Oxidation of non-phenolic β-*O*-aryl-lignin model dimers catalysed by lignin peroxidase. Comparison with the oxidation induced by potassium 12-tungstocobalt(III)ate

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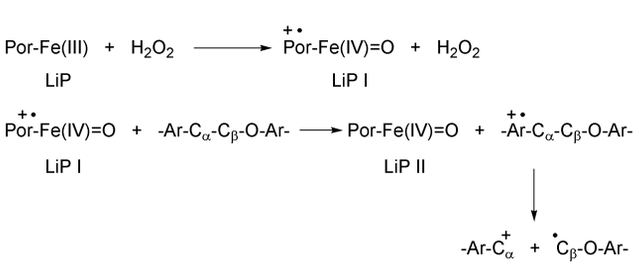
The H_2O_2-promoted oxidations of the non-phenolic β-*O*-aryl-lignin model dimers 1-(3,4-dimethoxyphenyl)-2-phenoxyethanol (**1**) and 2-(4-methoxyphenoxy)-1-phenylethanol (**2**) catalysed by LiP at pH = 4.0 have been studied. The oxidation of **1** mainly leads to the corresponding ketone, indicating that the prevailing reaction of the intermediate radical cation $\mathbf{1}^{+\bullet}$ is Cα–H deprotonation. The oxidation of **2** forms 2-(4-methoxyphenoxy)-2-phenylethanol (**7**, an isomer of **2**), 2-phenyl-1,4-dioxaspiro[4.5]deca-6,9-dien-8-one (**8**) and products coming from the cleavage of the C–C bond γ to the more electron rich ring. The formation of all these products can be rationalised by assuming that the main reaction of the intermediate $\mathbf{2}^{+\bullet}$ is a nucleophilic attack of the alcoholic OH group on the ring bearing the positive charge. This leads to a spirocyclohexadienyl radical, which either is then oxidised to the dioxaspirodecadienone **8** or undergoes ring opening to give an alkoxyl radical from which the isomer of **2** and the C–C bond cleavage products may form. Support for this mechanism has been provided by a study of the oxidation of 4-$MeOC_6H_4OCH_2CD_2OH$ and by comparing the results with those obtained when the alkoxyl radical 4-$MeOC_6H_4OCH_2CD_2O^{\bullet}$ was generated from 4-$MeOC_6H_4OCH_2CD_2OO$*t*-Bu. The oxidation of **1** induced by the genuine one-electron oxidant potassium 12-tungstocobalt(III)ate at pH = 4.0 confirms the results obtained with LiP. However, under the same conditions, no fragmentation products were observed in the oxidation of **2**, probably due to a fast oxidation, by potassium 12-tungstocobalt(III)ate, of the spirocyclohexadienyl radical.

Introduction

Lignin, a three-dimensional phenolic polymer built from phenylpropane units linked together by different bonds, is one of the most abundant biopolymers on earth. The presence of lignin mixed with hemicelluloses within the cellulosic fibre wall of wood cells creates a naturally occurring composite material which imparts strength and rigidity to trees and plants, providing protection from oxidative processes and from attacks by microorganisms.[1,2]

Studies concerning the oxidative degradation of lignin raise continuous interest for the following two reasons. First, this process can convert lignin into low molecular weight aromatic compounds, thus making this polymer a renewable source for the industrial preparation of a number of chemicals.[3] Second, and much more important, the selective degradation of lignin and its removal from the carbohydrate component is a key process in the pulp and paper industry.[4]

Recently, particular attention has been given to the possibility of degrading lignin with fungi, which would represent a process with low environmental impact and energy consumption.[5–8] Among the various fungi performing this task, the white rot basidiomycetous *Phanerochaete chrysosporium*, which secretes the ligninolytic enzyme lignin peroxidase (LiP), has attracted the most interest.[9] LiP is a ferric hemoprotein able to catalyse the oxidative degradation of lignin by the transfer of one electron from an aromatic ring of the substrate to LiP Compound I (LiPI), the active species of the enzyme (an iron(IV)-oxo porphyrin radical cation [$Por^{+\bullet}Fe(IV)=O$]), formed by oxidation of the native enzyme by H_2O_2. This electron transfer leads to the formation of LiP Compound II (LiPII), [Por-Fe(IV)=O], and the substrate radical cation. The latter should then undergo non-enzymatic side-chain fragmentation reactions[10] (some exceptions are however possible[11,12]) involving the cleavage of C–C and C–H β bonds (β with respect to the ring bearing the SOMO), thus initiating the degradation of lignin. A very simplified representation of this process is shown in Scheme 1, where only two aromatic rings are present, linked



Scheme 1

through a β-*O*-aryl bond,[13] which is the most common linkage in lignin.[1]

