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# Oxidative Dimerization of (*E*)- and (*Z*)-2-Propenylsesamol with $O_2$ in the Presence and Absence of Laccases and Other Catalysts: Selective Formation of Carpanones and Benzopyrans under Different Reaction Conditions

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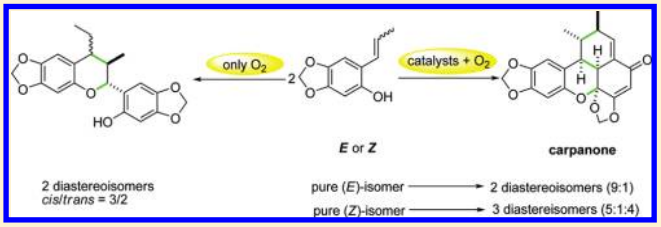
Supporting Information

**ABSTRACT:** The oxidative dimerization of 2-propenylsesamol to carpanone with $O_2$ as the oxidant, which probably proceeds as a domino phenol oxidation/*anti*-$\beta$,$\beta$-radical coupling/intramolecular hetero Diels–Alder reaction, can be efficiently catalyzed by laccases. Experiments with laccases and other catalysts like a Co(salen) type catalyst and $PdCl_2$ clearly demonstrate that the diastereoselectivity of the carpanone formation does not depend on the catalyst but on the double-bond geometry of the substrate. With (*E*)-2-propenylsesamol as the substrate, carpanone and a so far unknown carpanone diastereoisomer are formed in a 9:1 ratio. When (*Z*)-2-propenylsesamol is used as starting material, carpanone is accompanied by two carpanone diastereoisomers unknown so far in a 5:1:4 ratio. All three carpanone diastereoisomers have been separated by HPLC, and their structures have been elucidated unambiguously by NMR spectroscopy, DFT calculations, and spin work analysis. When the oxidation of 2-propenylsesamol with $O_2$ is performed in the absence of any catalyst two diastereoisomeric benzopyrans are formed, probably as the result of a domino oxidation/intermolecular hetero Diels–Alder reaction. Under these conditions, carpanone is formed in trace amounts only.




## INTRODUCTION

Carpanone (**1a**) is the most well-known member of a small group of naturally occurring benzoxanthenones which belong to the lignans and are characterized by an *O*-containing tetracyclic basic skeleton. The natural product with its highly oxygenated hexacyclic ring system and five contiguous stereogenic centers was isolated from the bark of the carpano tree (*Cinnamonum* sp., family Lauraceae) as a racemate (Figure 1).[1a] Aside from carpanone (**1a**), isocarpanone and carpananone were isolated from the carpano tree. Their structures show great similarities with carpanone (**1a**) but have not been elucidated completely.[1a] In addition, carpacin (**2**) was obtained from the same plant. This styrene derivative might be considered as a biosynthetic precursor of carpanone (**1a**).[1]

Later, some additional naturally occurring benzoxanthenones were isolated. Among them are several sauchinones from *Saururus chinensis*[2] as well as the polemannones A–C (**4**–**6**) from *Polemannia Montana* (Figure 2).[3] As sauchinone (**3**) exhibits various biological activities, such as hepatoprotective effects,[2a] antihypertension effects,[2b] immunosuppressive effects,[2c] and anti-inflammatory effects,[2d] the synthesis of natural as well as unnatural benzoxanthenones has become a field of growing interest in medicinal chemistry in recent years.

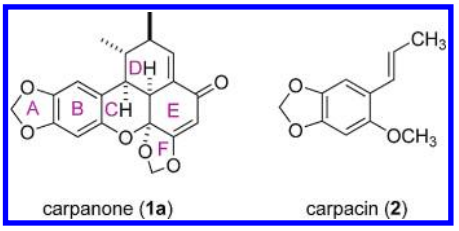



**Figure 1.** Natural products isolated from the carpano tree (*Cinnamonum* sp., family Lauraceae).

Most synthetic studies of both natural and unnatural benzoxanthenones can be traced back to the fundamental contribution from Chapman et al. who recognized that it is possible to synthesize carpanone (**1a**) by an intramolecular hetero-Diels–Alder reaction of the *o*-quinone methide **9**, which in turn is accessible from an *anti*-selective $\beta$,$\beta$-phenol coupling of two molecules (*E*)-**7** (Scheme 1).[4]

