Journal of Fluorescence, Vol. 12, No. 1, March 2002 (© 2002)

Fluorescence Emitted During the Autooxidation of 2,3,4,6-tetrahydroxy-5H-benzocyclohepten-5-one

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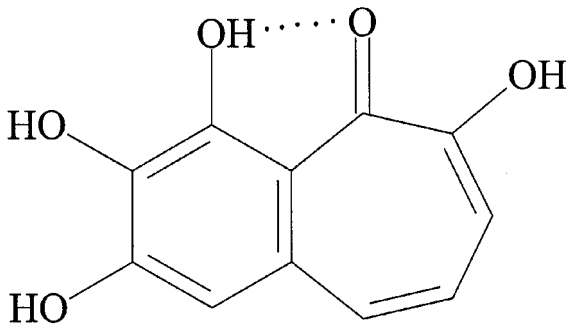
Received September 27, 2001; revised January 2, 2002; accepted January 3, 2002

Data suggest that the purpurogallin (2,3,4,6-tetrahydroxy-5H-benzocyclohepten-5-one, hydroxybenzotropolone) and its analogues formed from the polyphenols can be precursors of the most complex natural compounds—humic acids and melanin-like polymers. Therefore, to confirm this possibility, the autooxidation of purpurogallin to humus-like substances has been performed. The oxidation process has been assayed by means of fluorescence and UV/VIS absorption spectroscopy. The obtained results indicate that purpurogallin undergoes a free-radical–mediated autooxidation via purpurogallinquinones to humus-like polymers. The scheme of the complex transformation of purpurogallin has been proposed.

KEY WORDS: Hydroxybenzotropolone; humic acids; emission/absorption; ecological aspects.

INTRODUCTION

Purpurogallin (PPG = 2,3,4,6-tetrahydroxy-5H-benzocyclohepten-5-one) is an unusual, hydroxybenzotropolone-type compound. The molecule of PPG contains the tropolone 7-C-element aromatic ring (number of delocalized electrons = 6, equal to the number required for fulfillment of the Hückel rule 4n + 2), condensed with the 1,2,3-trihydroxybenzene ring (pyrogallol) and the hydrogen bond between C=O and −OH groups:



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This compound occurs in nature as a purple pigment in galls (*Cecidia, Galla*) on the leaves of *Quercus penduculata, Salix fragilis, Ciprinus betulus*, and other plants (e.g., in certain barks in the form of glucosides).

PPG reveals certain interesting physicochemical properties, such as strong absorption A over a wide spectral range (UV/VIS-IR), fluorescence Fl and phosphorescence Ph as a result of efficient intersystem crossing, chemiluminescence CL, diamagnetism in solid state, and microbiological resistance [1–7]. Recently it has been found that PPG may be a particularly promising antioxidant, scavenging reactive oxygen species ROS generated by polymorphonuclear leukocytes [4] and by three types of human cardiovascular cells [5]. It also plays the role of an active cytoprotector [6], effectively chelates Fe^{2+} ions, and suppresses the formation of the $OH^{\cdot}$ radical in the Fenton reaction. PPG oxidized enzymatically or in an alkaline solution exhibits relatively strong chemiluminescence and a deep-blue stable free radical of the semiquinone type [1–3,7]. Recently it has been shown that the 3,4,6-trihydroxy-5H-benzocyclohepten-5-one radical plays a dominant role in the transformation of theaflavins and their physiological activity [8].

There are data suggesting that PPG and its analogues formed from plant polyphenols can be precursors of the