As it is of interest to understand the detailed mechanism by which these fragmentations occur and also the structural factors that play a role in determining the type of bond that undergoes the cleavage, we have now investigated the LiP-promoted oxidation of the lignin model compounds **1** and **2**, both containing a β-*O*-aryl bond. These models possess differ-

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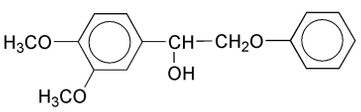
ent substitution patterns of the two aromatic rings and should therefore form two radical cations that differ in the position of the positive charge, which is located in the ring with two alkoxy substituents.[14] Thus, unambiguous information on how the position of the positive charge in the radical cation influences the cleavage of the C–C and C–H bonds can be obtained. The results of the enzymatic study have also been compared with those obtained when $\mathbf{1}^{+\bullet}$ and $\mathbf{2}^{+\bullet}$ have been generated with the genuine one-electron oxidant $K_5[Co(III)W_{12}O_{40}]$, from now on simply indicated as Co(III)W.[15,16]



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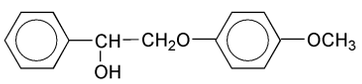

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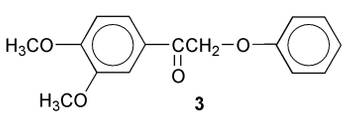
Results

The reactions of **1** and **2** with LiP were carried out in an Ar-saturated, 50 mM sodium tartrate buffer solution (pH = 4.0) containing 5% CH_3CN as cosolvent, at 25 °C. An equimolar amount of H_2O_2 in the buffer solution was gradually added over 1 h using an infusion pump. After the usual work-up, products and yields were determined by GC, GC-MS and ^1H NMR. In the reaction of **1** the major product observed was the corresponding ketone **3**, accompanied by a small amount of the Cα–Cβ bond cleavage product, 3,4-dimethoxybenzaldehyde (**4**). In contrast, with **2**, the Cα–Cβ bond cleavage products, benzaldehyde (**5**) and 4-methoxyphenol (**6**), were formed in substantial amounts along with 2-(4-methoxyphenoxy)-2-phenylethanol (**7**, an isomer of **2**)[17] and 2-phenyl-1,4-dioxaspiro[4.5]deca-6,9-dien-8-one (**8**).[18] In the absence of the oxidant or in the absence of LiP no products were detected.



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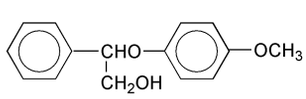

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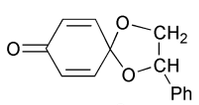

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The Co(III)W-induced oxidations of **1** and **2** were carried out at pH = 4.0 (50 mM sodium tartrate buffer solution, containing 5% CH_3CN as cosolvent) using equimolar amounts of oxidant and substrate, under an argon atmosphere, at 70 °C and for 72 hours. In the reaction of **1** the main product found was the corresponding ketone **3**, together with a small amount of 3,4-dimethoxybenzaldehyde **4**, as observed in the LiP-catalysed reaction. The oxidation of **2** showed low efficiency and led only to **8**, which was obtained with a 5% yield with respect to the starting material.[18] In the absence of the oxidant no products were detected. All of the results are collected in Table 1.

Discussion

It is reasonable to attribute the formation of **3** and **4** in the LiP-catalysed oxidation of **1** to the decay of the radical cation $\mathbf{1}^{+\bullet}$ derived from the one-electron oxidation of **1** by LiPI. Once formed, $\mathbf{1}^{+\bullet}$ undergoes one of the most typical reactions of alkylaromatic radical cations, *i.e.* the cleavage of a bond β to the ring bearing the positive charge.[19] The cleavage of the Cα–H bond (Scheme 2, path *a*), leads to an α-hydroxybenzylic radical, which, by oxidation followed by proton loss, produces the observed ketone (path *b*). The cleavage of the β C–C bond leads to 3,4-dimethoxybenzaldehyde (path *c*).[20] From the relative yields of ketone and aldehyde, it clearly appears that the main pathway followed by $\mathbf{1}^{+\bullet}$ is Cα–H deprotonation. C–C bond breaking is a minor route, probably due to the fact that such a cleavage forms a primary carbon radical, which is much less stable than the radical formed in the deprotonation pathway.[21]

Table 1 Products and yields in the LiP–H_2O_2 and Co(III)W-catalysed oxidation of **1** and **2**

Substrate	Products (yield, %)[a]	
	LiP–H_2O_2	Co(III)W
1	**3** (31)	**3** (26)
	4 (3)	**4** (2)
2	**5** (32)	**8** (5)
	6 (23)	
	7 (12)	
	8 (18)	

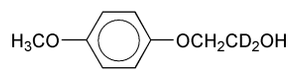
[a] Yields are given with respect to the substrate that is equimolar with the oxidant, H_2O_2. Average of at least three determinations. The material balance was >95% in all the experiments.

