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RADIATION CHEMISTRY

Interaction of Tryptophan and Its Derivatives with Oxygen- and Nitrogen-Centered Radicals

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Abstract—The interaction of tryptophan and its derivatives with the nitrogen-centered 2,2-diphenyl-1-picrylhydrazyl radical and oxygen-centered radicals formed during the radiolysis of oxygen-saturated ethanol and its aqueous solutions has been studied. It has been found that unlike pyrrole, indole, or melatonin, tryptophan, 5-hydroxytryptophan, serotonin, harman, harmine, and harmaline are capable of reducing oxygen-centered radicals by electron transfer from the lone pairs of nitrogen atoms. It has been shown that serotonin and 5-hydroxytryptophan reduce the 2,2-diphenyl-1-picrylhydrazyl radical via the transfer of hydrogen atoms from the hydroxyl groups of the molecules. In this case, pyrrole, indole, tryptophan, melatonin, harman, harmine, and harmaline, which do not contain phenolic hydroxyl groups in their structures, do not interact with the 2,2-diphenyl-1-picrylhydrazyl radical. The final molecular products of the reaction of serotonin and 5-hydroxytryptophan with 2,2-diphenyl-1-picrylhydrazyl radicals have been identified by chromatography–mass spectrometry.

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The reactive oxygen species (ROS) and reactive nitrogen species (RNS) participate in many processes of damage to biomolecules under the action of different factors [1]. These species can be generated by the action of ionizing radiation, by the metabolism of xenobiotics, and in the course of immune response or normal metabolic reactions [1, 2]. The amount and nature of free radicals in the human body are controlled by the so-called antioxidant system, the simplest components of which are low-molecular-weight regulators of homolytic processes [1]. Therefore, the substances capable of regulating the direction and intensity of free-radical processes frequently possess useful pharmacological properties [3, 4].

The lifetime of the radical forms of ROS and RNS in the body is short because of their high reactivity [5]. They are consumed in reactions with biologically important molecules; as a result of this, less active and, hence, more long-lived radicals are formed. In the majority of cases, these radicals are localized on the carbon atom. The subsequent processes with the participation of carbon-centered radicals are responsible for the pathophysiological and regulatory action of free radicals in the human body [1, 2, 6–10].

Previously [11, 12], we found that a number of classical antioxidants synthesized in the body from tryptophan possess high reactivity toward carbon-centered radicals. We established that tryptophan, 5-hydroxytryptophan, serotonin, and (to a lesser degree) melatonin are capable of adding α-hydroxyethyl radicals to prevent their further transformations, whereas β-carboline alkaloids inhibit the recombination of α-hydroxyethyl radicals by their oxidation. In continuation of the previous studies, we investigated in this work the interaction of tryptophan and its derivatives with oxygen- and nitrogen-centered radicals, which induce the development of the "oxidative stress." The establishment of a relationship between the structure and reactivity of tryptophan and its derivatives toward carbon-, nitrogen-, and oxygen-centered radicals can form the basis for seeking new effective regulators of free-radical reactions in the human body that possess radioprotective properties.

We studied the interactions of tryptophan and its derivatives with oxygen-centered radicals by the continuous radiolysis of ethanolic and aqueous ethanolic solutions saturated with oxygen. The irradiation of the oxygenated ethanolic solutions makes it possible to generate oxygen-centered radicals structurally related to those formed in living organisms. Therefore, a model of this kind has been widely used for studying the reactivity of different classes of substances toward oxygen-centered radicals [13–16].

The reactivity of tryptophan and its derivatives toward nitrogen-centered radicals and the reaction mechanisms were studied using the stopped-flow method: we measured the rate constants of pseudo-first-order reactions with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The final molecular products of the reactions of the test substances with DPPH

were identified by liquid chromatography–mass spectrometry. This model was chosen because DPPH is commonly used for determining the antiradical activity of low-molecular-weight antioxidants from different classes [17–20].

EXPERIMENTAL

Pyrrole (**I**), indole (**II**), tryptophan (**III**), 5-hydroxytryptophan (**IV**), serotonin (**V**), melatonin (**VI**), harman (**VII**), harmine (**VIII**), harmaline (**IX**), Trolox (**X**), α-tocopherol (**XI**), δ-tocopherol (**XII**), ascorbic acid (**XIII**), cysteine (**XIV**), and 2,2-diphenyl-1-picrylhydrazyl (**XV**) from Sigma-Aldrich were used without preliminary purification (Fig. 1). The purity of the compounds was no lower than 98%.

Solutions of the test compounds for the radiation-chemical experiment were prepared by dissolving their accurately weighed portions in 96 vol % ethanol and a 1 M ethanol solution. Doubly distilled water and foodstuff ethanol of Lux grade (96 vol %), which was purified by distillation on a rectifying column before use, were used for the preparation of the solutions.

