) 135<br>(+) 137 (95 [$M + H - C_3H_6$]) | 4-Isopropylphenol (initial substrate) |
| 17.6 | – | – | (+) 222 (177, 149) | Diethyl phthalate |
| *22.7* | *18.9* | 1266 | (–) 269 (225 [$M - H - C_3H_8$]) | 4-Isopropyl-3-(4-isopropylphenoxy)phenol |
| *24.1* | – | – | (+) 279 (205, 149) | Dibutyl phthalate |

[a] Retention times of major components of the reaction mixtures are indicated in italic.

17.6 and 24.1 min (regime I) were assigned to diethyl and dibutyl phthalates, widely used plasticizers [9] getting into the specimens from distilled water stored in plastic tare. However, those components contained no functional groups with labile hydrogen atoms and were observed only in the positive ions detection mode. Another example of such sort was given by two hydrophilic components with retention times 1.5 and 1.7 min (regime I); their molecular mass ($M$ = 150) suggested that they could be assigned to isomeric products of two-stage oxidation of 4-isopropylphenol: $136(C_9H_{12}O) - 2(2H) + 16([O]) = 150(C_9H_{10}O_2)$. Alternatively, the second stage of their formation involved addition of water followed by oxidation, $136(C_9H_{12}O) - 2(2H) + 18(H_2O) - 2(2H) = 150(C_9H_{10}O_2)$. Theoretically, three possible structures could be suggested for such products: *ortho*-quinone and two quinone methide one, 2-hydroxy-4-(1-methylethenyl)-cyclohexa-2,5-dien-1-one being the most favorable [ground state energy $E$(MM+) 13.1 kJ/mol, $E$(AM-1) – 2221.2 kJ/mol]. However, the made suggestions could not be clarified using the available analytical data. Since the content of those components strongly depended (down to complete disappearance) on the concentration of 4-isopropylphenol in the solution, they could be assigned to readily oxidized products, quinone methides.

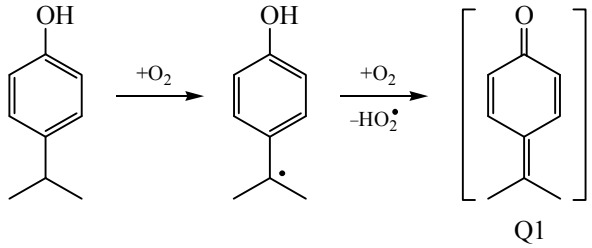
Starting 4-isopropylphenol was clearly detected in both regimes of chromatographic separation (I and II) during detection of positive as well as negative ions. After 4 h of bubbling air (~2 L/min) through 100 mL of the solution, products of 4-isopropylphenol oxidation were not detected. They appeared only after adjustment of the solution pH to ~10 by addition aqueous ammonia solution, followed by air bubbling. Hence, the oxidation with air oxygen became noticeable only when the solution pH approached the p$K_a$ value of 4-isopropylphenol (p$K_a$ 10.25).

The only component found in the mixture of products of 4-isopropylphenol oxidation under mild conditions is a hydrophobic compound with $t_R$ 22.7 (regime I) and 18.9 min (regime II), formally corresponding to the $[136(C_9H_{12}O) - 1(H)]\cdot 2$ dimer with molecular mass 270 Da (detected only in the negative ions mode). The formation of such dimer in fairly dilute aqueous solutions (0.77–1 mg/mL = 5.7–7.4 mmol/L) should be commented in more detail.

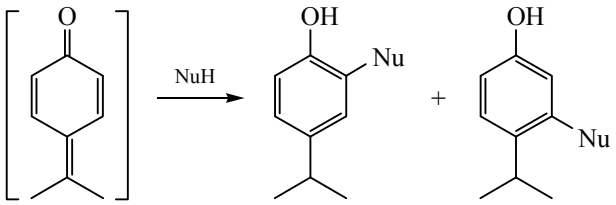
According to generally accepted views, the first stage of the interaction of the compounds containing labile hydrogen atoms at tertiary carbon (RH) is the formation of stable radicals ($R^{\cdot}$) further transformed into peroxide radicals ($RO_2^{\cdot}$), hydroperoxides ($RO_2H$), and other products (Scheme 1). 4-Isopropylphenol molecule contains such hydrogen atom, the α-hydrogen of the isopropyl group. It could be suggested that the primary radicals formed from 4-alkylphenols could be stabilized through the formation of unstable quinone methide intermediates [in the case of 4-isopropyl-phenol, 4-(1-methylethenyl)cyclohexa-2,5-dien-1-one (Q1)] (Scheme 3).

