First Evidence of Pheomelanin-UVA-Driven Synthesis of Pummerer’s Ketones by Peroxidase-Mediated Oxidative Coupling of Substituted Phenols

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ABSTRACT: Photoexcitation of pheomelanin produces high-energy singlet oxygen and the superoxide anion, which are reactive species in damage of cellular targets. In principle, these species can be involved in processes of synthetic utility when adequate experimental conditions are defined. Here, we describe that pheomelanin performs as a selective UVA antenna for the horseradish peroxidase oxidative coupling of substituted phenols to biologically active Pummerer’s ketones under 2-methyltetrahydrofuran/buffer biphasic conditions. In this system, singlet oxygen is scavenged by conversion of 2-methyltetrahydrofuran into the corresponding organic hydroperoxide, while the superoxide anion is dismutated into hydrogen peroxide. Both these intermediates are able to oxidize the active site of horseradish peroxidase triggering the oxidative coupling reaction. Trimer derivatives, produced by addition of phenoxy radicals on preformed Pummerer’s ketones were also isolated, suggesting the possibility to further improve the structural complexity of the reaction products.

INTRODUCTION

Pheomelanin is a product of random polymerization of cysteinyl-dopa precursors via benzothiazole and benzothiazine intermediates. It is commonly considered as a UVA photosensitizing agent in cellular damage, as a consequence of the generation of singlet oxygen and the superoxide anion and other radical centered oxygen species (ROS). Alternatively, excited pheomelanin can be reduced by accepting electrons from biological reductants (e.g., NAD(P)H and glutathione) and organic substrates. Examples of generation of singlet oxygen by pheomelanin at a higher value of wavelength than UVA are reported as in the case of blue-light photoreactions of meso-tetraphenyl porphyrin (meso-TPP) is selectively trapped by 2-methyltetrahydrofuran (2-MeTHF) to produce the corresponding organic hydroperoxide in the activation of horseradish peroxidase (HRP). This procedure avoided the inhibition of HRP by an excess of H₂O₂ under experimental conditions simpler than photochemical reduction of dioxygen by natural or synthetic cofactors. Moreover, the superoxide anion is responsible for the transformation of the ferric heme-prosthetic group (native form of HRP) to inactive compound III (oxy-
ketones, which represent synthons for different natural products, are synthesized by oxidative coupling of substituted phenols in a protective group-free procedure by pheomelanin-UVA-driven activation of HRP. In this study, 2-methyltetrahydrofuran (2-MeTHF) was used as a sustainable solvent to contemporarily transformed to H$_2$O$_2$ under acidic conditions. We compared the photocatalytic efficiency of two synthetic pheomelanins synthesized from l-cysteine and l-Dopa by chemical oxidation with sodium permanganate (PM-K) or, in alternative, by mushroom tyrosine enzymatic polymerization (PM-T). The pheomelanin samples showed a different efficacy in the oxidative coupling of phenols probably due to their specific content of photoactive aromatic subunits. In particular, the synthesis of Pummerer’s ketones was highly efficient and selective at pH 6 and room temperature in the presence of PM-K, with the yield of desired products being higher in biphasic conditions (2-MeTHF/buffer) than in the monophasic counterpart (2-MeTHF).