A different situation arises in the LiP-catalysed oxidation of **2** where substantial amounts of C–C bond cleavage products (benzaldehyde **5** and 4-methoxyphenol **6**) are formed together with the isomerization product **7** and the 2-phenyl-1,4-dioxaspiro[4.5]deca-6,9-dien-8-one, **8**.

The C–C bond cleavage in the radical cation $\mathbf{2}^{+\bullet}$, formed by oxidation of **2** with LiPI, is very surprising as it involves a bond which is γ with respect to the ring bearing the positive charge in $\mathbf{2}^{+\bullet}$. Accordingly, no interaction between this bond and the SOMO of the radical cation can be envisaged. As far as we know, no example of the direct cleavage of a γ bond in alkylaromatic radical cations has hitherto been reported.

A possible explanation, strongly supported by the formation of products **7** and **8**, is that for $\mathbf{2}^{+\bullet}$ the most important reaction pathway is the intramolecular nucleophilic attack of the alcoholic OH group on the oxygen-substituted position of the positively charged ring to form the spirocyclohexadienyl radical **9** (Scheme 3, path *a*), a possibility which has been previously proposed by Gold and co-workers[17] and by Gilbert and McCleland in similar systems.[22] As suggested by these authors, opening of this spirocyclohexadienyl radical can involve both geminal C–O bonds leading to the alkoxyl radicals **10** and **11** (Scheme 3, paths *b* and *c* respectively). By hydrogen-atom abstraction, **10** can form the starting material **2** whereas **11** produces the isomerized product **7** (Scheme 3, paths *e* and *g* respectively). In addition, we now suggest that **10** and **11**, in competition with hydrogen-atom abstraction, can also undergo a β C–C bond cleavage, a well known process for alkoxyl radicals.[19] From **10**, benzaldehyde and the phenoxy-substituted carbon radical **12** may form (Scheme 3, path *f*), the latter being eventually converted (by oxidation and hydrolysis) to 4-methoxyphenol. From **11**, formaldehyde and the secondary carbon radical **13** can be formed (Scheme 3, path *h*). Oxidation of **13** followed by hydrolysis forms again benzaldehyde and 4-methoxyphenol. It is very likely that compound **8** is also derived from **9**, by oxidation followed by water addition and methanol loss (Scheme 3, path *d*).

In order to test the mechanism presented in Scheme 3 we have also studied the oxidation with LiP of the isomerized product **7** and of the partially deuterated compound **14**. The results are shown in Table 2.