In the absence of substituents in the *para* position with respect to hydroxyl group, phenols form *para*-benzoquinones upon oxidation [10]. Quinone methides intermediates are well known in lignin chemistry [11]; they are chemically similar to quinones [12, 13], being prone to the addition of nucleophilic agent (so called nucleophilic coupled addition). Quinone methides are

**Scheme 3.**




**Scheme 4.**




sometimes recognized as cross-linking agents [14] (Scheme 4).

Aqueous solutions of 4-isopropylphenol contain two potential nucleophilic agents: water and 4-isopropylphenol. The addition of water to quinone methide Q1 leads to dihydroxyisopropylbenzene with *M* 152 (detected under conditions of electrochemical oxidation), further oxidation of which explains the formation of the above-mentioned components with *M* = 150. In the case of 4-isopropylphenol as the nucleophile, isomeric 4-isopropyl-3- (**A1**) and 4-isopropyl-2-(4-isopropylphenoxy)phenol (**A2**) with *M* = 270 are formed, as it is illustrated by Scheme 5.

If the oxidation products contain only one of these isomers (as was observed in this study), its structure elucidation by means of chromato–mass spectrometry analysis is impossible. If both products were present, they could be assigned from the elution order (in view of hydrophobicity) or from the data on their relative stability (for example, the correspondence between their fractions in the mixture and the ground state energies).

**Electrochemical oxidation of 4-isopropylphenol in aqueous solutions.** As exemplified by quercetin, the composition of the products of free-radical (with air oxygen dissolved in water, also known as auto-oxidation) and electrochemical oxidation processes are very similar [6], enabling the comparison of the formed products. Therefore, it was reasonable to consider electrochemical oxidation of 4-isopropylphenol in this study. Supposing that the corresponding quinone methide was the primary intermediate, the process could be described by Scheme 6.

Phenols (and, therefore, natural flavonoids) are electrochemically active compounds, and electrochemical detectors can be used for their detection in HPLC [15, 16]. In contrast to the chemical processes, the rate of oxidation can be tuned by changing the potential *U* of the electrochemical cell (detector). We used a ROXY cell designed to be incorporated in an HPLC–MS system and enabling the detection and identification of the oxidation products during their generation [17–20]. It was found that the potential up to 1.5 V was not enough to provide the reasonable rate of 4-isopropylphenol transformation, whereas its increase to 3.0 V allowed detection of more than ten oxidation products in the negative ions detection mode, independently of the solution pH.

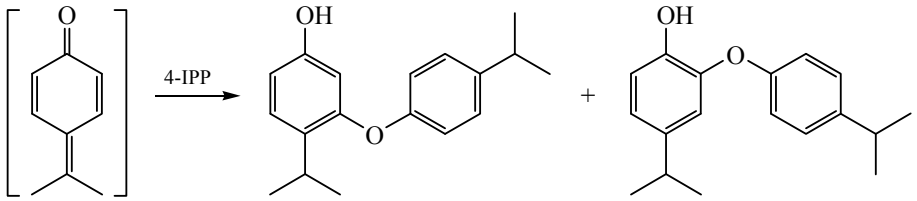
Table 2 lists the retention times of the products of electrochemical oxidation of 4-isopropylphenol (separation regime II), their retention indices ranging from 820 to 1340, mass numbers of the $[M - H]^-$ ions and other strong signals, possible identifications, and the calculated (ACD/Labs software) hydrophobicity parameters log *P* used to analyze the elution order. Experimental log *P* value is given for the starting substrate, whereas the isomers are characterized by the parameter range. Retention times of the minor products are given in *italic* in the first column to show the relative contents of the components.