## RESULTS AND DISCUSSION

### Preparation of Pheomelanin Samples.

PM-K and PM-T were synthesized starting from l-cysteine and l-Dopa by a modification of previously reported procedures\(^{29,30}\) (experimental details are in Supporting Information S1 and S2, respectively). UV–vis analysis of samples (Figure S1) showed the absorption bands expected for pheomelanin. Field-emission scanning electron microscopy (FESEM) of PM-K confirmed the formation of spherical particles with an average diameter of 100 nm (Figure S2, panel A). Instead, PM-T showed particles with an irregular morphology (Figure S2, panel B). Energy-dispersive X-ray elemental analysis (EDX) quantified the incorporation of cysteine in the samples in the amounts of 15.46 and 6.56 wt % of sulfur atoms (Table S1; EDX of PM-K is in Figure S3). Electron paramagnetic resonance (EPR) spectra of PM-K were characterized by a \(g\) value of 2.004 in accordance with the literature (Figure S4, panel A).\(^{31}\) Instead, the EPR signal of PM-T was not well-resolved (Figure S4, panel B). The UHPLC–MS analysis (Figure S5, panel A) of PM-K showed 4-methylthiazole as a repetitive fragment (m/z 100) typical of the benzothiazole subunit of pheomelanin, while the pyrrole fragment (m/z 68), associated to DHICA subunits of eumelanin, prevailed in the case PM-T (Figure S5, panel B). Finally, the C–S stretching vibration mode at 700–600 cm\(^{-1}\) was detected in the FT-IR analysis of both samples (Figure S6, panel A and panel B). Overall, PM-K was characterized by higher pheomelanin character than PM-T. These data were in accordance with the low content of aromatic subunits previously reported for PM-T with respect to PM-K.\(^{28}\)

### Synthesis of Pummerer’s Ketones.

The activity of HRP was initially evaluated by the pyrogallol assay in dark conditions and in the presence of PM-K and PM-T at pH 6 and pH 7, respectively.\(^{22,35}\) As reported in Table 1 (entry 1 versus entries 4 and 6), the enzymatic activity was not significantly modified with respect to the reference (HRP alone) in the presence of pheomelanin samples at pH 7, while a slight decrease of the enzymatic activity was observed at pH 6, probably due to the occurrence of supramolecular interactions between HRP and pheomelanin (Table 1, entry 2 versus entries 5 and 7).\(^{34,35}\) In a similar way, HRP retained the original activity after UVA irradiation at pH 7 for 24 h at 27 °C (Table 1, entry 3 versus entry 1).\(^{36}\)

We started our investigation analyzing the oxidation of para-cresol 1 in the presence of PM-K under biphasic conditions.\(^{20}\) The schematic representation of the biphasic system is in Figure 2. It includes (i) the photoactivation of pheomelanin and intercrossing system decay to the triplet state and successive generation of singlet oxygen (pathway A); (ii) the formation of the organic hydroperoxide I by selective carbon-alpha insertion of singlet oxygen into the tertiary carbon atom of 2-MeTHF and diffusion of hydroperoxide I from the organic layer to the buffer (pathway B); (iii) the activation of HRP and successive oxidative radical coupling (pathway C).

In a similar way, HRP retained the activity after UVA irradiation at pH 7 for 24 h at 27 °C (Table 1, entry 3 versus entry 1).\(^{36}\)

| entry | photosensitizer | pH | activity (U/mg) |
|-------|----------------|----|----------------|
| 1     | none           | 7  | 126.1          |
| 2     | none           | 6  | 90.2           |
| 3     | none           | 6  | 118.7          |
| 4     | PM-K           | 7  | 132.1          |
| 5     | PM-K           | 6  | 90             |
| 6     | PM-T           | 7  | 122.6          |
| 7     | PM-T           | 6  | 104.1          |

\(^{a}\)Experiments were performed after UVA irradiation of HRP. All the reactions were conducted in triplicate.

Note that the reaction was ineffective in a dark reactor, under anaerobic conditions (argon atmosphere), and in the absence of HRP, as well as in the presence of alternative organic solvents lacking a tertiary C–H bond, such as petroleum ether or n-hexane (S3). Pummerer’s ketone