Indeed, when Chapman et al. reacted (*E*)-**7** with 50 mol % of $PdCl_2$ under air they isolated 46% racemic carpanone (**1a**) after crystallization. The structure of **1a** was established by

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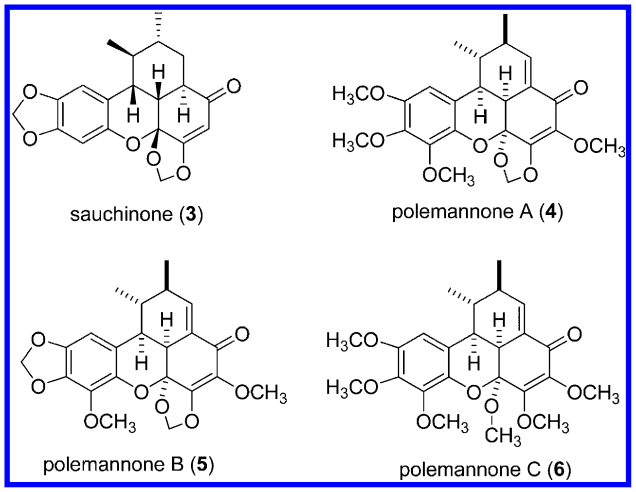



**Figure 2.** Some natural products with a benzoxanthenone skeleton.

X-ray structure analysis.[4] It is noteworthy that Chapman et al. achieved the synthesis of a comparatively complex natural product with five stereogenic centers in a highly elegant fashion and in a single synthetic operation. The synthesis is also an impressive example for the application of Diels–Alder reactions in the total synthesis of natural products.[5] Possibly, Chapman's approach to carpanone also represents a biomimetic synthesis of **1a** because the potential biosynthesis precursor carpacin (**2**) has also been isolated from the carpano tree. Meanwhile, the preparation of racemic carpanone (**1a**) has been performed successfully with a number of transition-metal catalysts and reagents. Kuroda and Matsumoto reported that the synthesis of racemic **1a** can be achieved by reaction of (*E*)-**7** using a catalytic amount of transition metal complexes like Co(II)salpr, Co(II)salen, Fe(II)salen, and Mn(II)salen as catalysts and $O_2$ as the oxidant.[6] With 2.7 mol % of Co(II)salen 94% of **1a** was isolated. However, they also reported the additional formation of small amounts of a second compound, whose structure could not be elucidated. Trivedi et al. used 2 equiv of $Ag_2O$ for the oxidation of (*E*)-**7** and observed the formation of racemic **1a** in 48% yield.[7] Ley et al. reported on the synthesis of racemic carpanone (**1a**) employing different reagents.[8] The best results were obtained when a polymer-bound Co(II)salen complex was used for the transformation of (*E*)-**7**. Even under these conditions **1a** was not produced as the sole product. Ley et al. observed the additional formation of small amounts of another compound, and it was assumed that this compound might be an isomer of carpanone (**1a**).[8a] Lindsley et al. reported that the oxidative dimerization of (*E*)-**7** can be achieved with 10 mol % of $CuCl_2$/10 mol % of (−)-sparteine and air to deliver racemic **1a** as the sole product in 91% yield.[9] It was Poli et al. who studied the effect of the double bond geometry of the substrate on the course of the reaction for the first time.[10] Unfortunately, they did not use (*E*)-**7** and (*Z*)-**7** with the free phenolic OH groups as starting materials. Instead, the TBS-protected phenols (*E*)-**10** and (*Z*)-**10**, which were deprotected in situ with TBAF, were chosen as substrates (Figure 3). With (*E*)-**10** as the substrate the reaction with 10 mol % of $PdCl_2/O_2$ exclusively delivered racemic **1a** in 82% yield. A similar result was obtained with $CuCl_2/O_2$. The oxidative dimerization of (*Z*)-**10** with 10 mol % of $PdCl_2/O_2$ also resulted in **1a** as the sole product (77% yield), but when the oxidation was performed in the presence of 10 mol % of $CuCl_2/O_2$ as the catalyst 40% of a 63:37 mixture of carpanone (**1a**) and a carpanone diastereoisomer whose structure was assigned to **1b** was obtained.[10] The obvious discrepancies between the results of the different groups prompted us to investigate the oxidative dimerization of diastereoisomerically pure (*E*)-**7** and (*Z*)-**7** under different reaction conditions.