The components listed in Table 2 include all the compounds from Table 1 detected in the negative ions mode. On top of them, 4-isopropylpyrocatechol (the product of water addition to the quinone methide, *M* = 152) and two dimeric products **A1** and **A2** (major) with *M* = 270 were detected. They were assigned using the ground state energies determined using the MM+ and AM-1 methods.

| Isomer | **A1** | **A2** |
|---|---|---|
| *E*(MM+), kJ/mol | 24.6 | 24.2 |
| *E*(AM-1), kJ/mol | –4396.1 | –4397.6 |
| log *P* | 6.33±0.32 | 5.94±0.33 |

The obtained data showed that the minor isomer **A1** ($t_R$ 15.7 min, RI 1097) was eluted before the major isomer **A2** ($t_R$ 18.9 min, RI 1266), since the *E*(MM+) and *E*(AM-1) values were somewhat lower for the

**Scheme 5.**




**A1** **A2**

latter one. The log *P* values were not informative, as their difference (0.39) did not exceed the estimated accuracy (0.65).

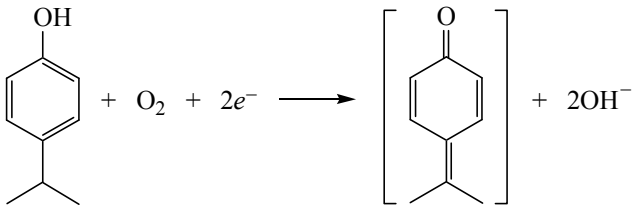
Besides the mentioned components, the mixture of products of the electrochemical oxidation contained three isomeric dihydroxyisopropyl-(4-isopropylphenoxy)benzene with *M* 286. Six such isomers were theoretically possible, and the presence of only two of them could be due to either different probabilities of their formation or overlapping of their peaks in the chromatogram. They could be formed via water addition to the products of oxidation of dimers **A1** and **A2** (or vice versa) and were characterized by the log *P* values of 5.01–5.78. Finally, the products contained seven compounds with *M* = 420, isomeric dihydroxyisopropylbis-(4-isopropylphenoxy)benzenes formed via a series of oxidation–nucleophilic addition processes. The last detected component with *M* = 404 corresponded to one of hydrophobic isomeric 4-isopropylbis-(4-isopropylphenoxy)phenols. It should be noted that the two compounds eluted the last could not be characterized by the retention indices, since their retention times fell outside the linear gradient of acetonitrile in the eluent (20 min) and corresponded to the final isocratic region. The detection of the reference and characterized components in different elution regimes is impossible due to systematic inaccuracy in the retention indices.

Oxidation of 4-isopropylphenol under conditions of scanning voltammetry gave the values of the lowest potential corresponding to the formation of certain products. That allowed the estimation of the order of the products formation (that is, determine the primary and secondary components). For example, the formation of the products with *M* = 270 was observed at potential of at least *U* = 0.7 V, the compounds with *M* = 286 and 420 were formed at *U* = 0.95 V, and the compound with *M* = 152 appeared at *U* = 1.4 V. The highest yield of the oxidation products was observed at *U* of 1.5 to 3.0 V.

To verify the identification of the oxidation products with unambiguously elucidated structure, it was reasonable to use the independent criteria, the simplest one being the correlation of the retention indices in the reversed-phase HPLC with the hydrophobicity parameters log *P*. Since there were only three such compounds (Table 2) [4-isopropylpyrocatechol (RI 822, log *P* 2.22), initial 4-isopropylphenol (RI 929, log *P* 2.90), and 4-isopropyl-2-(4-isopropylphenoxy)phenol (RI 1266, log *P* 5.94)], the parameters of the corresponding linear regression equation could be estimated using the three points: *a* 117±7, *b* 575±28, *r* 0.998, $S_0$ 20. Nevertheless, good linear relationship RI = *a*log *P* + *b* was observed, thus confirming the correct identification of those compounds.