\(^{20}\) 2-MeTHF is a green and water-immiscible solvent deprived of any toxic effect.\(^{37,38}\) The solution was gently stirred (200 rpm) at 27 °C for 48 h in an air atmosphere under UVA irradiation (365 nm). Irrespective of the experimental conditions, 4a,9b-dihydro-8,9b-dimethyl-3(4H)-dibenzo[ab]naphthene 2 (Pummerer’s ketone) was obtained as the main reaction product, in addition to 2,2′-dihydroxy-5,5′-dimethyl-diphenyl 3 (ortho–ortho dimer) as a side product (Scheme 1 and Table 2, entry 1), highlighting the effective capacity of PM-K to act as a photosensitizer in the synthesis of the Pummerer’s ketone.
showed all the characteristic NMR signals expected for the tricyclic structure. In particular, the presence of the H-4 hydrogen signal at 4.71 ppm (multiplet) coupled with the adjacent \( \text{CH}_2 \) group (double doublet centered at 3.05 ppm) confirmed the closure of the central tetrahydrofuran ring. In this latter case, the oxidative coupling proceeded by coupling of the \( \text{ortho} \)- and \( \text{para} \)-radical mesomeric forms of the substrate followed by internal Michael addition (Figure 2, panel A).

As an alternative, dimer 3 was derived from \( \text{ortho} \)-\( \text{ortho} \) oxidative coupling.

Compounds 2 and 3 were previously obtained by oxidation of compound 1 with HRP and an excess of \( \text{H}_2\text{O}_2 \) in a lower yield. The effective formation of hydroperoxide I during UVA irradiation of PM-K was evaluated by the pyrogallol assay performed at pH 7 and pH 6 and at different reaction times (1, 4, 24, and 48 h). As reported in Figure S7 (panel A), the concentration of hydroperoxide I increased by increasing the irradiation time, reaching the highest value after 48 h (47.5 and 49.4 \( \mu \text{mol/mL} \), respectively). A similar trend was observed in the case of PM-T (Figure S7, panel B). Once formed, hydroperoxide I recognized the ferric heme (Fe(III)) of HRP by neighboring group participation of the endocyclic oxygen followed by delivery of the oxygen atom and regeneration of 2-MeTHF.

The reaction was repeated in the absence of HRP using coumarin as a selective OH scavenger (“coumarin assay”) to exclude the formation of hydroxyl radicals (OH). No trace amounts of OH were detected in the reaction mixture confirming the concerted nature of the transfer of the oxygen atom in the formation of the hydroperoxide I (Figure S8).

From the synthetic point of view, the Pummerer’s ketone was obtained in the highest yield in the presence of 17 units of HRP (Table 2, entry 3 versus entries 1 and 2). The low mass balance observed in the presence of 68 units of HRP was probably due to the occurrence of oligomerization of

### Table 2. Pheomelanin UVA-Driven HRP-Mediated Oxidative Coupling of \( \text{para} \)-Cresol 1 in Biphasic Conditions

| entry | photosensitizer (amount % w/w) | pH | HRP (U/mg) | conversion (%) | product(s) | yield (%)<sup>a</sup> |
|-------|-------------------------------|----|------------|----------------|------------|---------------------|
| 1     | PM-K (1.0%)                   | 7  | 68         | 73             | 2(3)       | 30.1(5)             |
| 2     | PM-K (1.0%)                   | 7  | 34         | 70             | 2(3)       | 38.7(11)            |
| 3     | PM-K (1.0%)                   | 7  | 17         | 66             | 2(3)       | 41.8(16)            |
| 4     | PM-K (1.0%)                   | 6  | 17         | 76             | 2(3)[4]    | 50.7(16.3)[1.1]    |
| 5     | PM-K (0.5%)                   | 6  | 17         | 71             | 2(3)[4]    | 28.7(12)[1.6]      |
| 6     | PM-K (0.25%)                  | 6  | 17         | 49             | 2(3)       | 18.0(2.1)           |
| 7     | PM-T (1.0%)                   | 6  | 17         | 37             | 2(3)       | 34(4)              |
| 8     | PM-T (0.5%)                   | 6  | 17         | 23             | 2(3)       | 26.4(3)            |
| 9     | PM-T (0.25%)                  | 6  | 17         | 12             | 2(3)       | 22(2)              |

<sup>a</sup>Yield was calculated based on the amount of the converted substrate. All the reactions were conducted in triplicate.
compound 3 to high polar derivatives not isolated under our experimental conditions, in accordance with data previously reported. This effect was explained on the basis of the thermodynamic requirement of the successive oxidation of compound 3 in order to complete the single cycle of catalytic turnover of compound I (HRP-I) to compound II (HRP-II) and native HRP, a process that is operative in the presence of a relatively high amount of the enzyme.