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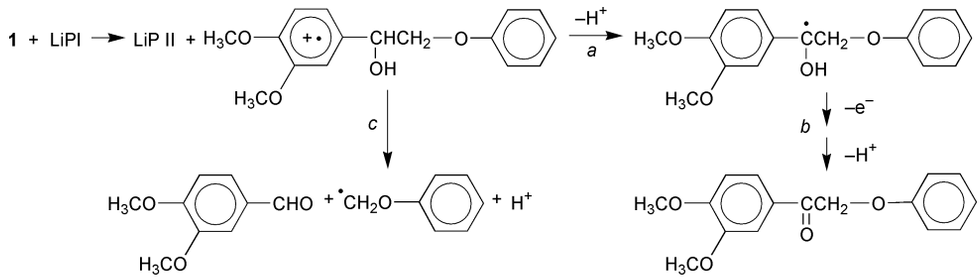
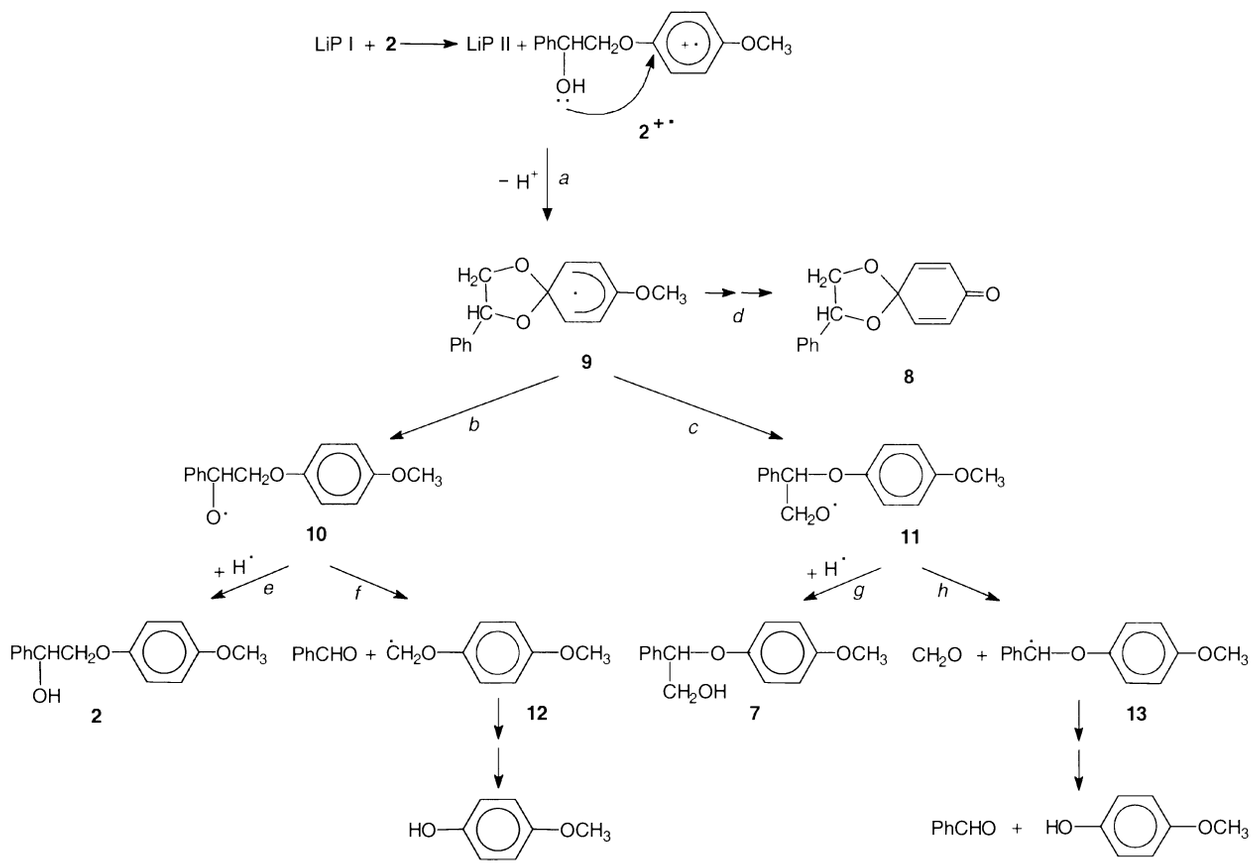


Scheme 2 Fragmentation pathways for $\mathbf{1}^{+\bullet}$.

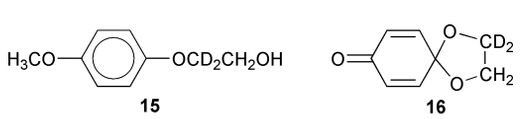


Scheme 3 Proposed mechanism for the decomposition pathways of $\mathbf{2}^{+\bullet}$.

Table 2 Products and yields in the LiP-catalysed oxidation of **7** and **14**

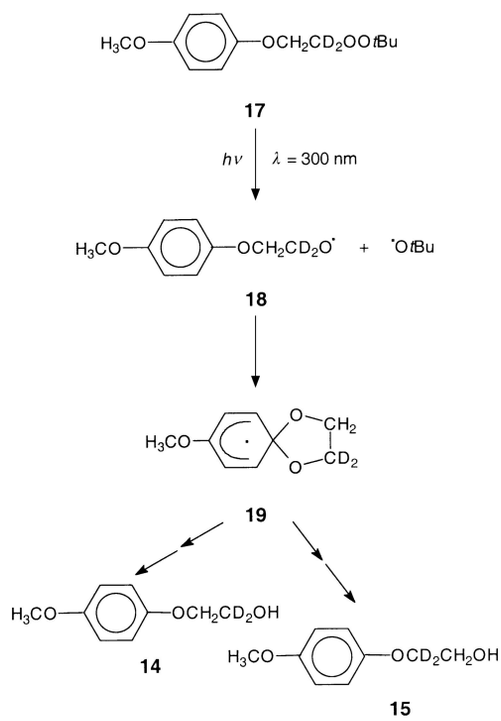
Substrate	Products (yield, %)[a]
7	**2** (11)
	5 (16)
	6 (14)
	8 (11)
14	**15** (30)
	16 (28)

[a] Yields are given with respect to the substrate that is equimolar with the oxidant, H_2O_2. Average of at least three determinations. The material balance was >95% in all the experiments.