1053-0509/02/0300-0083/0

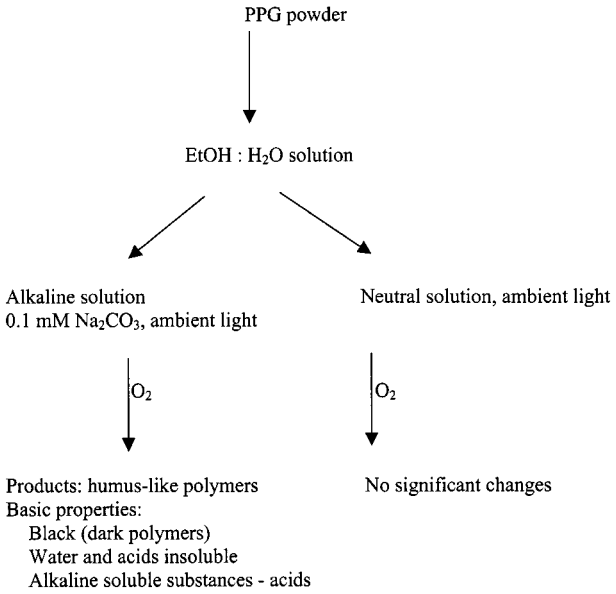
most complex [10] and ubiquitous compounds in the biosphere—humic acids (HA) and melanin-like polymers [1–3,7,9,15,17,18,21]. To prove this possibility, we performed the auto-oxidation of PPG to humus-like substances.

MATERIALS AND METHODS

The oxidation process of PPG in neutral/alkaline solution (PPG in EtOH : H_2O = 1:10 v/v, [PPG] = 0.1 mM) with/without VIS-range light and/or 0.1 mM Na_2CO_3 by atmospheric oxygen ([O_2] = 0.7 mM) to macromolecular humus-like polymers was performed. The molecular spectroscopy techniques fluorescence and UV/VIS absorption have been used for investigation of the reaction progress.

The technical-grade commercial PPG compound (Aldrich P-5590-2, 15 g) was purified by slow crystallization from diethyl ether saturated solution. Finally, 2.3 g sample of the purificated PPG powder was obtained. The purity of the PPG was confirmed by the absorption UV/VIS spectrum measurement and comparison with literature data [1–3]. Other reagents were purchased from POCh Gliwice, Poland (puriss. grade).

The experiment was performed according to the following scheme:



Aliquots of the alkaline PPG solution were analyzed by spectrofluorometry using the emission/excitation mode (Shimadzu RF 510 PC) and UV/VIS spectrophotometry (Jasco V-530).

RESULTS AND DISCUSSION

Fluorescence

It is known [1–3] that non-irradiated, pure PPG in neutral solution ([PPG] = 0.1 mM, EtOH : H_2O = 1:10 v/v) exhibits very weak fluorescence emission intensity I_{em} (Fig. 1 p. 1i). In alkaline environment (0.1 mM PPG in 0.1 mM Na_2CO_3, EtOH : H_2O = 1:10 v/v) I_{em} significantly increases during the reaction progress (Fig. 1 p. 1ii). It should be noted that the observed fluorescence of PPG during autooxidation is a new phenomenon.

The fluorescence of PPG occurs in the excitation/emission wavelength region 200–400/300–600 nm, respectively. In fluorescence measurements of strongly absorbing PPG, the self-absorption effect may be important, and therefore the intensity values (I_{em}, I_{ex}) were corrected using the following formula:

$$I_0 = \frac{IA}{1 - \exp(-A)} \qquad (1)$$

where I_0 indicates the true fluorescence intensity, I is the observed intensity, and A half of the measured absorbance. There is no considerable difference between values of the corrected and uncorrected results beyond 300 nm, because the concentration of PPG used in the fluorescence measurement was low (0.1 mM), and the self-absorption (inner filter) has an imperceptible effect.

In the course of auto oxidation of PPG in alkaline solution a blue-green fluorescence emission does appear. Several fluorescers, which have never been exactly iso-