Because of the high volatility of ethanol, solutions were purged with high-purity (99.9%) oxygen for 60 min in volumetric flasks in order to prepare the oxygenated solutions of the test compounds; thereafter, the evaporated solvent volume was compensated with oxygenated ethanol, and the solutions were stirred. The ampoules preliminarily blown with oxygen were filled with the solutions and sealed. The concentration of the tryptophan derivatives was 10^{-3} mol/L (5×10^{-4} mol/L for tryptophan). The solutions of the test substances in 1 M ethanol at pH 7 were prepared with the use of a phosphate buffer solution (0.01 M, KH_2PO_4 and $K_2HPO_4 \cdot 3H_2O$). The solutions were purged with oxygen in ampoules for 60 min; thereafter, the ampoules were sealed. The concentration of the tryptophan derivatives was 2×10^{-4} mol/L (2×10^{-5} mol/L for harman and harmine).

Irradiation was performed on an MRKh-γ-25M unit with a ^{60}Co source. The dose rate was 0.29 ± 0.008 Gy/s. The absorbed dose range was 0.10–0.52 kGy. Acetaldehyde (the product of the radiation-chemical transformations of ethanol) and changes in the concentration of pyrrole were determined on a Shimadzu GC-17A chromatograph in accordance with a previously published procedure [11].

A reagent spectrophotometric method based on the formation of a yellow complex of hydrogen peroxide with titanyl sulfate in a sulfuric acid solution was used to determine hydrogen peroxide [16]. As in the case of the determination of indole concentrations, a Specord S600 spectrophotometer (Analytik Jena, Germany) was used for the measurements.

The concentrations of tryptophan, 5-hydroxytryptophan, serotonin, melatonin, harman, harmine, and harmaline were determined and the products of their radiation-induced transformations were identified using a Shimadzu LCMS-2020 liquid chromatograph with a mass-spectrometric quadrupole detector in accordance with a published procedure [12].

The radiation-chemical yields (G, molecule/100 eV) of the formation of reaction products and the consumption of the test compounds were calculated from the linear portions of the dose dependences of the concentrations of substances. The results of two or more independent experiments were used to calculate the radiation-chemical yields. Errors in the determination of the radiation-chemical yields were calculated by the least squares method at a confidence level of 0.95.

The interaction of the test substances with DPPH was studied using the stopped-flow method. For this purpose, accurately weighed portions of the test substances were dissolved in technical-grade ethanol (96 vol %) twice distilled on a rectifying column. The concentration of the solutions of the test compounds was 10^{-3} mol/L, and the DPPH concentration was 10^{-4} mol/L. A tenfold excess of the test compounds with respect to the radical was used to maintain the conditions for pseudo-first-order reaction kinetics. The solutions were mixed in a 1 : 1 volume ratio using a Pro-K.2000 rapid kinetics system with a stopped-flow mixing accessory (Applied Photophysics, Great Britain). Before mixing, the solutions were thermostated at 25°C for 10 min in the Pro-K.2000 system with the aid of the external circulation circuit of an F12-ED thermostat (Julabo, Germany). The isothermal reaction conditions (25°C) were maintained with the use of a Peltier element (Analytik Jena, Germany) embedded in the cell holder.

The concentration of DPPH during the course of its reactions with the test substances was measured on the Specord S600 spectrophotometer. The instrument operation was computer controlled with the aid of the WinASPECT Version 2.3.1.0 software. The analysis conditions were the following: measured wavelength range, 400–800 nm; single measurement duration, 90 ms; number of averaged spectra measured in 90 ms, 2; number of test solution measurements, from 20 to 300; and the total duration of measurements, from 1 to 500 s depending on reaction rate.

The reaction rate order was monitored independently by the method of substitution and the differential van't Hoff method [21]. The pseudo-first-order reaction rate constants were calculated graphically. The measurement results were represented as the plot of ln ($[DPPH]_0/[DPPH]$) versus time t. The portions sections of this dependence were described by the following equation of the $y = kx$ type:

$$\ln([DPPH]_0/[DPPH]) = k_{pfo}t, \quad (1)$$

where ($[DPPH]_0/[DPPH]$) is the ratio between the initial ($[DPPH]_0$) and current ($[DPPH]$) radical concentrations; k_{pfo} is the slope of a straight line—the