In summary, we found that the free-radical as well as electrochemical oxidation of alkyl-substituted phenols was accompanied by the formation of dimeric (or, generally, oligomeric) products. The nature of the products was fully in line with the suggested generation of quinone methide intermediates. Other ways of oxidation of alkyl-substituted phenols, for example, with iron (III) chloride [21] have led to the formation of dimeric and oligomeric products, possibly via the same mechanism. Moreover, it could be suggested that the transformations of 4-alkyl-substituted phenols (including 4-nonylphenols [8]) in aqueous media are strongly affected by the reactions of quinone methide intermediates with nucleophilic agents present in such media (for example, humic acids in the case of natural water reservoirs).

**Scheme 6.**




Q1

**Table 2.** Components detected in the products of electrochemical oxidation of 4-isopropylphenol in an aqueous solution at pH ~ 7.4 (separation regime II)[a]

| $t_R$, min | RI | $m/z$ $[M - H]^-$, ($m/z$ of fragmentation ions) | Compound | log $P$ |
|---|---|---|---|---|
| *9.2* | 822 | 151 | 4-Isopropylpyrocatechol[b] | 2.22 |
| 12.1 | 929 | 135 | 4-Isopropylphenol (starting)[b] | 2.90 |
| 12.3 | 937 | 285 (267 [$M - H - H_2O$]) | Isomer of dihydroxyisopropyl-(4-isopropylphenoxy)benzene | 5.01–5.78 |
| 12.8 | 959 | 419 (203) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| *13.4* | 986 | 285 (242 [$M - H - C_3H_7$]) | Isomer of dihydroxyisopropyl-(4-isopropylphenoxy)benzene | 5.01–5.78 |
| *15.7* | 1097 | 269 (226) | 4-Isopropyl-3-(4-isopropylphenoxy)phenol | 6.33 |
| *17.0* | 1164 | 285 (267 [$M - H - H_2O$]) | Isomer of dihydroxyisopropyl-(4-isopropylphenoxy)benzene | 5.01–5.78 |
| *17.5* | 1191 | 419 (401 [$M - H - H_2O$]) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| 18.0 | 1217 | 419 (383) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| 18.9 | 1266 | 269 (225 [$M - H - C_3H_8$]) | 4-Isopropyl-2-(4-isopropylphenoxy)phenol[b] | 5.94 |
| 19.4 | 1294 | 419 (376) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| *20.0* | 1327 | 419 (401 [$M - H - H_2O$]) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| *20.2* | 1339 | 419 (267) | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| *21.9* | –[c] | 419 | Isomer of dihydroxyisopropylbis-(4-isopropylphenoxy)benzene | 8.04–8.93 |
| 23.6 | –[c] | 403 (385 [$M - H - H_2O$]) | Isomer of 4-isopropylbis-(4-isopropylphenoxy)phenol | |

[a] Retention times of major components of the reaction mixtures are indicated in italic. [b] Characteristics of those compounds were used for the calculation of parameters of regression equation RI = $a$log $P + b$. [c] Retention times of two components eluted last were outside the linear gradient (20 min); the change in the elution regime made impossible the calculation of their retention indices.

The obtained data could reasonably explain the formation of numerous dimeric products with mass [2$M$ – 2] ($M$ being the mass of starting substrates) upon oxidation of quercetin and other flavonoids in aqueous media. The primary oxidation products included quinones as well as quinone methides existing in different tautomeric forms [5, 6], the major pathway of their further transformations involving nucleophilic addition of the starting flavonoids. That resulted in the formation of dimers containing flavonoid moieties linked by oxygen bridges.

## EXPERIMENTAL

Electrochemical oxidation of a solution of 4-isopropylphenol (50–100 μg/mL) was performed in mixtures of an aqueous solution of ammonium acetate (pH ~7.4) and acetonitrile (3 : 1 v/v) in a ROXY electrochemical cell (Antec, Netherlands) equipped with a Magic Diamond boron-doped electrode at 37°C and flow rate 10 μL/min. The electrode potential was varied between 2.5 and 3.5 V monitoring the oxidation process by measuring the current. The solution of the oxidation products was acidified with $HCO_2H$ (~0.1% in the analyzed specimen) prior to the analysis.

Electrochemical oxidation of 4-isopropylphenol with mass-spectrometric detection of the products was performed in the scanning voltammetry mode at the potential scanning rate 2 mV/s at $U$ from 0 to 1.5 (a) and from 1.0 to 4.4 V (b).

