Oligomerization of Humic Phenolic Monomers by Oxidative Coupling under Biomimetic Catalysis

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Three humic phenolic monomers, catechol (CAT), caffeic acid (CAFF), and p-coumaric acid (COUM), were subjected to oxidative coupling catalyzed by biomimetic water-soluble iron−porphyrin (Fe(TDCPPS)Cl) in either separate or mixed solution, and the reaction products were characterized by gas chromatography−mass spectrometry (GC−MS) and electrospray−mass spectrometry (ESI−MS). The GC−MS analysis proved the formation of C−C and C−O dimers, whereas the ESI−MS/MS analysis also suggested trimerization for all the monomers and tetramerization for CAT. On the basis of mass spectra, molecular structures were assigned to the observed oligomers. In the phenolic separate solutions, dimers represented about 65%, 44%, and 30% of reaction products for CAT, CAFF, and COUM, respectively, whereas trimers were from 4 to 5%. A relevant part of the products were unidentified oligomers and several degradation compounds, mostly aromatic aldehydes and alcohols and aromatic or aliphatic carboxylic acids. When all three humic phenolic monomers underwent the catalyzed coupling reaction in one mixed solution, 14% of the reaction products were identified as C−C dimers of CAT. Although no other C−O dimers of CAT, nor any dimers of COUM and CAFF, could be identified, some other structurally unknown oligomers were present among the reaction products of the mixed solution. However, no oligomers larger than tetramers were formed in either separate or mixed solutions. This work indicates the essential role of biomimetic metal−porphyrins in catalyzing the oxidative coupling of humic phenolic monomers in aqueous media, thereby promoting the polymerization of natural organic matter.

Introduction

Humic substances (HS), otherwise referred to as natural organic matter (NOM), belong to the most widely occurring natural products on the earth surface. Despite their various important roles in environmental processes, the mechanism of HS formation is still one of the least understood aspects of humus chemistry (1, 2) because of their high complexity and heterogeneous nature. However, existing literature hypothesizes that various oxidoreductases, such as peroxidase or polyphenoloxidase, and inorganic catalysts may contribute to the formation of newly formed humic molecules by favoring the oxidative coupling of phenolic compounds derived from the decomposition of biomass (3−6).

Naturally occurring phenols have attracted much attention as molecular models to study the processes leading to the stabilization of organic matter in soil (2, 7). Peroxidase-like catalysts were mostly applied to reactions of methoxy-substituted derivatives of benzenes, such as ferulic acid, and sinapyl aldehyde (8−11). In contrast, there is a lack of evidence for peroxidase-like catalyzed coupling of OH-substituted phenolic compounds, namely, dihydroxybenzenes and hydroxy-substituted cinnamic acids. The reaction of these phenolic monomers occurs in aqueous solutions and may contribute to the humification processes. Moreover, further understanding of naturally occurring oxidative coupling and its practical application under mild and environment-friendly conditions is of high interest for the removal of toxic phenolic compounds from soils and wastewaters (12).

Unless encapsulated or immobilized, natural peroxidases are susceptible to both denaturation and microbial degradation and suffer from being unstable in the presence of excess oxidants (13). Some of these disadvantages may be overcome by using their synthetic biomimetic alternatives containing metal−porphyrin rings sterically stabilized by bulky substituents, such as 2,6-dichloro sulfonatophenyl groups (14, 15). Metal−porphyrins, such as iron−porphyrin, are believed to couple phenolic compounds by way of radical mechanism. In brief, these catalysts are thought to undergo an oxidation giving highly reactive iron(IV)porphyrin cation radical species, which can catalyze the oxidation of phenolic moieties into free radicals (16, 17). The generated radicals may be further stabilized by spontaneous mutual coupling without additional involvement of a catalyst (7) thus resulting into oligo- or polymers of larger molecular weights, low solubility, and increased stability against further biological degradation.

It is the aim of this work to subject three different humic monomeric precursors catechol, caffeic, and p-coumaric acid to oxidative polymerization induced by hydrogen peroxide and a biomimetic catalyst such as a water-soluble iron−porphyrin. The extent of polymerization of the humic phenolic precursors and their mixture was assessed by mass spectrometry.