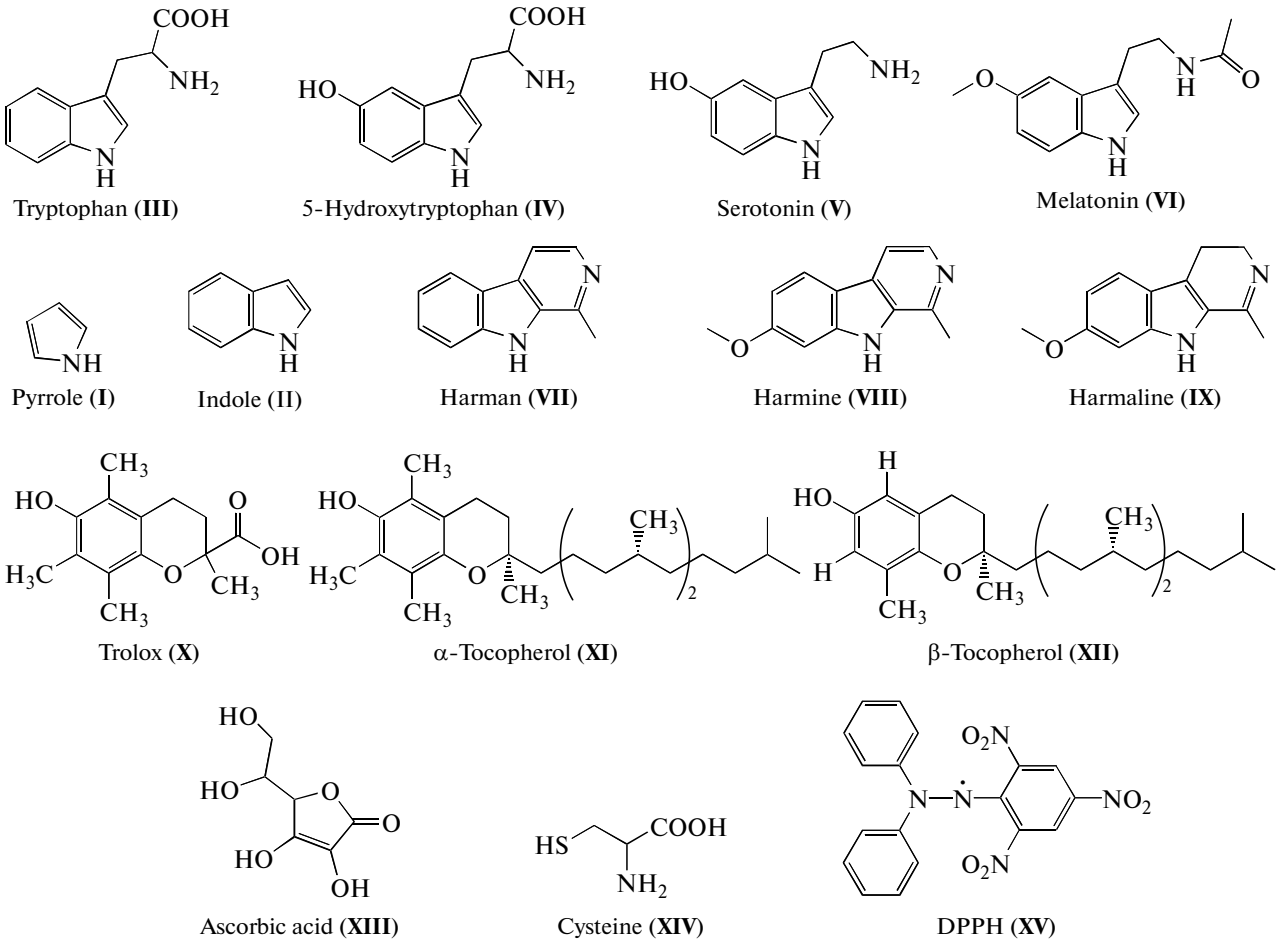


Fig. 1. Structural formulas of the compounds used in this work.

pseudo-first-order reaction rate constant, s^{-1}; and t is the reaction time, s.

In the calculation of the pseudo-first-order reaction rate constant, the results of two or more independent runs were averaged. The mathematical processing of data and the calculation of errors were performed using the OriginPro 8.5.1 software.

RESULTS AND DISCUSSION

During the radiolysis of oxygen-saturated 96 vol % and 1 M solutions of ethanol, the α-hydroxyethyl radicals formed in reactions (3) and (5) add oxygen at a diffusion-controlled rate (reaction (6)). The fragmentation of the generated oxygen-centered radicals (**XVI**) leads to the formation of acetaldehyde and hydroperoxyl radicals [22, 23]:

$$CH_3CH_2OH \xrightarrow{\gamma} CH_3CH_2OH^{\oplus\cdot} + e^{\ominus}_{solv}, \quad (2)$$

$$CH_3CH_2OH^{\oplus\cdot} + CH_3CH_2OH \rightarrow CH_3CH_2OH_2^{\oplus} + CH_3\dot{C}HOH, \quad (3)$$

$$H_2O \xrightarrow{\gamma} {}^{\cdot}OH, {}^{\cdot}H, e^{\ominus}_{aq}, H_2O_2, \quad (4)$$

$$CH_3CH_2OH \xrightarrow[-H_2O\,(-H_2)]{{}^{\cdot}OH\,({}^{\cdot}H)} CH_3\dot{C}HOH, \quad (5)$$

$$CH_3\dot{C}HOH \xrightarrow{O_2} CH_3CH(OO^{\cdot})OH \rightarrow CH_3CHO + HO_2^{\cdot}. \quad (6)$$

(XVI)