In order to optimize the yield of Pummerer’s ketone, the reaction was repeated at pH 6 in the presence of 17 units of HRP and different amounts of PM-K (1.0, 0.5, and 0.25% w/w with respect to the substrate). Again, the Pummerer’s ketone was isolated as the main reaction product, in addition to dimer 3 and a low amount of 1,1′-dimethyl-[1,1′-bi(cyclohexane)]-2,2′,5,5′-tetraene-4,4′-dione 4 (para–para dimer) (Scheme 1) in the presence of 1.0 and 0.5% w/w of PM-K (Table 2, entries 4 and 5). The HRP-mediated synthesis of compound 4 was not previously described. Examples of the formation of para–para dimers by oxidative coupling of phenol derivatives have been reported only in the case of para-unsubstituted derivatives, with the only exception of α-cumyl. Note that Pummerer’s ketone 2 was obtained in a higher yield at pH 6 than pH 7 (Table 2, entry 4 versus entry 1), confirming the beneficial effect of the acidic medium. pH 6 also favored a high value of the overall mass balance of the reaction. On the other hand, the yield of compound 2, the conversion of the substrate, and the overall mass balance of the reaction were found to be decreased by decreasing the amount of PM-K (Table 2), highlighting the fundamental role played by pheomelanin in the reaction. Next, we studied the oxidation of compound 1 with 17 units of HRP and PM-T (1.0, 0.50, and 0.25% w/w) at pH 6. As a general trend, PM-T afforded compound 2 in the conversion of the substrate and with the yield of the product lower than PM-K (Table 2, entries 7–9 versus entries 4–6). Again, the yield of compound 2 and the conversion of the substrate decreased by decreasing the amount of PM-T.

In order to generalize the procedure, the reaction was repeated using a panel of phenols, including 3,4-dimethyl phenol 5, 4-ethyl phenol 6, 4-methoxy phenol 7, and chlorophenol 8, characterized by substituents with different steric hindrance and stereoelectronic effects. The oxidation of

| entry | substrate R | R1 | pH | conversion (%) | product(s) | yield (%) |
|-------|-------------|----|----|----------------|------------|-----------|
| 1     | Me Me H     | 6  | 58 | 5a(5b)[5c]     | 38.6(6.3)[18.1] |
| 2     | Me Me H     | 7  | 50 | 5a(5b)[5c]     | 26.7(3.2)[13.7] |
| 3     | Me Me H     | 6  | 43 | 5a[5c]         | 23.1[9.3]   |
| 4     | Et H H      | 6  | 70 | 6a(6c)[6d]     | 41.4[7.2](6.8) |
| 5     | Et H H      | 6  | 86 | 6a(6b)[6c][6d] | 35.4(16.3)[4.3](4.1) |
| 6     | OMe H H     | 6  | 30 | 7b[7e]        | 6.12[21.2]  |
| 7     | Cl H H      | 6  | 60 | 8b            | 27.2       |

Yield was calculated based on the amount of the converted substrate. All the reactions were conducted in triplicate. Reaction performed with PM-K. Reaction performed with PM-T.

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5, 6a, 5b, 5c: R=Me; R2=H; 6, 6a, 6b, 6c, 6d: R1=R2=H, R=Et; 7, 7b, 7e: R1=R2=H, R=O-Me; 8, 8b: R1=R2=H, R=Cl
compound 5 performed at both pH 6 and pH 7 afforded 4a,9b-dihydro-1,7,8,9b-tetramethyl-3(4H)-dibenzo[1,3]dioxophenone 5a as the main reaction product, in addition to 2,2′-dihydroxy-5,5′-6,6′-tetramethyldiphenyl 5b, and the spirobenzo[1,3]-dioxo trimer 5c (Scheme 2 and Table 3, entries 1 and 2). The formation of compound 5c was unexpected. It showed a structure similar to compound II (Scheme 2, highlighted structure) previously isolated after anodic oxidation of 2,4-dimethylenephene. In accordance with these data, compound 5c was probably formed by successive addition of the phenoxyl radical on the enol form of Pummerer’s ketone 5a followed by monocentric transfer and formation of a carboxylation intermediate.