Experimental

Biomimetic Iron−Porphyrin. Information on the synthesis, structure, and characterization of the water-soluble meso-tetra-(2,6-dichloro-3-sulfonatophenyl)-porphyrinate of Fe(III) [Fe(TDCPPS)Cl] used here as biomimetic catalyst (FeP) is reported elsewhere (15).

Humic Phenolic Monomers. 1,2-Dihydroxybenzene or catechol (CAT), 3-(3,4-dihydroxyphenyl)acrylic acid or trans-caffeic acid (CAFF), and 3-(4-hydroxyphenyl)acrylic acid or trans-p-coumaric acid (COUM) were purchased by Sigma-Aldrich (Germany), with purity ranging between 97 and 99%. All phenolic monomers were used without further purification.

Reaction Solutions. Stock solutions (500 ppm) were prepared by dissolving each humic monomeric compound first in methanol until complete dissolution and then in MilliQ grade water, reaching a final methanol/water ratio of approximately 5:95 (v/v). A 100 ppm solution of each phenolic monomer, as separate substrate, was prepared by dissolving the appropriate aliquot of the stock solution in 20 mL of
MilliQ grade water (Millipore, United States), adding 0.30 μmol of Fe(TDCPPS)/Cl (2.75 mL of a 1.09 × 10⁻⁴ M solution), 1.0 mL of toluene (Sigma-Aldrich, Germany; 97% purity) as a bacteriostatic agent, and reaching 25 mL with MilliQ water. After mixing the solution thoroughly, 117 μL of a 0.017 M solution of H₂O₂ (Ashland Chemical, Italy) was added to reach a final concentration of 0.08 mM in hydrogen peroxide. A mixed solution containing 100 ppm of each phenolic monomer (CAT + CAF + COUM) as mixed substrate was prepared in a similar way as the separate substrate solutions, including the same amount of catalyst and H₂O₂. An additional mixed solution was similarly prepared, but without catalyst, to obtain a noncatalyzed (blank) control solution. All solutions (prepared in duplicates) were mixed thoroughly, were kept at room temperature in stoppered flasks, and were exposed to daylight. After an optimum reaction time (i.e., the time when reaction products reached a maximum concentration, for details see ref 18 and Supporting Information), the reaction solutions were brought to pH 2 with 6 M HCl and were freeze-dried.

**Derivatization.** Freeze-dried samples of reaction solutions were resolubilized in methanol and were dried again under N₂ flux. The dried residues were redissolved in 50 μL of pyridine and were silylated by adding 50 μL of BSTFA/TMCS (99:1) and by heating at 60 °C for 30 min.

**Gas Chromatography–Mass Spectrometry (GC/MS).** GC/MS analyses were conducted on a Perkin-Elmer Autosystem XL gas chromatograph, equipped with a Perkin-Elmer Turbomass-Gold mass spectrometer. The derivatized samples (1 μL) were manually injected into the gas chromatograph equipped with (1) a capillary injector operated in splitless mode and maintained at a temperature of 250 °C and (2) a Restek Rtx-5MS fused silica capillary column (30 m per 0.25 mm i.d., 0.25-μm film thickness). Helium was used as the carrier gas at a flow rate of 1.8 mL min⁻¹. The column oven was programmed with an initial temperature of 80° for 1 min, followed by a gradient of 80–180 °C at 4 °C/min, 180–320 °C at 10 °C/min, and a final temperature of 320 °C held for 10 min. The mass spectrometer was calibrated with tris-(perfluoro-heptyl)-s-triazine and was operated in the full scan mode, scanning in the range of m/z 50–1200 and using the electron-impact ionization energy of 70 eV at a scan rate of 1.0 s/scan. Identification of phenolic monomers was achieved by comparing their mass spectra to standard mass spectra reported in the NIST MS library. Percentage distributions of reaction products were derived from their relative areas in total ion chromatogram.