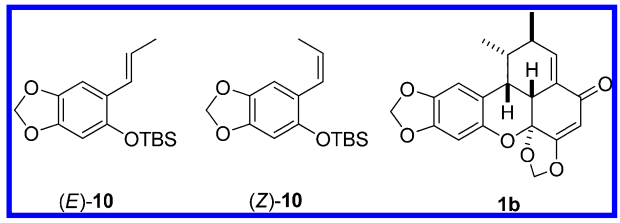
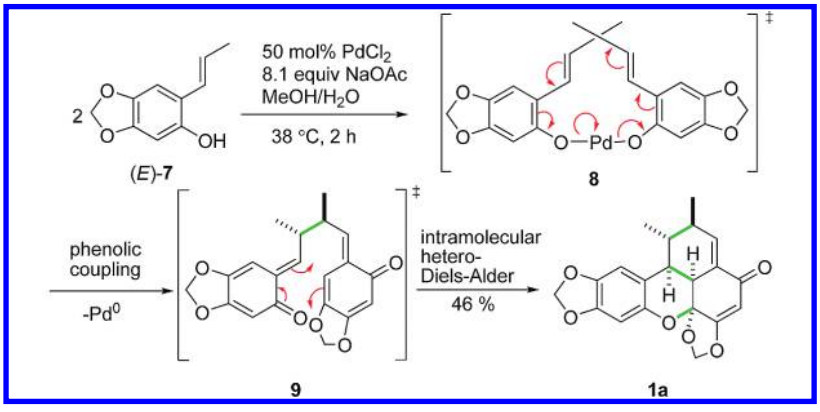



**Figure 3.** Silyl ethers (*E*)-**10** and (*Z*)-**10** used as precursors for the formation of carpanones; structure **1b** as proposed by Poli et al.[10]

Chapman's approach to the synthesis of benzoxanthenones is not restricted to the synthesis of carpanone (**1a**) itself but can also be applied to the construction of other benzoxanthenones.[9,11,12] As an example, Lindsley et al. have achieved the preparation of diastereoisomerically pure polemannones B (**5**) and C (**6**) by treatment of suitable substituted precursors with 10 mol % of $CuCl_2$/10 mol % of (−)-sparteine.[12]

Astonishingly, there is no report so far that the formation of carpanone (**1a**) by domino phenol oxidation/*anti*-*β*,*β*-radical coupling/intramolecular hetero-Diels–Alder reaction can be catalyzed enzymatically. As it is generally believed that the formation of **9** proceeds via radical dimerization of (*E*)-**7**, it seemed reasonable to try the synthesis of carpanone (**1a**) with enzymes known to be capable to initiate oxidative phenol couplings. We expected laccases to be suitable for this purpose

**Scheme 1. Synthesis of Racemic Carpanone (1a) According to Chapman et al.[4]**





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as a number of oxidative phenol couplings have been catalyzed successfully by this type of enzymes.