When the reaction was repeated in the presence of PM-T at pH 6, compounds 5a and 5c were obtained as the only recovered products in the conversion of the substrate and with the yield of the product lower than PM-K (Table 3, entry 3). The oxidative coupling of compound 6 with PM-K at pH 6 afforded 4a,9b-dihydro-8,9b-diethyl-3(4H)-dibenzo[1,3]dioxophenone 6a as the main reaction product, in addition to the spirobenzo[1,3]-dioxo trimer derivative 6c and the para–para dimer 6d (Scheme 2 and Table 2, entry 4). In this latter case, the ortho–ortho dimer was not isolated probably due to the occurrence of oligomerization side processes. Low amounts of compounds 6a, 6c, and 6d were obtained in the presence of PM-T under similar experimental conditions (Table 3, entry 5), with the ortho–ortho dimer 6b being detected albeit in a low amount. A different behavior was observed in the oxidation of 4-methoxyphenol 7 with PM-K at pH 6, in which case, 5,5′-dimethoxy-[1,1′-biphenyl]-2,2′-diol 7e was isolated as the main reaction product, in addition to the ortho–ortho dimer 7b (Table 2, entry 6). Probably, the dibenzofuran derivative 7e was obtained from the corresponding Pummerer’s ketone by elimination of MeOH from the adjacent 4a and 9b positions. The oxidation of chlorophenol 8 afforded 5,5′-dichloro-[1,1′-biphenyl]-2,2′-diol 8b in a low yield and overall mass balance (Table 3, entry 7), probably due to the occurrence of dehalogenation processes.

Finally, the oxidation of compounds 1, 5, and 6 was studied in monophasic-like conditions by reducing the amount of the buffer in the reaction mixture just to attain the hydration shell of the enzyme. As a general procedure, the selected substrate (0.5 mmol) was dissolved in 2-MeTHF (3.0 mL) in the presence of PM-K (1% w/w) followed by irradiation for 48 h at 25 °C. Note that PM-K was solubilized only in part in this system. Under these experimental conditions, the substrates were converted in a lower amount than a previously reported biphasic system to afford desired products in a low yield, suggesting the loss of activity of HRP associated to the low dispersion of PM-K in the reaction medium (Table 4, entries 1–3). In the case of compounds 1 and 5, the Pummerer’s ketones 2 and 5a were again obtained as the main reaction products, in addition to ortho–ortho dimers 3 and 5b, respectively, while compound 6 afforded the para–para dimer 6d as the only recovered product. These results confirmed the detrimental effect shown by tolune in the oxidative coupling of compound 1 with HRP and an excess of H₂O₂.

## Conclusions

We developed a novel photobiocatalytic system in which pheomelanin works as an antenna for the UVA-driven activation of HRP in the oxidative coupling of substituted phenols to yield bioactive Pummerer’s ketones. Examples of natural substances bearing the Pummerer’s ketone-like tricyclic structure are galantamine, codeine, and usnic acid. In the designed system, singlet oxygen produced by pheomelanin was converted into 2-MeTHF hydroperoxide, while the superoxide anion was dismutated in H₂O₂. PM-K was more efficient than PM-T, probably due to a higher amount of aromatic subunits. The optimal experimental conditions included the use of 1.0% w/w PM-K, 17 U of HRP, and pH 6, in order to facilitate the dismutation of the superoxide anion. The yield of Pummerer’s ketone was higher in the biphasic system with respect to the monophasic counterpart, suggesting the partial loss of activity of HRP as a consequence of the reduction of the amount of the buffer, associated to the low solubility shown by pheomelanin in 2-MeTHF. Note that the Pummerer’s ketones were obtained in yield and selectivity higher than those of electrochemical, enzymatic, and chemical approaches. Dimers and spirobenzo[1,3]-dioxo trimer were also isolated from the reaction mixture, with the latter compounds being produced by successive addition of the phenoxyl radical on the preformed Pummerer’s ketone. The synthesis of trimers from Pummerer’s ketones was previously reported only by an electrochemical process. Overall, these results open a new entry for the use of pheomelanin in HRP-mediated photochemical synthetic transformations, avoiding the use of tedious reducing cofactors and the occurrence of undesired and unselective radical side reactions.