Reactions (7) and (8) lead to the formation of hydrogen peroxide, another main product of radiolysis:

$$HO_2^{\bullet} + CH_3CH_2OH \longrightarrow H_2O_2 + CH_3\dot{C}HOH, \quad (7)$$

$$2HO_2^{\bullet} \longrightarrow H_2O_2 + O_2. \quad (8)$$

The radiolysis of the test substances in the oxygenated ethanol solutions makes it possible to assess their antioxidant properties by studying the reactions of the additives with oxygen-centered radicals (**XVI**) and hydroperoxyl radicals, which occur in equilibrium with superoxide radical anions (reaction scheme (9)):

$$HO_2^{\bullet} \rightleftharpoons O_2^{\bullet-} + H^+. \quad (9)$$

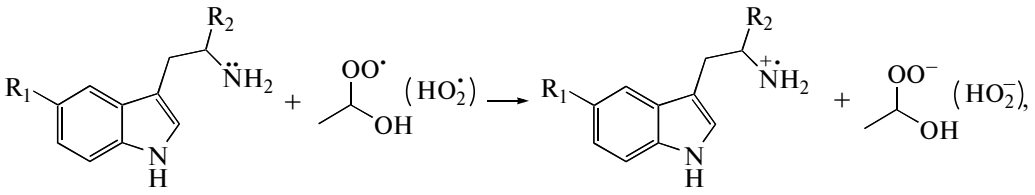
Interactions of this type will manifest themselves as an effect of the additives on changes in the G values of acetaldehyde and hydrogen peroxide. Tables 1 and 2 summarize the radiation-chemical yields of acetaldehyde and H_2O_2 formed in the absence and the presence of the test substances during the radiolysis of 96 vol % and 1 M solutions of ethanol, respectively.

In the case of radiolysis of the solutions of the test substances in oxygenated ethanol (96 vol %), pyrrole (**I**) and melatonin (**VI**) exerted an insignificant effect on G(acetaldehyde) and $G(H_2O_2)$. This fact indicates the inability of these substances to affect the radiation-chemical oxidation of ethanol. In the presence of indole (**II**), the yields of acetaldehyde and H_2O_2 increased; this was likely due to the ability of **II** to sensitize the oxidation process. Compounds **III–V** decreased G(acetaldehyde) and $G(H_2O_2)$, as compared with those in the additive-free system, and β-carboline alkaloids (**VII–IX**) decreased only $G(H_2O_2)$, whereas the yield of acetaldehyde remained at the level of the additive-free system (Table 1). Thus, substances **III–V** and **VII–IX** inhibit the oxidation of ethanol. The common features of the test compounds were their low degradation yields and the absence of their radiation-chemical transformation products, which we attempted to detect using HPLC with mass-spectrometric detection. The absence of conversion products and the low yields of decomposition of the test substances indicate that compounds **II–V** and **VII–IX** are capable of regeneration through interaction with oxygen-centered radicals.

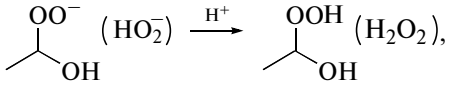
The indole ring is the base structural unit of compounds **III–IX**; however, indole itself does not inhibit the oxidation of ethanol. It is likely that differences in the reactivity of **III–IX** are due to the presence and structure of the substituent groups in these molecules.

Of the test compounds, serotonin exhibited the most pronounced antioxidant properties, which can be due to the presence of the OH group in its molecular structure. The OH group can reduce oxygen-centered radicals to inhibit the ethanol oxidation process. At the same time, tryptophan, which does not contain hydroxyl groups, exhibited antioxidant activity. The common feature of the test compounds is the presence of one or two nitrogen atoms in their structures. In our opinion, the nitrogen-containing structural units of the molecules are responsible for the effect of the test compounds on radiation-chemical processes in oxygenated ethanol, and the observed differences in the yields of formation of the final products are caused by the structural peculiarities of the compounds.

Tryptophan and its derivatives **IV** and **V** can enter electron transfer reactions, e.g., reactions (10)–(12). Aminyl radical cations (**XVII**) generated in reaction (10) are unstable and exhibit oxidizing properties; it is likely that they are consumed in reactions with superoxide radical anions or hydroperoxyl radicals [reaction (13)]. The occurrence of this reaction leads to the regeneration of the initial compounds, a fact that is confirmed by the low degradation yields of the test substances. In this case, the radiation-chemical yields of acetaldehyde and H_2O_2 decrease as a result of the termination of the short-chain process of ethanol oxidation.


(10)

(XVII)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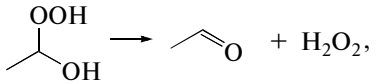
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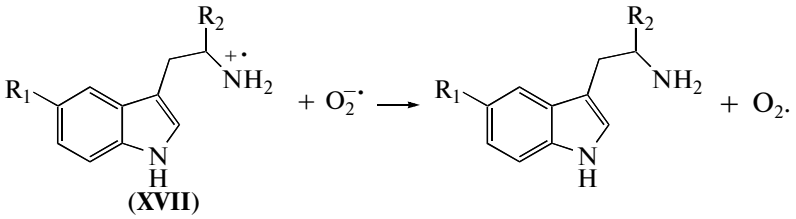


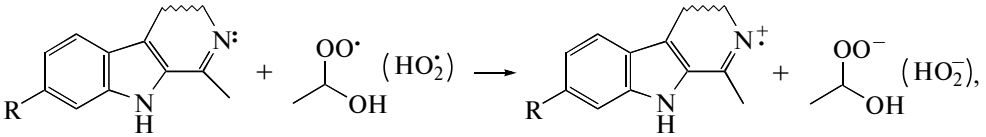
(13)

(XVII)

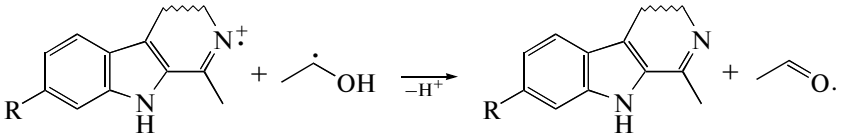
Tryptophan (**III**): $R_1 = H$, $R_2 = COOH$.
5-Hydroxytryptophan (**IV**): $R_1 = OH$, $R_2 = COOH$.
Serotonin (**V**): $R_1 = OH$, $R_2 = H$.