**Electrospray–Mass Spectrometry (ESI–MS/MS).** Mass spectra of samples were obtained using a TSQ 7000 mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with an ESI source operating in negative mode. For ESI, the best conditions were found to be ionization voltage at 4 kV, transfer capillary temperature at 220 °C, and tube lens and skimmer at 60 V. Nitrogen was employed as both the drying and nebulization gas. The ionization current was fixed at 5 mA and the detector voltage was at 1.3 kV. The mass spectrometer was used in the single scan mode, and the first quadrupole was scanned in the mass range of 120–1500 amu at a scan speed of 2 s in centroid mode. The collision energies (CE) applied for ESI–MS/MS analysis are shown in Table S2 (in Supporting Information).

Sample injection (20–50 μL) by direct infusion was performed with a GINA 50 autosampler (Gynkotek) and with a Gynkotek P 580 HDG HPLC pump. The injection flow rate was 0.1 mL min⁻¹ with a solution of 0.1 M ammonium acetate—acetonitrile (9:1). Infusion measurements were made in duplicates. The relative intensities of the base peaks in ESI mass spectra were between 4–7 × 10⁴ and 1–4 × 10⁶.

**Results and Discussion**

The formation of a phenoxy radical from variously substituted phenols by metal–porphyrins catalysis can theoretically lead to their polymerization. The evaluation of polymerization among phenolic monomers, as either separate or mixed substrates, and the identification of oxidation products were attempted by different approaches. The reaction products were (1) silylated and analyzed by GC–MS, (2) redissolved in methanol and directly injected into an ESI–MS/MS system, and (3) fractionated by semipreparative HPLC and the separated materials characterized by GC–MS analysis. However, the latter approach gave limited information, which is discussed in the Supporting Information (SI).

While GC–MS analysis of derivatized compounds enabled to distinguish between C–C and C–O coupled reaction products, the absolute molecular mass of the parent oligomer was obtained from mass spectra of derivatized compounds by ESI–MS. Furthermore, the formation of characteristic fragment ions in the ESI–MS/MS mode allowed the identification of the substituted position and the overall ring structure of the oligomeric products (19).

Though the combination of GC– and ESI–MS analyses may be useful for structural elucidation of coupling products, the exact bonding position may not always determined univocally, thus leaving a possible uncertainty on the chemical structure of the reaction products. However, since the mass spectra of coupling products were not provided by the NIST library, their recognition and characterization were enabled by comparing their semi-fragments patterns to that of monomeric compounds. The usefulness of this method was demonstrated when new lignin oligomers had to be characterized (20). The mass–ion fragments identified for phenolic monomers and relative oligomers by both GC–MS and ESI–MS/MS techniques are reported in Table S1 and S2 (in SI), respectively.

**Biomimetic Oxidation of Phenolic Compounds.** The MS analysis of reaction products showed in all cases the presence of polymerized compounds together with side chain oxidation products. The mass spectra of oligomeric coupling products by GC–MS and ESI–MS and their proposed molecular structure are shown in Figure 1 (structures 1–10A) and Figure 2 (structures 1–10B), respectively. Main fragments, collision energies, and relative abundances of oligomeric products proposed in Figure 2 are reported in Table S2 (in SI).

**Catechol.** The occurrence of coupling reactions was inferred from GC–MS total ion chromatograms (TIC). The reaction products with 31.6, 32.3, and 33.8 min of retention time showed all a molecular mass of 506 Da and corresponded to the C–C coupling dimers of CAT (1A). The mass spectra of these products revealed a similar fragment distribution, though the mass intensities varied. The presence of dimeric compounds with similar mass spectra but of different intensities can be explained by C–C couplings occurring among different resonance forms of the dehydrogenated CAT radical, as shown in Figure 3. Furthermore, a hydroxylated derivative of the C–C CAT dimer (m/z 594) was detected at 32.8 min in the TIC. Besides the C–C coupling products, a C–O dimer (m/z 434) was related to the peak with 30.4 min of retention time (2A). A trimeric oligomer with molecular mass of m/z 686, that resulted from both C–C and C–O couplings, was identified in the compound with 37.1 min of retention time (3A). In all cases, the presence of specific ions and mass fragments, which are identical or similar to those found for the CAT monomer, suggested that the parent compounds may well be CAT oligomers (see Table S1 in SI).