### Experimental Section

**Materials and Methods.** Horseradish peroxidase (EC 1.11.1.7), reagents, and solvents were obtained from a commercial supplier Merck KGaA, Darmstadt, Germany. UV–visible (UV–vis) spectra were recorded using a Cary 60 UV–vis spectrophotometer, Agilent, Santa Clara, USA. Chemical reactions were monitored using thin-layer chromatography on precoated aluminum silica gel Merck 60 F254 plates, and a UV lamp (λ_max = 254 nm) was used for visualization. A Merck silica gel 60 (230–400 mesh) was used for flash chromatography applying the indicated mobile phase. All products were dried in high vacuum (10−3 mbar). 1H NMR and 13C NMR were recorded on a Bruker Avance DRX-400 (400 MHz/100 MHz) spectrometer. Chemical shifts for protons and carbons are reported in parts per million (δ scale) and internally referenced to the CDCl₃ signal at 7.0 ppm. Coupling constants (J) are reported in Hz. Multiplicities are reported in the conventional form: s = singlet, d = doublet, t = triplet, dd = double of doublets, and m = multiplet. HPLC analysis was performed by an Ultimate 3000 Rapid Resolution UHPLC system (Thermo Fisher Scientific) equipped with an Alltima C18 (250 mm × 4.6 mm, 5 mm) column and a...
multimwavelength detector. The UVA apparatus consisted of four lamps (wavelength, 365 nm).

**HRP Activity Assay.** The enzymatic activity of HRP was determined spectrophotometrically by the pyrogallol assay. The analysis was conducted in a quartz cuvette containing a mixture of pyrogallol (0.8 mmol), phosphate buffer (0.1 M, pH 6), H₂O₂ (30%), and a suitable amount of the enzyme (0.45, 0.67, and 0.75 U/mL). The oxidation of pyrogallol was followed by an absorbance increase at 420 nm. One unit activity of HRP is the amount of the enzyme that transforms 1.0 μmol of pyrogallol per minute at pH 6 and pH 7 at 25 °C.

**Evaluation of Enzymatic Activity of HRP in the Presence of PM-K.** The enzymatic activity of HRP in the presence of PM-K was determined spectrophotometrically by the pyrogallol assay. The analysis was conducted in a quartz cuvette containing a mixture of pyrogallol (0.8 mmol, 50 mg/mL), phosphate buffer (0.1 M, pH 6), H₂O₂ (30%), and PM-K (1% mol), and a suitable amount of the enzyme (0.45, 0.67, and 0.75 U/mL). The oxidation of pyrogallol to obtain purpurigallin was followed by absorbance increase at 420 nm at 25 °C.

**Coumarin Assay.** Coumarin (0.2 mmol) was dissolved in 2-MeTHF (4.0 mL); then, 2.0 mL volumes of PBS (0.1 M, pH 6.0) and PM-K/PM-T were added, and the mixture was stirred under UVA irradiation at 25 °C for 48 h under an air atmosphere. At scheduled times (1, 24, and 48 h), the organic phase (100 μL) was picked up and analyzed by a UV−vis spectrophotometer with coumarin and 7-hydroxy-coumarin as references. Results are reported in Figure S8.