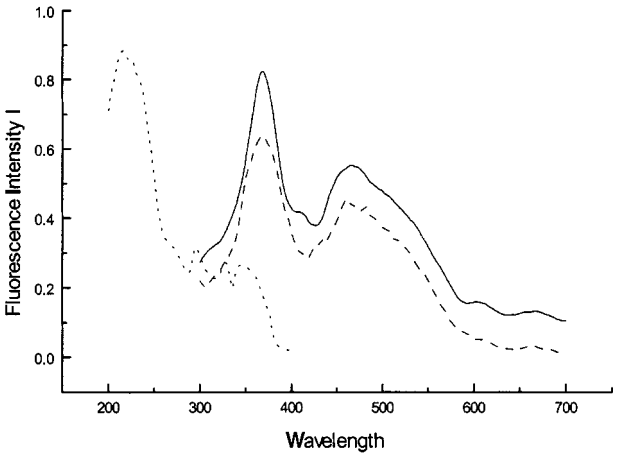


Fig. 1. 1. PPG fluorescence emission spectra: (i) Neutral solution (dash line, EtOH : H_2O = 1:10 v/v, [PPG] = 0.1 mM) (ii) Alkaline solution (solid line, 0.1 mM PPG in 0.1 mM Na_2CO_3, EtOH : H_2O = 1:10 v/v) 10 minutes after the reaction initiation, λ_{ex} = 220 nm. 2. PPG fluorescence excitation spectrum in wavelength region 200–400 nm (dots).

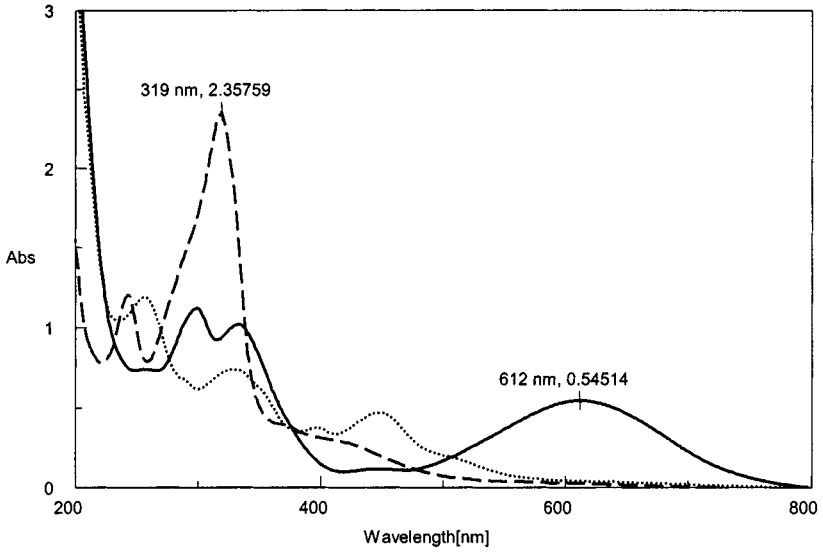


Fig. 2. 1. Absorption spectrum of purpurogallin (PPG) in EtOH : H_2O = 1:10 v/v solution, [PPG] = 0.1 mM (dash line). 2. PPG + 0.7 mM O_2 + 0.1 mM Na_2CO_3 solution 3 minutes after the redox reaction initiation. The absorption maximum at 612 nm corresponds to the deep-blue PPG-semiquinones (solid line). 3. Absorption spectrum 60 minutes after the reaction initiation, resembling that of humic acids (dots).

lated and identified, have to account for the observed broad fluorescence emission spectrum with characteristic maxima at 370 and 470 nm. In such a case, this spectral shape (see Fig. 1 p. 1ii) suggests that these products might be considered as tropolone derivatives containing polar groups [3]. With increasing time of reaction progress, the intensity and shape of the fluorescence spectrum become generally similar to those of previously [11,15,19,20] measured HA.

The fluorescence excitation spectrum of the PPG in neutral solution displays maxima at 220, 300, 335, and 355 nm. The short-wavelength excitation maximum at 220 nm is the strongest one, and this is why we used that wavelength as λ_{ex} in all fluorescence emission measurements. Because the excitation spectra do not look like the absorption spectra (Fig. 1 p. 2 and Fig. 2 p. 1), one can deduce that quenching and excitation energy transfer (EET) processes play a significant role in the spectroscopic behavior of irradiated PPG.