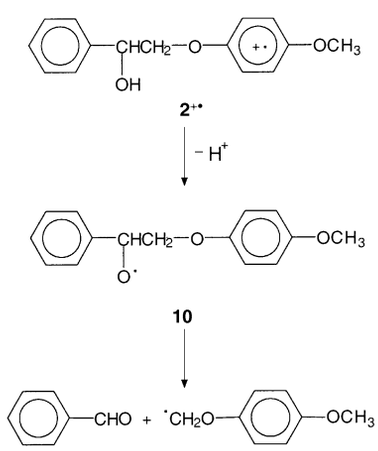


We can see that the LiP-catalysed oxidation of **7** leads to its isomer **2** which is formed together with the same products (the dioxaspirodecadienone **8**, benzaldehyde **5** and 4-methoxyphenol **6**) observed when **2** was the starting substrate. This result is fully consistent with the mechanism shown in Scheme 3 as in $\mathbf{7}^{+\bullet}$, the intramolecular nucleophilic attack by the OH group leads to the same spirocyclohexadienyl radical formed from $\mathbf{2}^{+\bullet}$. The study of the oxidation of **14** also provided results in line with the proposed mechanism. Accordingly, the oxidation of **14** led to its isomer **15**, where the CH_2 and CD_2 groups have inverted their relative position, and the dioxaspirodecadienone **16**.[18] In order to find further evidence for the intermediacy of the alkoxyl radical in the isomerization process, the peroxide **17** was synthesised and its photolysis was carried out (see Experimental). It was observed that such a reaction produces **14** and its isomer **15**. Since the photolysis of **17** leads to the alkoxyl radical **18**, by O–O bond cleavage, this result clearly shows that **18** is an intermediate *en route* to **14** and **15** (Scheme 4). From **18** no β fragmentation products are obtained, probably since the β fragmentation of **18** leads to formaldehyde and the primary carbon radical **12** and it is therefore much less favoured with respect to that of **10** and **11**.

There is, however, another mechanistic possibility whereby an alkoxyl radical could be formed from $\mathbf{2}^{+\bullet}$ without the intervention of the spirocyclohexadienyl intermediate. This is based on our recent observations that arylalkanol radical cations exhibit oxygen acidity up to when the OH group is in the γ-position.[23,24] Thus, the alkoxyl radical **10** might also be formed by OH deprotonation in $\mathbf{2}^{+\bullet}$ as shown in Scheme 5. However, we feel that this possibility is unlikely as the radical cations gener-



Scheme 4 Photolysis of peroxide **17**.



Scheme 5 Fragmentation reaction induced by OH deprotonation of $2^{+\bullet}$.

ally display OH acidity in basic media, whereas LiP-catalysed reactions take place at pH 4. Some specific interaction between an amino acid residue of the enzyme with the OH group in $2^{+\bullet}$ might be envisaged, but at present no evidence in this respect is available.

In summary, the mechanism illustrated in Scheme 3 appears to satisfactorily account for the formation of **7** and **8** from $2^{+\bullet}$, as well as for that of the products derived from C–C bond cleavage. If this mechanism is correct, an interesting conclusion is that the possibility of forming a spirocyclohexadienyl radical in β-*O*-aryl substructures seems to be an important factor with respect to oxidative C–C bond cleavage in lignin.

The oxidation of **1** with Co(III)W, a well recognised one-electron oxidant,[15,16] has given results very similar to those observed in the LiP-catalysed oxidation, Table 1. The ketone **3** is the predominant product, accompanied by small amounts of 3,4-dimethoxybenzaldehyde. Clearly, Cα–H deprotonation is the main reaction of $1^{+\bullet}$, whereas the cleavage of the β C–C bond is a minor route, as observed in the LiP-promoted reaction. This suggests that the behaviour of $1^{+\bullet}$ is independent of its mode of generation (enzymatic or chemical) and fully supports the intervention of a *free* radical cation in the LiP-catalysed oxidation.

Chemical and enzymatic oxidations seem instead to behave differently in the case of **2** (Table 2). Accordingly, the oxidation of **2** by Co(III)W leads exclusively to the formation of the dioxaspirodecadienone **8** and does not produce the isomerization or fragmentation products observed with LiP. However, we suggest that this difference is only apparent because in the oxidation promoted by Co(III)W the spirocyclohexadienyl radical, once formed, preferentially undergoes oxidation to **8**, rather than ring opening to form an alkoxyl radical (Scheme 3). Thus, no isomerization and fragmentation products are formed. Accordingly, the strong capacity of Co(III)W to oxidise carbon radicals is well known.[25]

Conclusion

The results reported in this paper have shown that the oxidation of the lignin model compound **1** catalysed by LiP leads to a radical cation that preferentially undergoes deprotonation at the Cα–H bond. Cleavage of the β carbon bond is a minor route. Similar results have been obtained by using the *bona fide* one-electron oxidant Co(III)W.