The inability of melatonin (**VI**) to enter into reactions (10)–(13) may be due to the delocalization of electrons from the nitrogen atom over the acyl moiety of its molecule.

In the process of the biosynthesis of β-carboline alkaloids, the aliphatic amino group of tryptamine or tryptophan participates in the formation of six-membered nitrogen heterocycles of different degrees of saturation, for example, pyridine in the case of harman or harmine and dihydropyridine in the case of harmaline. The lone electron pair of the nitrogen atoms in the pyridine and dihydropyridine rings of the test β-carboline alkaloids is not involved in the aromatic system of the molecules; therefore, it can enter into electron transfer reactions by analogy with compounds **III–V** (reaction (14)). The experimentally observed absence of changes in *G*(acetaldehyde) can be caused by the ability of nitrogen-centered radical cations to oxidize α-hydroxyethyl radicals (reaction (15)) by analogy with the reaction scheme proposed earlier [11] for the interaction of β-carboline alkaloids with α-hydroxyethyl radicals produced in the radiation- and peroxide-induced transformations of deaerated ethanol. Reaction (15) leads to the regeneration of the initial alkaloid molecules and, hence, to the low degradation yields observed in the experiments.



(14)



(15)

Harman (**VII**): R = H, ~~ = ═
Harmine (**VIII**): R = CH_3O–, ~~ = ═
Harmaline (**IX**): R = CH_3O–, ~~ = —

It is likely that the lower antioxidant activity of the test β-carboline alkaloids, as compared with that of compounds **III–V**, is caused by the effect of the aromatic system of the molecules on the lone electron pair of the nitrogen atom; this conclusion is confirmed by the stronger basic properties of saturated nitrogen-containing heterocycles as compared with those of the unsaturated counterparts.

If the mechanism proposed for the interaction of substances **III–V** and **VII–IX** with oxygen-centered radicals is true, the incorporation of the lone electron pair of the nitrogen atoms of the test substances in the formation of a chemical bond or the delocalization of these electrons will cause a loss of antioxidant properties by these compounds.

To test this hypothesis, we performed the radiolysis of solutions of the test substances in oxygen-saturated 1 M ethanol at pH 7. Under these conditions, the amino groups in compounds **III–V** and the nitrogen atoms in the six-membered rings of **VII–IX** are protonated; therefore, they cannot be electron donors in reactions with oxygen-centered radicals. From the experimental data (Table 2), it follows that none of the test compounds exerted a considerable effect on the *G* values of acetaldehyde and H_2O_2 under the specified conditions (Table 2). We also couldn't detect the conversion products of the test substances.

Thus, the presence of nitrogen atoms with a lone electron pair in the structures of tryptophan and its derivatives is responsible for the ability of these compounds to reduce oxygen-centered radicals by the electron transfer mechanism and to serve as an antioxidant.

Table 1. Effect of tryptophan derivatives on the radiation-chemical yields of the radiolysis products of oxygen-saturated ethanol

Test compound	Radiation-chemical yield (G), molecule/100 eV		
	acetaldehyde	H_2O_2	additive decomposition
Additive-free	5.85 ± 0.46	5.81 ± 0.42	–
Pyrrole (**I**)	4.79 ± 0.42	6.58 ± 0.92	–0.28 ± 0.07
Indole (**II**)	7.41 ± 0.72	7.64 ± 0.86	–0.14 ± 0.13
Tryptophan (**III**)	2.91 ± 0.29	3.68 ± 0.56	–0.24 ± 0.17
5-Hydroxytryptophan (**IV**)	2.62 ± 0.29	3.48 ± 0.39	–0.05 ± 0.09
Serotonin (**V**)	2.41 ± 0.25	2.42 ± 0.24	–0.04 ± 0.05
Melatonin (**VI**)	5.09 ± 0.43	5.75 ± 0.57	–0.15 ± 0.08
Harman (**VII**)	5.82 ± 0.72	4.75 ± 0.76	–0.08 ± 0.09
Harmine (**VIII**)	5.34 ± 0.48	3.69 ± 0.44	–0.13 ± 0.11
Harmaline (**IX**)	5.25 ± 0.52	3.29 ± 0.28	–0.19 ± 0.10

Table 2. Effect of tryptophan derivatives on the radiation-chemical yields of the radiolysis products of a 1 M solution of ethanol saturated with oxygen at pH 7