UV/VIS absorption

The absorption spectrum of PPG in neutral solution (EtOH : H_2O = 1:10 v/v, [PPG] = 0.1 mM) shows maxima at 245 nm (Abs = 1.2) and 319 nm (strongest maximum, Abs = 2.35) wavelengths (Fig. 2 p. 1). The origin of the strongest maximum at wavelength 319 nm can be explained as the following. As a result of the conjugation of the electrons from the C=O chromophore with the π-delocalized electrons from the tropolone and benzene aromatic rings, a bathochromic shift takes place, and absorption at this wavelength indicates a $\pi -> \pi^*$ transition in C=O chromophore. Without conjugation with the π-delocalized electrons, $\pi -> \pi^*$ transition in C=O occurs at 250 nm.

PPG auto oxidized in the alkaline solutions (0.1 mM PPG in 0.1 mM Na_2CO_3, EtOH : H_2O = 1:10 v/v) 3 minutes after the redox reaction initiation, forming a deep-blue anion of PPG^- of the semiquinone type [1–3,9,17,18] of stable free radical $SQ^{\cdot}$ (Fig. 2 p. 2), strongly absorbing at 612 nm. In this step, the OH groups of the phenolic ring of PPG undergo oxidation, leading to the intermediate $SQ^{\cdot}$.

The absorption spectrum (Fig. 2 p. 3, reaction solution, 60 minutes after the reaction initiation) characterizes a shape generally resembling that of HA or melanins [11,12,15,19–21]. It is known that absorption in the range 250–385 nm is characteristic for carbonyl derivatives of tropolone [1–3].

It should be noted that a distinct isosbestic point at 378 nm (Abs = 0.35) was obtained. The total absorbance value at this wavelength consists of at least absorbances of the substrate PPG, deep-blue $SQ^{\cdot}$ and products: humus-like polymers.

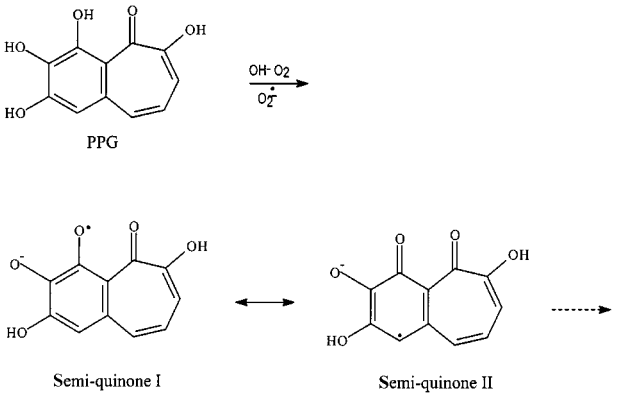
The oxidation process of the PPG, leading to humus-like polymers, proceeds by several steps. This seems to

be confirmed by the fact that several types of absorbing species are formed in the course of PPG oxidation, as described above.

CONCLUSIONS

Taking all of these considerations into account, we propose the following multi-step scheme of the PPG redox conversions to humus-like polymers, that proceeds via free-radical mechanism:

Stage I. Semiquinone radical ($SQ^{\cdot}$) generation from the PPG in the reaction of oxygenation:



Semi-quinone I and semi-quinone II are two resonance forms.

Stage II.

first possibility:

$SS^{\cdot} + SQ^{\cdot}$ (recombinant)

$\rightarrow$ dimer (SQ-SQ) [without PPG polymers]

second possibility:

$SQ^{\cdot} + SQ^{\cdot}$ (dismutation) $\rightarrow$ PPG + PPG-quinone

Stage III. After stage II, further polymerization is possible:

$$\text{PPQ-quinone} \xrightarrow{\text{polymerization}} \text{PPG polymers resembling HA}$$

It is worthwhile noting that these processes may play a significant ecological role as polyphenols and flavonoids are the major plant constituents undergoing transformation to HA [2,7–9,15]. It is very possible that these processes proceed via the hydroxybenzotropolone moiety. Therefore further adequate, detailed investigations are still required and the exact mechanisms remain to be established.

ACKNOWLEDGMENTS

The work was financially supported by the Polish State Committee for Scientific Research by the grant KBN 7 T09B 057 20 and by the Poznan University of Technology grant BW 31-015/2001.

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