A somewhat different situation is observed when **2** is the substrate. In this case, the LiP-promoted oxidation forms substantial amounts of the C–C bond cleavage products together with the isomerization product **7** and dioxaspirodecadienone **8**. Evidence has been obtained that all these products are derived from the spirocyclohexadienyl radical **9**, formed by an intramolecular nucleophilic attack of the alcoholic OH group at the positively charged ring in the radical cation. Radical **9** can then undergo ring opening to form an alkoxyl radical from which the isomer **7** and the products of C–C bond cleavage are formed. Evidence for the intermediacy of an alkoxyl radical has been obtained. Oxidation of **9** can also occur to produce the dioxaspirodecadienone **8**. The latter reaction is the only one observed when Co(III)W is used as oxidant.

The different behaviours of $1^{+\bullet}$ and $2^{+\bullet}$ with respect to the fragmentation pathway can be rationalised on the basis of the different structural features of the two radical cations. In $1^{+\bullet}$ the easily scissile C–H and C–C β bonds are present and accordingly the radical cation decays by cleavage of these bonds. No such bonds are present in $2^{+\bullet}$ in which, however, intramolecular nucleophilic attack of the alcoholic OH group on the oxygen-substituted position of the positively charged aromatic ring is possible, leading to a spirocyclohexadienyl radical from which all the observed products are formed.

Experimental

Instrumentation

^{1}H NMR spectra were recorded on a Bruker AC300P spectrometer in $CDCl_3$. GC-MS analyses were performed on an HP5890 GC (OV1 capillary column, 12 m × 0.2 mm) coupled with an HP5970 MSD. GC analyses were carried out on a Varian 3400 GC (OV1 capillary column, 25 m × 0.2 mm). UV–Vis measurements were performed on a Perkin Elmer Lambda 18 spectrophotometer. Melting points were recorded using a Büchi 510 melting point apparatus.

Substrates and reagents

All the reagents and solvents were of the highest purity available and used without further purification (unless otherwise specified). The concentration of H_2O_2 (Carlo Erba Reagents) was determined by titration with permanganate.[26] Co(III)W was prepared using the literature procedure[15] with some modifications.[16] LiP was prepared and purified as described in the literature.[27] The concentration of the enzyme solution was determined spectrophotometrically [ε(409 nm) = 169 mM^{-1} cm^{-1}].[28]

1-(3,4-Dimethoxyphenyl)-2-phenoxyethanol (1)

2-Bromo-1-(3,4-dimethoxyphenyl)ethanone. To a stirred solution of 3,4-dimethoxyacetophenone (Aldrich) (5.0 g, 27.8 mmol) in chloroform (20 mL) a solution of bromine (C. Erba) (1.4 mL, 27.5 mmol) in chloroform (7 mL) was added. After refluxing the solution for 3 hours, the solvent was removed under reduced pressure. Recrystallization from methanol gave 3.2 g (12.3 mmol) of the brominated derivative, 44% yield. ^{1}H NMR ($CDCl_3$): δ 3.93 (3 H, s, OCH_3), 3.95 (3 H, s, OCH_3), 4.43 (2 H, s, CH_2), 6.88–6.93 (1 H, m, Ar), 7.52–7.53 (1 H, m, Ar), 7.58–7.63 (1 H, m, Ar).

1-(3,4-Dimethoxyphenyl)-2-phenoxyethanone. To a stirred solution of phenol (C. Erba) (1.2 g, 12.7 mmol) in acetone (25 mL) K_2CO_3 (Fluka) (1.8 g, 13.0 mmol) and 2-bromo-1-(3,4-dimethoxyphenyl)ethanone (3.2 g, 12.3 mmol) were added. After 1 hour the inorganic salts were filtered off and washed with acetone. The combined filtrates were concentrated under reduced pressure. The residue was dissolved with dichloromethane, the solution was washed with water, then with brine and dried over anhydrous $MgSO_4$. The product was purified by column chromatography on silica gel using ethyl acetate–petroleum ether 1 : 3 as eluent, 77% yield. ^{1}H NMR ($CDCl_3$): δ 3.91 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 5.21 (2 H, s, CH_2), 6.87–6.91 (1 H, m, Ar), 6.92–7.00 (3 H, m, Ar), 7.22–7.31 (2 H, m, Ar), 7.55–7.56 (1 H, m, Ar), 7.62–7.67 (1 H, m, Ar).

1-(3,4-Dimethoxyphenyl)-2-phenoxyethanol (1). To a stirred solution of $NaBH_4$ (Aldrich) (1.1 g, 29 mmol) in methanol (10 mL) a solution of 1-(3,4-dimethoxyphenyl)-2-phenoxyethanone (2.6 g, 9.5 mmol) in methanol (10 mL) was added. After 2 hours 30 mL of water–acetic acid (5 : 1) were added. The reaction mixture was extracted with dichloromethane, the organic layer was washed with water, with $NaHCO_3$ solution and then dried over anhydrous $MgSO_4$. The product was purified by column chromatography on silica gel using dichloromethane as eluent, 92% yield. Mp 95–97 °C (from dichloromethane). ^{1}H NMR ($CDCl_3$): δ 2.77 (1 H, s, OH), 3.89 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 3.96–4.13 (2 H, m, CH_2), 5.05–5.11 (1 H, m, CH), 6.86–7.02 (5 H, m, Ar), 7.24–7.34 (3 H, m, Ar). MS *m*/*z* (%): 274 (10), 167 (100), 139 (65), 77 (30).