The oligomeric coupled compounds were about 92% of all reaction products present in the TIC and were accounted by dimers (65%), trimers (4%), and other unknown coupling products with molecular mass between 484 and 863 Da (23%).
Some of the unknown oligomers showed fragments characteristic of cinnamic or hydroxycinnamic acids, thereby suggesting to have resulted from coupling reactions between the CAT monomer and the modified CAT intermediates. Ring-opened products, such as 2-butenedioic acid, formed less than 3% of the reaction products, whereas hydroxylation and side chain oxidation products, such as 1,2,3-trihydroxybenzene or 1,2-benzenedicarboxylic acid, represented about 5% of reaction products.

The ESI−MS/MS spectra of underivatized reaction products confirmed the formation of the CAT C−C dimers ([M−H]− with m/z 217) (1B) and C−C and C−O trimers ([M−H]− with m/z 325) (2B), as suggested by GC−MS. Furthermore, ESI−MS/MS spectra indicated the presence of oligomers with larger molecular mass, such as a tetramer ([M−H]− with m/z 399) (3B), that was not detected by GC−MS. Even though the fragmentation of the tetramer appeared somewhat different from that of the CAT trimer, the presence of ions with m/z 216 and 109 indicated close similarities to the CAT structure.

Caffeic Acid. The GC−MS analysis of CAFF oxidation products revealed a compound with the same molecular mass and fragmentation pattern as that of the standard trans-caffeic acid. However, this compound showed a TIC retention time 2 min shorter than the CAFF standard and was identified as the CAFF cis-isomer (21, 22). Since this cis-caffeic acid was also found in the blank (i.e., noncatalyzed) solution, it must have been the result of a photoisomerization of the cinnamic acid (23) rather than a product of the FeP-catalyzed oxidation. Another compound, that reached 40% of all TIC reaction products, was identified as 6,7-dihydroxycoumarin ether (Figure S5 in SI), that, being structurally very similar to CAFF, had a retention time between those of the cis- and trans-CAFF isomers.
The coupling reactions among the different resonance forms of caffeoyl radicals (Figure 3) resulted mostly in dimers, which represented about 44% of TIC reaction products. Most dimers had the molecular mass of m/z 646 (40.5 and 41.2 min), 674 (37.0 min), 718 (39.9 min), and 790 (38.5 min). All of them were recognized as CAFF coupling products because of the detection of ions and masses, which are identical or very similar to the ones found for monomeric CAFF (see Table S1 in SI). On the basis of existing literature regarding caffeic oligomers (24–26), the molecular mass of 790 Da can be assigned to the C–C dimer (4A), while that of 674 Da can be attributed to the decarboxylated C–C coupled dimer shown as compound 5A (Figure 1). The oligomeric product with the molecular mass of 718 Da may well correspond to the dehydrodimerized structure indicated in 6A. The molecular mass of 646 Da may be explained with the furfuran-type caffeic dimer (7A), sometimes referred to as dehydrocaffeic acid dilactone, resulting from the loss of conjugation between the aromatic ring and the alkene double bond. Furthermore, the products of CAFF partial degradation or hydroxylation, such as ring-opened fragments (pentanedioic acid, 2-butenedioic acid), degraded compounds (1,2-dihydroxybenzene, 4-hydroxycinnamic acid, 4-hydroxybenzaldehyde, 2,3,4-trihydroxycinnamic acid), and other unidentified oxidized compounds, were detected by GC–MS in low amounts.

The ESI–MS/MS spectra of underivatized reaction products showed a major component with molecular mass of 178 Da.
Da (4B) (Figure 2). Even though its mass corresponded to that of 6,7-dihydroxycoumarin ether, that was detected by GC–MS as one of the main products, its fragmentation ions (m/z 177, 133, 105) were not those of aromatic ethers. In the latter compounds, a primary cleavage occurs at the β bond to the ring, and the elimination of a CO group is expected as the first step in fragmentation (27). Conversely, in our spectra, the elimination of the CO group followed the loss of a COO group, thereby suggesting that the [M–H]− ion with m/z 177 may hardly represent an aromatic ether. This oxidation product may rather be the 3-(3′,4′-dioxo-1′,5′-cyclohexadienyl) propenoic acid, otherwise referred to as the o-quinone of caffeic acid (Figure S5 in SI). This compound is often observed as one of the main intermediates during the oxidative coupling of caffeic substrates (28–30). However, the mass of the corresponding silylated o-quinone (250 Da) was not detected by GC–MS.