Test compound	Radiation-chemical yield (G), molecule/100 eV		
	acetaldehyde	H_2O_2	additive decomposition
Additive-free	4.23 ± 0.42	3.31 ± 0.42	–
Pyrrole (**I**)	3.71 ± 0.44	3.94 ± 0.67	–0.17 ± 0.11
Indole (**II**)	3.96 ± 0.14	3.20 ± 0.33	–0.32 ± 0.18
Tryptophan (**III**)	4.29 ± 0.32	3.52 ± 0.30	–0.02 ± 0.15
5-Hydroxytryptophan (**IV**)	4.41 ± 0.50	3.97 ± 0.16	–0.31 ± 0.28
Serotonin (**V**)	4.49 ± 0.44	4.01 ± 0.44	–0.25 ± 0.18
Melatonin (**VI**)	4.31 ± 0.42	3.50 ± 0.24	–0.17 ± 0.18
Harman (**VII**)	3.89 ± 0.62	3.34 ± 0.26	–0.01 ± 0.03
Harmine (**VIII**)	4.17 ± 0.35	3.25 ± 0.27	–0.08 ± 0.23
Harmaline (**IX**)	4.17 ± 0.50	3.51 ± 0.11	–0.25 ± 0.04

To evaluate the reactivity of tryptophan and its derivatives toward nitrogen-centered radicals, we studied the kinetics of their interaction with DPPH using the stopped-flow method. For comparing the reactivity of tryptophan derivatives and classical antioxidants, we also studied the reactions of ascorbic acid, cysteine, Trolox, α-tocopherol, and δ-tocopherol with DPPH. Under the experimental conditions, the reactions of all of the test substances with DPPH strictly followed the pseudo-first-order rate law. Therefore, we used the pseudo-first-order reaction rate constants obtained upon the treatment of kinetic curves as a quantitative measure of the reactivity of compounds toward DPPH (Tables 3 and 4).

From the experimental data, it is evident that substances **I–III** and **VI–IX** are characterized by low rate constants of reactions with DPPH, which are at the level of the experimental error (Table 3). We couldn't detect their conversion products by chromatography–mass spectrometry. Consequently, substances **I–III** and **VI–IX** do not interact with nitrogen-centered radicals.

Among the nitrogen-containing test compounds, only 5-hydroxytryptophan (**IV**) and serotonin (**V**) exhibited pronounced reactivity toward DPPH; this finding indicates the determining role of the phenolic hydroxyl group in the formation of the radical-inhibiting properties of these compounds. It is likely that a considerable increase in the reaction rate constant on going from serotonin (**V**) to 5-hydroxytryptophan (**IV**) is due to the presence of the carboxyl group in the structure of the latter. Taking into account the weakly

Table 3. Reaction rate constants of the DPPH radical with the test tryptophan derivatives

Test compound	Pseudo-first-order rate constant $k \times 10^4$, s^{-1}
Pyrrole (**I**)	2.0 ± 0.1
Indole (**II**)	3.6 ± 0.1
Tryptophan (**III**)	2.2 ± 0.1
5-Hydroxytryptophan (**IV**)	388.6 ± 2.3
Serotonin (**V**)	50.2 ± 0.3
Melatonin (**VI**)	2.7 ± 0.1
Harman (**VII**)	1.7 ± 0.1
Harmine (**VIII**)	2.6 ± 0.1
Harmaline (**IX**)	3.2 ± 0.1

basic properties of DPPH, we cannot exclude the possibility of electrostatic interactions between its protonated form and the anion of 5-hydroxytryptophan (**IV**).

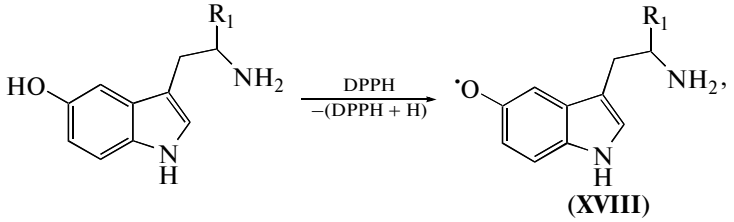
A comparison with the reaction rate constants of classical antioxidants (Table 4) clearly indicates that serotonin (**V**) and 5-hydroxytryptophan (**IV**) have moderate reactivity toward nitrogen-centered radicals. Thus, the reaction rate constant of 5-hydroxytryptophan (**IV**), which is the most reactive of the pair of compounds **IV** and **V**, was found to be four to five times lower than the rate constant for α-tocopherol (**XI**), comparable with the constant measured for δ-tocopherol (**XII**), and higher than that obtained for cysteine (**XIV**) by a factor of above 2. In this case, the reaction rate constants of substances **IV** and **V** were found to be lower than the rate constants measured for Trolox (**X**) and ascorbic acid (**XIII**) by one or two orders of magnitude. Note that the presence of the carboxyl group in the Trolox (**X**) molecule considerably increases the rate constant of its reaction with DPPH as compared with those of its natural structural analogs α- and δ-tocopherols. As in the case of 5-hydroxytryptophan (**IV**) and serotonin (**V**), the higher rate constant is most likely due to the electrostatic interaction of the Trolox anion with the protonated form of DPPH.