2-(4-Methoxyphenoxy)-1-phenylethanol (2)

2-Bromo-1-phenylethanone. 2-Bromo-1-phenylethanone was prepared as previously described for 2-bromo-1-(3,4-dimethoxyphenyl)ethanone. ^{1}H NMR ($CDCl_3$): δ 3.74 (3 H, s, OCH_3), 4.41 (2 H, s, CH_2), 6.88–6.92 (2 H, m, Ar), 7.47–7.65 (3 H, m, Ar).

2-(4-Methoxyphenoxy)-1-phenylethanone. To a stirred solution of 4-methoxyphenol (Aldrich) (6.9 g, 55.6 mmol) in acetone (100 mL), NaOH (2.2 g, 55.0 mmol) and 2-bromo-1-phenylethanone (11.0 g, 55.1 mmol) were added. After the solution had been refluxed for 1 hour the solvent was removed under reduced pressure. The residue was dissolved with dichloromethane, and the solution was washed with water, then with NaOH (1 mM) and dried over anhydrous $MgSO_4$. 2-(4-Methoxyphenoxy)-1-phenylethanone (9.6 g, 39.6 mmol, 72% yield) was purified by column chromatography on silica gel using dichloromethane–petroleum ether in an elution gradient of 1 : 20–1 : 1. ^{1}H NMR ($CDCl_3$): δ 3.76 (3 H, s, OCH_3), 5.23 (2 H, s, CH_2), 6.81–6.84 (2 H, m, Ar), 6.88–6.92 (2 H, m, Ar), 7.47–7.65 (3 H, m, Ar), 7.98–8.01 (2 H, m, Ar).

2-(4-Methoxyphenoxy)-1-phenylethanol (2). To a stirred solution of $NaBH_4$ (Aldrich) (4.5 g, 120 mmol) in methanol (60 mL) a solution of 2-(4-methoxyphenoxy)-1-phenylethanone (9.6 g, 39.6 mmol) in methanol (100 mL) was added. After 2 hours 300 mL of water–acetic acid 5 : 1 were added. The reaction mixture was extracted with dichloromethane, the organic layer was washed with water, with $NaHCO_3$ saturated solution and then dried over anhydrous $MgSO_4$. The product **2** (8.8 g, 36.1 mmol, yield 91%) was purified by column chromatography on silica gel using dichloromethane as eluent. Mp 59–61 °C (from dichloromethane). ^{1}H NMR ($CDCl_3$): δ 3.77 (3 H, s, OCH_3), 3.92–4.09 (2 H, m, CH_2), 5.08–5.12 (1 H, m, CH), 6.81–6.89 (4 H, m, Ar), 7.31–7.48 (5 H, m, Ar). MS *m*/*z* (%): 244 (20), 124 (100), 109 (40), 77 (30).

2-(4-Methoxyphenoxy)-2-phenylethanol (7)

To a stirred solution of 4-methoxyphenol (Aldrich) (4.7 g, 37.9 mmol) and anhydrous K_2CO_3 (8.0 g, 58.0 mmol) in EtOH (60 mL) was added styrene oxide (Aldrich) (5.0 g, 41.7 mmol). After the solution had been refluxed for 2 hours water (20 mL) was added and the mixture was extracted with ether. The organic layer was washed with NaOH (1 mM) and dried over anhydrous Na_2SO_4. The product was purified by column chromatography on silica gel using ethyl acetate–petroleum ether in an elution gradient of 1 : 50–1 : 5 (3.9 g, 16.0 mmol, yield 38%). Mp 85–87 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.71 (3 H, s, OCH_3), 3.75–3.96 (2 H, m, CH_2), 5.14–5.19 (1 H, m, CH), 6.70–6.83 (4 H, m, Ar), 7.30–7.37 (5 H, m, Ar). MS *m*/*z* (%): 244 (6), 124 (100), 109 (36), 77 (12).