In agreement with GC–MS results, the ESI–MS/MS spectra revealed the presence of two dimeric compounds both with a [M–H]− ion at m/z 357 (5B and 6B), but showing a different fragmentation pattern (Figure 2). Conversely, a trimer having a [M–H]− at m/z 535 (7B), that could not be identified by GC–MS, was instead clearly detected by ESI–MS/MS. All of these oligomers repeatedly lost fragments of mass 44. Some of these fragmentations may indicate the presence of COOH groups within the molecule, whereas additional losses of 44 Da in the mass spectra of hydroxycinnamic dimers may be attributed to the elimination of a –CH2CHOH– fragment (31, 32).

In contrast to our results, incubation of CAFF monomer with natural peroxidase and H2O2, under the conditions generally used for the production of dehydrogenated polymers, failed to give any coupling products, and the starting material was recovered unchanged (33). Although synthetic porphyrins were developed to mimic the peroxidase activity (17), it is evident that, unlike natural peroxidase, the synthetic FeP catalyst did not show a negative selectivity toward the CAFF substrate.

**p-Coumaric Acid.** As in the case of CAFF, also the COUM cinnamic acid may undergo photochemical reactions (23). Thus, the compound having a TIC retention time of 24.2 min, that showed the same molecular weight and mass spectrum as the trans-p-coumaric acid standard with 27.6 min of retention time, was assigned to the cis-isomer of COUM. The formation of cis-isomer was enabled by exposing the reaction solution to the solar light. Since approximately 47% of the original substrate underwent photoisomerization, it cannot be excluded that both the original trans- and cis-COUM isomers were involved as substrates in the catalyzed oxidative coupling.

The reaction products, that were derived from catalyzed oxidative couplings, yielded 30% of COUM dimers and about 39% of unknown coupled compounds having aromatic character and molecular mass ranging from 456 to 510 Da. Different C–C dimeric products (8A), arising from the coupling of various p-coumaroyl radicals (Figure 3), had similar mass fragmentation and the same 614 Da molecular mass but different TIC retention times (37.0, 37.3, 38.0, and 39.3 min). The C–C dimers were identified by the presence of ions and mass peaks, which are identical or very similar to the ones found for the COUM monomer (see Table S1 in SI). The C–O dimers (37.7 and 37.8 min) with the molecular mass of 542 Da (9A), were also identified by the presence of specific ions and mass peaks (see SI). Compounds derived from degradation, hydroxylation, and side chain oxidation summed up to 31% of all reaction products present in the TIC, which was a larger percentage than for CAT and CAFF. One of the degradation products, 1,2-dihydroxybenzene (CAT), was found to form C–C dimers with molecular mass of 506 Da (1A) and TIC retention times of 31.6 min and 33.8 min. On the basis of this observation, the large number of unidentified catalyzed oxidation products with aromatic character may have resulted from the coupling among partially oxidized or degraded molecules of the COUM substrate.

The ESI–MS/MS spectra of the reaction products confirmed the formation of COUM dimer with [M–H]− ions at m/z 325 (8B). Moreover, a trimer (m/z 487), that had not been identified by GC–MS, was revealed by the ESI–MS spectra (9B). Both these oligomers produced a characteristic ionization pattern, because of the loss of a [M–H−COO]− ion in the primary fragmentation step, and formation of ions typical of the COUM monomer at m/z 163 and 119. Since the fragment distribution of the dimer was different from that of the trimer (see Table S2 in SI), the molecular structures of these two oligomers should not be related to the same dimer. Besides the oligomers, other degradation products,
mainly aldehydes and other unknown compounds, were found in the ESI−MS spectra.