Table 4. Rate constants of the reactions of the DPPH radical with classical antioxidants

Test compound	Pseudo-first-order rate constant $k \times 10^4$, s^{-1}
Trolox (**X**)	7633.0 ± 45.8
α-Tocopherol (**XI**)	1676.0 ± 10.1
δ-Tocopherol (**XII**)	300.3 ± 1.8
Ascorbic acid (**XIII**)	11585.0 ± 69.5
Cysteine (**XIV**)	168.2 ± 1.1

To establish the mechanism of the reactions of 5-hydroxytryptophan (**IV**) and serotonin (**V**) with DPPH, we studied their final molecular products using liquid chromatography coupled to mass-spectrometric detection. For 5-hydroxytryptophan (**IV**), we identified the molecular ions of products at m/z of $439 = 2M_{\text{5-hydroxytryptophan}} - 2M_H + M_{H+}$, $461 = 2M_{\text{5-hydroxytryptophan}} - 2M_H + M_{Na+}$, $657 = 3M_{\text{5-hydroxytryptophan}} - 4M_H + M_{H+}$, and $614 = M_{\text{5-hydroxytryptophan}} - M_H + M(DPPH) + M_{H+}$ (Fig. 2, b1). The molecular ions with m/z of $351 = 2M_{\text{serotonin}} - 2M_H + M_{H+}$, $373 = 2M_{\text{serotonin}} - 2M_H + M_{Na+}$, $525 = 3M_{\text{serotonin}} - 4M_H + M_{H+}$, and $570 = M_{\text{serotonin}} + M_H - M(DPPH) + M_{H+}$ corresponded to the conversion products of serotonin (**V**) (Fig. 2, b2).

The experimental data suggest that as phenolic antioxidants, 5-hydroxytryptophan (**IV**) and serotonin (**V**) can be hydrogen atom donors in the reactions with DPPH (reaction scheme (16)). As a result, a phenoxyl radical (**XVIII**) is formed. This radical can undergo dimerization, for example, via reaction (18) yielding the identified products with the molecular ions at m/z 439 and 461 for 5-hydroxytryptophan (**IV**) or 351 and 373 for serotonin (**V**). Because of the keto–enol tautomerism, the dimers can undergo rearrangement with the formation of hydroxyl groups (reaction scheme (19)). Therefore, the dimers can interact with one additional DPPH particle analogously to reactions (16)–(18). The recombination of the phenoxyl radicals of dimers and **XVIII** is a source of the accumulation of trimers with the molecular ions at m/z 657 and 525, which were identified for compounds **IV** and **V**, respectively.



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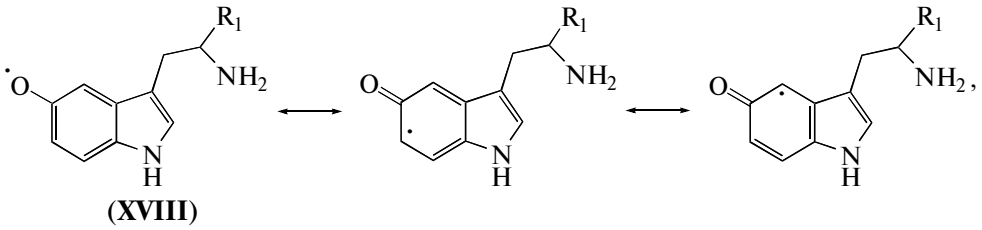
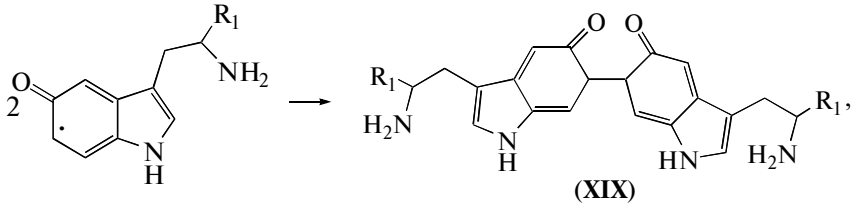
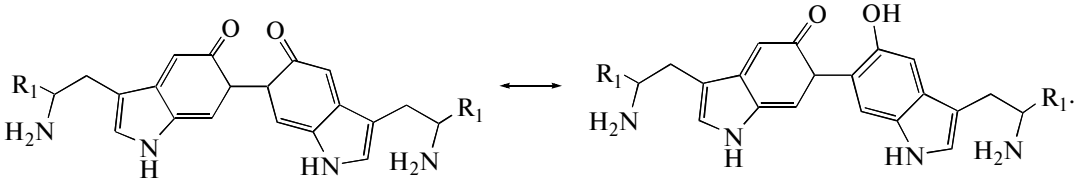


(XVIII) (17)



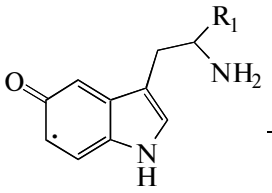
(XIX) (18)



(19)

5-Hydroxytryptophan (**IV**): R_1 = COOH.
Serotonin (**V**): R_1 = H.