**General Procedure for the Synthesis of Pummerer’s Ketones and Dimers and Derivatives under Biphasic Conditions.** Compounds 1 and 5−8 (1.0 mmol) were dissolved in 2-MeTHF (5.0 mL) and treated with the appropriate amount of HRP (17, 34, and 68 U) in PBS (5.0 mL) in the presence of PM-K or, in alternative, PM-T (5 mg; 1.0% w/w with respect to the substrate) at the appropriate pH value. The biphase system was gently stirred (200 rpm) under UVA irradiation and an air atmosphere at 25 °C for 48 h. The organic phase was separated, washed with brine (3 × 4 mL), dried over sodium sulfate, and evaporated under vacuum. The crude product was purified by flash chromatography. The original 1H NMR and 13C NMR spectra of reaction products are reported in the SI.

**4a,9b-Dihydro-8,9b-dimethyl-3(4H)-dibenzo- furanone 2.** Oil. Elemental analysis for C₁₀H₁₂O₄ expected values: C, 78.48; H, 6.59; O, 14.93; calculated values: C, 78.46; H, 6.59; O, 14.97. MS (EI, 70 eV) m/z 214.10 (100%). 1H NMR (400 MHz, CDCl₃): δ 7.08 (1H, s, CH), 6.73 (1H, d, J = 8.0 Hz, CH), 6.47 (2H, m, 2 × CH), 5.94 (1H, d, J = 8.0 Hz, CH), 5.95 (1H, d, J = 10.8 Hz, CH), 4.71 (1H, m, CH), 3.05 (1H, dd, J = 17.4 Hz, J = 3.6 Hz, CH₂), 2.80 (1H, dd, J = 17.4 Hz, J = 4.0 Hz, CH₂), 2.34 (3H, 2 × CH₃). 13C NMR (100 MHz, CDCl₃): δ 190.1, 152.6, 145.6, 126.2, 125.5, 121.7, 119.1, 82.4, 64.4, 40.9, 33.4, 26.7, 25.6.

**2,2′-Dihydroxy-5,5′-dimethylphenyl 3.** Oil. Elemental analysis for C₁₀H₁₂O₂ expected values: C, 78.48; H, 6.59; O, 14.93; calculated values: C, 78.48; H, 6.59; O, 14.95. MS (EI, 70 eV) m/z 214.10 (100%). 1H NMR (400 MHz, CDCl₃): δ 7.13 (2H, dd, J = 7.6 Hz, J = 1.6 Hz, CH), 7.04 (2H, d, J = 8.0 Hz, CH), 6.97 (2H, d, J = 8.0 Hz, CH), 3.23 (6H, s, CH₃). 13C NMR (400 MHz, CDCl₃): δ 125.6, 133.4, 131.3, 124.3, 123.0, 118.1, 42.3.

**1,1′-Dimethyl-[1,1′-bi(cyclohexane)]-2,2′,5,5′-tetraene-4,4′-dione 4.** Oil. Elemental analysis for C₁₀H₁₂O₂ expected values: C, 78.48; H, 6.59; O, 14.93; calculated values: C, 78.43; H, 6.59; O, 14.93. MS (EI, 70 eV) m/z 214.10 (100%). 1H NMR (400 MHz, CDCl₃): δ 7.01 (4H, d, J = 8.0 Hz), 6.61 (4H, d, J = 8.4 Hz), 2.22 (s, 6H). 13C NMR (100 MHz, CDCl₃): δ 182.7, 146.6, 126.3, 40.9, 25.6.
1,1′-Diethyl-[1,1′-bicyclohexane]-2,2′,5,5′-tetrade-4,4′-dione 6d. Oil. Elemental analysis for C_{10}H_{12}O_{2} expected values: C, 79.31; H, 7.49; O, 13.20; calculated values: C, 79.31; H, 7.49; O, 13.20. MS (EI, 70 eV) z/242.13 (100%). 1H NMR (400 MHz, CDCl_{3}): δ 7.06 (2H, d, J = 8 Hz, CH), 6.71 (1H, d, J = 8.3 Hz, CH), 6.51 (1H, d, J = 4.3 Hz, CH), 3.78 (3H, s, OCH$_3$). 13C NMR (100 MHz, CDCl$_3$): δ 191.1, 152.7, 123.0, 35.0, 24.3, 11.9.