**Mixed Solution.** The GC−MS analysis of reaction products from the mixed solution of the three phenolic monomers showed the presence of some compounds, which were already detected when each of the substrates was catalyzed in separate solutions. Among these, the cis-isomers of both COUM and CAFF and 6,7-dihydroxy coumarine ether were observed. Many chromatographic peaks with TIC retention times of 31.6, 31.9, 32.3, and 33.8 min corresponded to the C−C dimer of CAT (1A) with molecular mass of 506 Da. The C−C dimer of CAT, with the retention time of 31.9 min, was not detected when CAT underwent the catalyzed oxidation as separate substrate. A hydroxylated derivative of the 1A structure (Figure 1) with molecular mass of 594 Da was detected at the retention time of 32.8 min.

While the C−C dimers of CAT formed 14% of TIC reaction products from the mixed solution, surprisingly, no C−O dimers of CAT, nor any dimers of COUM and CAFF, were found among the reactions products of the mixed solution. Conversely, unknown compounds, with TIC retention times of 34.3, 36.8, and 37.0 min, had a mass of 532 Da that was related to the fragmentation of molecular ions with the following m/z 562 (35.4 min), 607 (37.6 min), 620 (35.1 min), 712 (39.1 min), and 728 (37.5 min). In fact, the ion with a mass of 532 was found in more than half of all observed oligomers (72% of products) for this mixed catalyzed reaction. The presence of mass peaks at m/z 166, 237, and 254 suggested that part of reaction products must have derived from dihydroxybenzene-like structures, such as that of CAT, whereas the presence of other specific masses (see Table S1 in SI) indicated a similarity to the fragmentation pattern of the CAFF/F monomer.

The ESI−MS/MS spectra showed fragments related to CAT and CAFF at m/z 109 and 135, respectively, which derived from a [M−H]− ion with m/z 243. By combining the information from both GC−MS and ESI−MS/MS spectra, the formation of a decarboxylated C−C coupling product between CAFF and CAT may be proposed as 10A (GC−MS) and 10B (ESI−MS/MS). Furthermore, the ESI−MS/MS spectra revealed unequivocally the presence of a CAT dimer in the reaction mixture, as suggested by a [M−H]− ion with m/z 217. This finding indicates that most of the oligomeric products of the mixed solution derived mainly from the coupling among CAT or CAT and decarboxylated CAFF molecules. This is in agreement with a previous work that reported the rates of oxidation for the same catalyzed reaction mixture (18). Those results suggested that FeP preferentially induces oxidation of molecules with a high electron-donor substitution on the aromatic ring, such as dihydroxybenzenes.

Other compounds detected among products of the mixed oxidative polymerization showed [M−H]− parent ions at m/z 419 and 459. Although these were most probably derived from CAT and COUM, respectively, no sufficient fragmentation similarities were found to suggest their possible structures. However, the main fragments and relative abundances are reported in Table S2 in SI. Moreover, degraded and hydroxylated compounds, such as 1,2,3-trihydroxybenzene or 2,3,4-trihydroxy cinnamic acid, as well as other products, mostly aldehydes and phenols, were additionally identified in the mixed solution, though only in low amounts (about 10% of reaction products).

The same amount of the FeP catalyst was used in both the mixed phenolic solution and in the separate phenolic solutions. A consequence is that the catalyst/substrate ratio for the mixed solution was lower than for the separate solutions. Since all oligomers detected in the mixed solution, except for 5% of the unrecognized oligomeric products, were formed by C−C coupling, whereas the C−O coupled products were only found for the separately catalyzed substrates, it may be suggested that the lower catalyst/substrate ratio favored the C−C coupling rather than the C−O coupling. A similar result was reported for some coupling reactions catalyzed by peroxidase (34, 35).