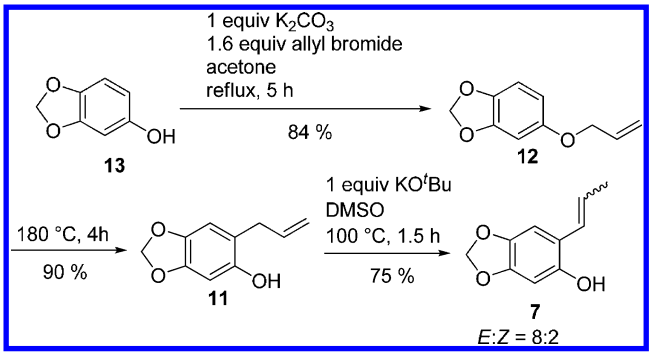
Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are widespread in nature.[13−16] Among the oxidoreductases there are several groups of biocatalysts, including dehydrogenases, oxidases, oxygenases, and peroxidases.[17] The laccases belong to the oxidases which catalyze the oxidation of a substrate using $O_2$ as the oxidant without incorporation of oxygen into the substrate. The laccases are similar to the ascorbate oxidases from plants and to the mammalian plasma protein ceruloplasmin. Together they form the small group of the blue copper oxidases.[18] Laccases catalyze four one-electron oxidations of organic substrate molecules to the corresponding four radicals using $O_2$ as the oxidant. Usually, the resulting radicals are highly reactive and undergo radical couplings with formation of dimers, oligomers, or polymers. The oxidation of the substrate is coupled with the four-electron reduction of $O_2$ to water.[19] In nature, laccases contribute to numerous processes. It is believed that plant laccases are involved in the synthesis of lignin[20] while fungal laccases are known for the degradation of lignin.[21] Because of their ability to oxidize organic substrates with $O_2$, laccases play an important role in industrial and biotechnological processes including applications from the field of food, paper, textile, and cosmetic industries. The application of laccases in soil remediation is also important.[22] Naturally occurring laccases normally exhibit redox potentials in the range of between 0.5 and 0.8 V.[13,20d,22a] As a result, the number of substrates which undergo laccase-catalyzed oxidation is limited. However, the redox potential of laccases can be extended by using mediators, allowing other classes of compounds to be oxidized. It has been demonstrated that oxidations catalyzed by laccases and laccase/mediator systems, respectively, can be employed to achieve a number of selective transformations,[17,22a,23] including the oxidation of aromatic methyl groups,[24] alcohols,[25] ethers,[26] benzylamines, and hydroxyl amines.[27] Laccases can also be used for the oxidation of phenolic substrates and for phenolic couplings. Examples for laccase-catalyzed oxidative couplings include the dimerizations of penicillin X,[28] bisphenol A,[29] 17-$\beta$-estradiol,[30] isoeugenol,[31] coniferyl alcohol,[32] *trans*-resveratrol,[33] and polyhydroxystilbene derivatives.[34] One-pot reactions which combine a laccase-catalyzed oxidation with a chemical reaction are highly attractive.[35] Recently, we have reported on laccase-initiated domino reactions of catechols and hydroquinones with 1,3-dicarbonyls.[36] The combination of the laccase-catalyzed oxidation of catechols and hydroquinones to the corresponding benzoquinones with intermolecular Diels−Alder reactions with normal electron demand is also known.[23a] The use of laccases in organic synthesis offers numerous advantages: (a) they can be easily isolated and some of them are commercially available, (b) laccase-catalyzed processes use $O_2$, which is the cheapest and most abundant oxidant, and water is the only byproduct formed, (c) laccases catalyze the transformations of a great number of substrates and there is no need for a cofactor and cofactor regeneration, (d) the redox potential of laccases can be extended by mediators, (e) laccase-catalyzed oxidations can be performed in aqueous solvent systems as well as mixtures of aqueous and organic solvents,[22a] and (f) laccases can be immobilized.[37] The recombinant production of laccases allows the targeted overexpression of specific laccases. On one hand, this facilitates the isolation of a specific laccase compared with its purification from a mixture of laccases, as formed by most natural producers of fungi. On the other hand, the heterologous production of laccase allows for the specific modification of the enzyme, which can result in improvements concerning activity, selectivity, stability, expression, or redox potential. So far, fungal laccases have been heterologous produced mainly in filamentous fungi like *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reseii* as well as in yeasts like *Pichia pastoris*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Klyveromyces lactis*.[38]

The focus of this study was to explore whether the oxidative dimerization of two molecules 2-propenylsesamol (**7**) to carpanone (**1a**) can be performed with laccase as the catalyst and $O_2$ as the oxidant. The obvious discrepancies between the results of different groups prompted us to study the influence of the double bond geometry of **7** on the stereoselectivity of the carpanone formation as well.

## RESULTS AND DISCUSSION

We started with the laccase-catalyzed reaction of an 8:2 mixture of (*E*)- and (*Z*)-2-propenylsesamol (**7**). This 8:2 mixture was obtained by column chromatography of the crude 7:3 mixture of (*E*)- and (*Z*)-**7** ($^1$H NMR) from the KO$^t$Bu-mediated isomerization of 1-propenylsesamol (**11**) (Scheme 2).

**Scheme 2. Synthesis of 2-Propenylsesamol (7)**




1-Propenylsesamol (**11**) was prepared by Claisen rearrangement of **12**, which in turn can easily be synthesized by allylation of **13**.[4,39,40]

Then, the laccase-catalyzed reaction of the (*E*)/(*Z*)-mixture of **7** was investigated. In a first experiment, it was found that the reaction of **1** mmol **7** (*E*/*Z* = 8:2) with 28 U laccase from *Trametes versicolor* in acetate buffer (0.1 M, pH = 5.0)/acetone (9:1) under $O_2$ at room temperature for 24 h delivered 46% of a product mixture after column chromatography (Scheme 3). $^1$H NMR analysis after column chromatography clearly demonstrated that carpanone (**1a**) was the major, but not the only, component of the product mixture. The structure elucidation unequivocally established that the two other components formed are the carpanone diastereoisomers **1c** and **1d**. This experiment clearly demonstrated that the oxidative dimerization of **7** can be catalyzed by a laccase. To study the influence of the double bond geometry of **7** on the stereoselectivity of the oxidative dimerization of **7** pure (*E*)-**7** and (*Z*)-**7** had to be prepared.