The adducts of phenoxyl radicals with DPPH are formed as a result of the combination of phenoxyl radicals (**XVIII**) with DPPH [reaction scheme (20)]:



(XVIII)

$\xrightarrow{\text{DPPH}}$ Radical recombination product. (20)

5-Hydroxytryptophan (**IV**): R_1 = COOH.
Serotonin (**V**): R_1 = H.

Thus, serotonin and 5-hydroxytryptophan reduce the DPPH radical via the transfer of hydrogen atoms from the hydroxyl groups of the molecules, as a result of which the dimers and trimers of the initial compounds and the adducts of phenoxyl radicals with DPPH are formed. Compounds **I–III** and **VI–IX** lacking in phenolic hydroxyl groups do not interact with the nitrogen-centered DPPH radical.

CONCLUSIONS

The experimental data suggest that tryptophan (**III**), 5-hydroxytryptophan (**IV**), serotonin (**V**), and the β-carboline alkaloids harman (**VII**), harmine (**VIII**), and harmaline (**IX**) inhibit the radiation-chemical oxidation of ethanol, with these compounds being not consumed. Compounds **III–V** and **VII–IX** lose their antioxidant properties upon the protonation of the amino groups. Thus, the antioxidant properties of tryptophan (**III**), 5-hydroxytryptophan (**IV**), serotonin (**V**), harman (**VII**), harmine (**VIII**), and harmaline (**IX**) are due to the ability of these compounds to reduce oxygen-centered radicals via to the reaction of electron transfer from the lone pair of nitrogen atoms.

We found that pyrrole (**I**), indole (**II**), tryptophan (**III**), melatonin (**VI**), harman (**VII**), harmine (**VIII**), and harmaline (**IX**) possess low reactivity toward the nitrogen-centered DPPH radicals. The presence of a phenol-type hydroxyl group in the molecules of 5-hydroxytryptophan (**IV**) and serotonin (**V**) endows these compounds with the ability to reduce DPPH via hydrogen atom transfer. In this case, the main products of their interaction with DPPH are the dimers and trimers of 5-hydroxytryptophan (**IV**) and serotonin (**V**) and adducts with DPPH. We have found that serotonin (**V**) and 5-hydroxytryptophan (**IV**) are characterized by moderate reactivity toward the nitrogen-

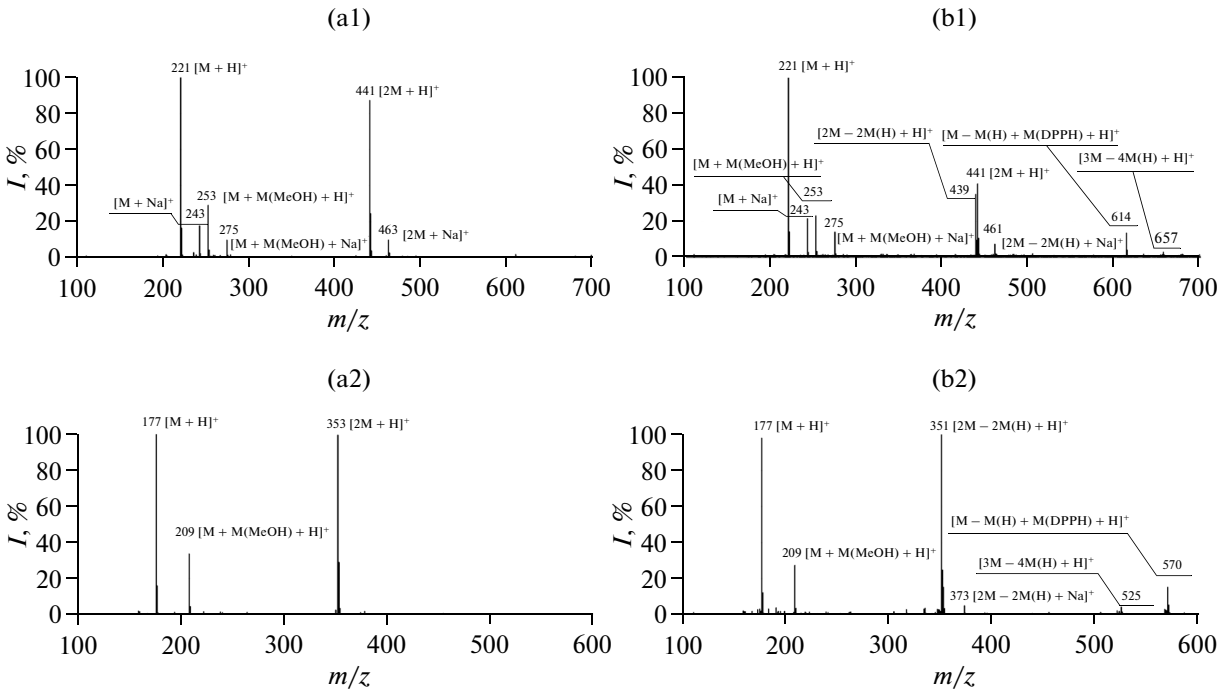


Fig. 2. Mass spectra of the (a1, b1) 5-hydroxytryptophan and (a2, b2) serotonin solutions obtained (a1, a2) before and (b1, b2) after a reaction with DPPH.

centered DPPH radical as compared with that of classical antioxidants.

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