A blank mixed phenolic solution was prepared without addition of the biomimetic catalyst. Though the total amount of the observed products was much lower than in the presence of the FeP catalyst, the GC−MS analysis of the derivatized blank solution showed the occurrence of coupling, hydroxylation and carboxylation reactions. The abundance of oligomeric products, that of hydro- or carboxylated products, was 45% and 34%, respectively. Only 6% of the oligomers was represented by the C−C dimers of CAT (1A) with a TIC retention time of 31.9, 32.3, and 33.8 min, while 4% of the detected compounds had ions with m/z 532 (10A, 36.8 min) and m/z 562 (35.4 min). No C−O dimers of CAT, nor any dimers or trimers of COUM and CAFF, were detected in the uncatalyzed mixed solution.

The presence of C−C dimers of CAT in the latter solution is in agreement with previous studies on CAT polymerization, where it was found that the oxidative coupling of CAT occurred to some extent in ethanol (2) and aqueous (18) solutions. In both these works, however, CAT dimerization was largely accelerated by the presence of a catalyst.

A large part (35%) of the coupling products present in the uncatalyzed mixed solution, with molecular mass ranging from 470 to 660 Da, remained unknown, because of no sufficient similarities to the fragmentation patterns of any of the original substrate present in the solution. The occurrence of these compounds might have resulted from spontaneous couplings among partially oxidized and either carboxylated or hydroxylated products.

The efficiency of the FeP catalyst in oligomer formation in the mixed phenolic solution is evident by comparing product yields. The overall occurrence of hydro- and carboxylated products was 10 times larger in the uncatalyzed mixed solution, whereas that of oligomers was 1.5 lower. Moreover, the abundance of CAT dimers, and of the oligomers showing the peak at m/z 532 (10A and 10B), was significantly larger in the catalyzed mixed solution. Therefore, the FeP catalyst not only increased the reaction rate of the oxidative coupling (18) but also promoted the formation of oligomeric couplings among substrates that would not have occurred without the catalyst.

The GC−MS mass spectra of derivatized reaction products proved the capacity of the water-soluble Fe(TDCCPP)Cl catalyst to promote the oxidative coupling of OH-substituted phenolic compounds in aqueous medium by forming both C−O and C−C intermolecular bonds. Although dimers were found to be the most abundant oligomeric products, the ESI−MS/MS analysis indicated also the occurrence of trimers for CAT, COUM, and CAFF and tetramers for CAT. None of the larger oligomers were observed when all three phenolic substrates underwent the catalyzed coupling reaction in one mixed solution.

The identification of several degradation products, mostly aromatic aldehydes and alcohols and aromatic or aliphatic carboxylic acids, and the occurrence of dimers, which were previously suggested only as intermediate reaction products (18), may indicate that some depolymerization processes take place with increasing reaction time. However, the same oligomeric products that were detected within 1−5 d were also present after 10 and 30 d from reaction start, thereby suggesting that even though some depolymerization reactions occurred, these were not extensive enough to fragment the already coupled molecules. Conversely, no significant evidence was found, within the time course of the reaction, for a progression in the oligomerization to larger coupled products (i.e., no pentamers or larger oligomers). The absence
of larger oligomers formed from previously coupled products suggests that the extent of the oxidative polymerization may be controlled by the stability of the catalyst, the amount of available oxygen, and the oxidative potential of the products. Since the Fe(TDCPPS)Cl catalyst was reported to be stable up to 10 months after preparation (36), the extent of oxidative polymerization catalyzed by FeP should rather depend on the oxidation potential of already coupled products or the amount of available oxygen donors present in solution. A recent work (13) indicated that phenoxyl radicals may attack the protoporphyrin ring when natural horseradish peroxidase is used as catalyst for phenol oxidation and lead to heme destruction and thus porphyrin inactivation. While a similar mechanism of porphyrin ring degradation may also occur on the sterically protected biomimetic FeP catalyst used in this work and thus reduce its catalytic activity, no experimental evidence is yet available to substantiate such a progressive catalytic inactivation. However, the FeP/H2O2 system used here was shown to successfully mediate oxidative oligomerization of phenolic monomers in aqueous media and thus may represent an environment friendly tool for either reducing the toxicity of phenol-rich wastewaters or binding OH-substituted aromatic toxic compounds into humic matter.

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Supporting Information Available
Experimental details and results on HPLC fractionation of reaction products and identification of fragment ions by GC–MS and ESI–MS/MS analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


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