5,5′-Dimethoxy-[1,1′-biphenyl]-2,2′-diol 7b. Oil. Elemental analysis for C_{14}H_{16}O$_2$ expected values: C, 68.28; H, 5.73; O, 25.99; calculated values: C, 68.29; H, 5.73; O, 26.03. MS (EI, 70 eV) z/253.99 (100%). 1H NMR (400 MHz, CDCl$_3$): δ 7.50–6.90 (2H, m, 2 × CH), 6.92 (1H, d, J = 8.8, CH), 6.60 (1H, d, J = 9.2, CH), 6.40 (1H, s, CH), 3.74 (3H, s, OCH$_3$). 13C NMR (100 MHz, CDCl$_3$): δ 145.2, 127.1, 124.4, 123.0, 118.1, 105.9, 45.12.

8-Methoxybenzol[6,7][1,4]furan-3-ol 7e. Oil. Elemental analysis for C$_{10}$H$_{12}$O$_2$ expected values: C, 68.28; H, 5.73; O, 25.99; calculated values: C, 68.29; H, 5.73; O, 26.03. MS (EI, 70 eV) z/246.09 (100%). 1H NMR (400 MHz, CDCl$_3$): δ 7.05–6.90 (2H, m, 2 × CH), 6.92 (1H, d, J = 8.8, CH), 6.60 (1H, d, J = 9.2, CH), 6.40 (1H, s, CH), 3.74 (3H, s, OCH$_3$). 13C NMR (100 MHz, CDCl$_3$): δ 162.8, 158.1, 151.9, 142.1, 136.7, 115.9, 113.3, 103.4, 101.3, 90.1, 29.5.

5,5′-Dichloro-[1,1′-biphenyl] 8b. Oil. Elemental analysis for C$_{14}$H$_{10}$Cl$_2$O$_2$ expected values: C, 56.50; H, 3.16; Cl, 27.81; O, 12.54; calculated values: C, 56.50; H, 3.16; Cl, 27.81; O, 12.50. MS (EI, 70 eV) z/253.99 (100%). 1H NMR (400 MHz, CDCl$_3$): δ 7.79–7.27 (2H, m, 2 × CH), 6.98 (1H, d, J = 8.8 Hz, CH). 13C NMR (100 MHz, CDCl$_3$): δ 152.7, 129.5, 127.1, 124.4, 123.0, 118.1.

General Procedure for the Synthesis of Pummerer's Ketones and Dimers and Derivatives under Monophasic Conditions. Compounds 1, 5, and 6 (0.5 mmol) were dissolved in 2-MeTHF (3.0 mL) and treated with hydrated HRP (17 U, 200 μL) and PM-K/PM-T (5 mg, 1.0% w/w with respect to the substrate) at pH 6. The final mixture was gently stirred (200 rpm) under UVA irradiation and an air atmosphere at 25 °C for 48 h. The organic phase was washed with brine (3 × 4 mL), dried over sodium sulfate, and evaporated under vacuum. The crude product was purified by flash column chromatography.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06584.

Procedures for the synthesis, UV–visible spectra, SEM images, elemental analysis (EDX spectra), electron paramagnetic resonance, UHPLC–MS, FTMS, ESI, and FTIR analyses of the pheomelanin samples; control experiments; evaluation of the concentration of organic hydroperoxide; UV–visible determination of •OH radicals; 1H NMR and 13C NMR spectra (PDF).

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**ABBREVIATIONS**

hydrogen peroxide, H$_2$O$_2$; 2-methyltetrahydrofuran, 2-MeTHF; horseradish peroxidase, HRP; superoxide dismutase, SOD; 2-methyltetrahydrofurfuran, 2-MeTFH; meso-tetraphenyl porphyrin, meso-TPP; pheomelanin from sodium permanganate, PM-K; pheomelanin from mushroom tyrosinase, PM-T; hydroxyl radicals, OH

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