2-(4-Methoxyphenoxy)[1,1-$^2H_2$]ethanol (14)

(4-Methoxyphenoxy)acetic acid. Bromoacetic acid (Aldrich) (10.5 g, 74.4 mmol) was added to a stirred solution of 4-methoxyphenol (Aldrich) (6.0 g, 48.3 mmol), NaOH (5.0 g, 125 mmol) and Bu_4NI (1.0 g, 2.7 mmol) in H_2O (100 mL) and benzene (50 mL). After reflux for 6 hours the solvent was partially removed under reduced pressure. After the addition of a saturated solution of Na_2CO_3 the reaction mixture was extracted with chloroform, then a dilute solution of HCl was added to the aqueous phase and the mixture was extracted with chloroform. The organic layers were dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure 4.5 g of (4-methoxyphenoxy)acetic acid were obtained (24.7 mmol, yield 51%). ^{1}H NMR ($CDCl_3$): δ 3.78 (3 H, s, OCH_3), 4.64 (2 H, s, CH_2), 6.86–6.87 (4 H, m, Ar).

2-(4-Methoxyphenoxy)[1,1-$^2H_2$]ethanol (14). At 0 °C a suspension of 2.1 g of $LiAlD_4$ (Aldrich) (50.0 mmol) in 15 mL of anhydrous THF was gradually added to a stirred solution of (4-methoxyphenoxy)acetic acid (4.5 g, 24.7 mmol) in 10 mL of anhydrous THF under an argon atmosphere. The reaction mixture was equilibrated at room temperature and, after 3 hours, 50 mL of H_2O were added to remove the unreacted $LiAlD_4$. The precipitate was removed by filtration and the solvent was evaporated under reduced pressure. A solution of Na_2CO_3 was added to the residue and the mixture was extracted with dichloromethane to give 3.7 g of product (21.7 mmol, yield 88%). Mp 69–72 °C (from dichloromethane). ^{1}H NMR ($CDCl_3$): δ 2.15 (1 H, s, OH), 3.77 (3 H, s, OCH_3), 4.02 (2 H, s, CH_2), 6.84–6.85 (4 H, m, Ar). MS *m*/*z* (%): 170 (55), 124 (100), 110 (20), 109 (85).

2-(4-Methoxyphenoxy)[1,1-$^2H_2$]ethyl *tert*-butyl peroxide (17)

2-(4-Methoxyphenoxy)bromo[1,1-$^2H_2$]ethane. To a stirred solution of 2-(4-methoxyphenoxy)[1,1-$^2H_2$]ethanol (**14**) (170 mg, 1.0 mmol) in anhydrous ether (3 mL) was added a solution of PBr_3 (Aldrich) (270 mg, 1.0 mmol) in anhydrous ether (1 mL) dropwise at 0 °C. After 6 hours the reaction mixture was extracted with water and dried over anhydrous Na_2SO_4. The product (0.4 mmol, yield 40%) was purified by column chromatography on Florisil using *n*-hexane as eluent. ^{1}H NMR ($CDCl_3$): δ 3.76 (3 H, s, OCH_3), 4.22 (2H, s, CH_2), 6.79–6.90 (4 H, m, Ar).

2-(4-Methoxyphenoxy)[1,1-$^2H_2$]ethyl *tert*-butyl peroxide (17). Compound **17** was prepared by reacting 2-(4-methoxyphenoxy)bromo[1,1-$^2H_2$]ethane with *t*-BuOOH as described in the literature.[29] Column chromatography on Florisil using *n*-hexane as eluent gave the pure product (yield 47%). ^{1}H NMR ($CDCl_3$): δ 1.25 (9 H, s, $C(CH_3)_3$), 3.77 (3 H, s, OCH_3), 4.13 (2 H, s, CH_2), 6.79–6.90 (4 H, m, Ar).

Enzymatic oxidation

The oxidant H_2O_2 (20 μmol) was added, over a period of 1 h by an infusion pump, to a magnetically stirred argon-degassed solution of the substrate (20 μmol) and LiP (0.63 units, 1.0 nmol) in 10 mL of a 50 mM sodium tartrate buffered solution, pH 4, with 5% of acetonitrile as cosolvent, at 25 °C. The products of the reaction were extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4.

Chemical oxidation

The oxidant Co(III)W (20 μmol) and the substrate (20 μmol) were magnetically stirred in 10 mL of an argon-degassed 50 mM sodium tartrate buffered solution, pH 4, with 5% of acetonitrile as cosolvent, at 70 °C. After 3 days the products of the reaction were extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4.

Product analysis

Reaction products were identified by GC, GC-MS and ^{1}H NMR by comparison with authentic specimens. Yields were determined by GC and ^{1}H NMR (using 4-methoxyacetophenone as the internal standard) with respect to the starting material. A good material balance (>95%) was observed in all the experiments.

Photochemical reaction

The peroxide **17** (40 μmol), in 5 mL of argon-degassed 50 mM sodium tartrate buffered solution, pH 4, with 5% of acetonitrile as cosolvent, was irradiated in a Rayonet photoreactor (16 × 21 W sunlight phosphor lamps, centre of emission 300 nm) at 25 °C for 1 hour. The reaction products were extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and analysed by ^{1}H NMR.

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