Chromatographic analysis of the aqueous solutions was performed using an Agilent 1290 Infinity chromatograph equipped with a mass-spectrometric detector. Separation regime I: column (150×2.1 mm) Zorbax Eclipse XDB-C8 (sorbent particles size 3.5 μm), precolumn filled with the same sorbent, gradient elution with mobile phases A (water–acetonitrile–formic acid, 99 : 1 : 0.1) and B (water–acetonitrile–formic acid, 10 : 90 : 0.1). Content of phase B in the eluent was linearly increased from 5 to 80% during 20 min, kept constant during 2 min, and then decreased to 5% during 1 min, and the column was conditioned during 2 min. Eluent flow rate (pH ~ 2–3) 0.2 mL/min,

applied specimen volume 10 μL, column thermostat temperature 30°C. Separation regime II: column (100×2.1 mm) Acquity UPLC HSS T3 (sorbent particles size 1.8 μm), pre-column filled with the same sorbent, gradient elution with mobile phases A (water–acetonitrile–formic acid, 99 : 1 : 0.1) and B (water–acetonitrile–formic acid, 10 : 90 : 0.1). Content of phase B in the eluent was linearly increased from 20 to 90% during 20 min, kept constant during 4 min, and then decreased to 20% during 1 min, and the column was conditioned during 2 min. Eluent flow rate (pH ~ 2–3) 0.2 mL/min, applied specimen volume 10 μL, column thermostat temperature 30°C.

Mass-spectrometric detection of the products of 4-isopropylphenol and quercetin oxidation was performed using a Bruker amaZON ETD spectrometer with ions trap (Germany) under electrospray ionization conditions in the positive and negative ions detection modes. Voltage on the capillary –4.8 kV, nitrogen as drying gas, 250°C, flow rate 9 L/min, mass scan range 70–1000 Da. Each spectrum was obtained by averaging five sequential scans. The detection was performed using the total ion current (AutoMS) as well as by isolation and fragmentation of ions with pre-selected masses (MRM).

Linear-logarithmic retention indices for the 4-isopropylphenol oxidation products in regimes I and II were calculated using retention times of the reference components: acetophenone [$t_R$ 13.0 min (I) and 8.4 min (II)], propiophenone [15.7 min (I) and 11.4 min (II)], and butyrophenone [17.9 min (I) and 13.7 min (II)]. Aqueous solution of thiourea was used to estimate the retention time of non-adsorbed component [$t_0$ 2.1 min (I) and 1.5 min (II)]. The calculations were performed using a QBasic program.

Hydrophobicity parameters (log *P*) used to determine the elution order of the components were calculated using ACD/Labs software. The energies of formation were simulated using the MM+ and AM-1 methods implemented in HyperChem Pro 6.0 software.

**Preparation 4-isopropylphenol solutions and the oxidation with dissolved air oxygen.** Solutions of 4-isopropylphenol {Sigma-Aldrich, mp 60–61°C (mp 62–63°C [22]), p$K_a$ 10.25} with concentration 0.77–1.0 mg/mL in a mixture of aqueous solution of $NH_4HCO_3$ (25 mM., pH ≈ 8.5) and acetonitrile (80 : 20 v/v) were prepared. After bubbling air through 100 mL of the solution (~2 L/min) during 4 h under scattered luminescent illumination, the solution was kept in air without stirring during 1 day; no oxidation products were detected in the mixture. Aqueous solution of ammonia (10%) was added to pH ~10, and air was bubbled through the solution during 1 h. Prior to the analysis, the specimens were acidified with formic acid (about 0.1%) to pH ~3. To exclude the signals of the admixtures as well as minor or unstable components, the starting substrate concentration in the solution was varied.

Samples preparation and oxidation conditions for quercetin has been described elsewhere [6].

## ACKNOWLEDGMENTS

Authors are grateful to the directorate of Research Institute of Hygiene, Occupational Pathology and Human Ecology, Federal Medico-Biological Agency of Russia (St. Petersburg) for the opportunity to perform the experiments.

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