It was found that (*E*)-**7** and (*Z*)-**7** can be obtained in diastereoisomerically pure form by repeated column chromatography ($SiO_2$; dichloromethane/cyclohexane = 5:2) of the mixture of (*E*)-**7** and (*Z*)-**7** obtained by isomerization of **11**. $^1$H NMR spectroscopy clearly indicated that (*E*)-**7** and (*Z*)-**7** were diastereoisomerically pure. To avoid any isomerization and/or oxidation the two diastereoisomers were reacted immediately.

First, we reacted (*E*)-**7** under the reaction conditions reported by Chapman et al.[4] When (*E*)-**7** was treated with 50 mol % of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**Scheme 3. Laccase-Catalyzed Oxidative Dimerization of an 8:2 Mixture of (*E*)- and (*Z*)-2-Propenylsesamol (7)**

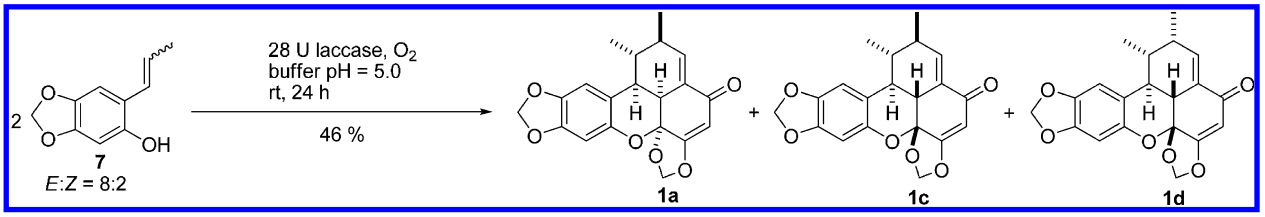
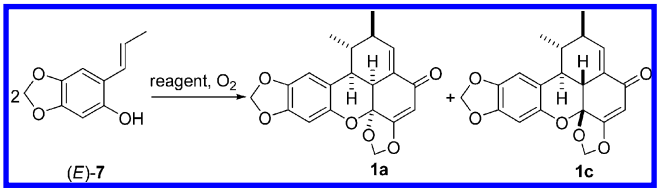



**Table 1. Oxidative Dimerization of (*E*)-7 with Transition-Metal-Based Reagents[a]**




| entry | reagent | *T* (°C) | time (h) | yield **1a** + **1c**[b] (%) | **1a**:**1c**[c] |
|---|---|---|---|---|---|
| 1 | 50 mol % $PdCl_2$[d] | 38; rt | 2; 1 | 42 | 9:1 |
| 2 | 10 mol % $PdCl_2$[e] | 50 | 4 | 47 | 9:1 |
| 3 | 2.6 mol % **14**[f] | rt | 4 | 79 | 9:1 |

[a] 1 mmol of (*E*)-7 was reacted. [b] Yields refer to yields after flash chromatography. [c] The ratio **1a**:**1c** was determined by $^1$H NMR spectroscopy after flash chromatography. [d] The reaction was performed in $MeOH/H_2O$ = 5:1 using air as the oxidant. [e] The reaction was performed in $MeOH/H_2O$ = 1:1 using $O_2$ as the oxidant. [f] The reaction was run in $CH_2Cl_2$ and $O_2$ was used as the oxidant.

$PdCl_2$ under air, we did not observe the exclusive formation of carpanone (**1a**) as reported by Chapman et al. but instead isolated 42% of a mixture of two carpanone diastereoisomers in a 9:1 ratio (Table 1, entry 1). Structure elucidation clearly established that the major diastereoisomer was the expected carpanone (**1a**) and that the minor diastereoisomer was **1c** (cf. Structure Elucidation). When the reaction was carried out under Poli's conditions using 10 mol % of $PdCl_2$ and $O_2$ as the oxidant, again a 9:1 mixture of **1a** and **1c** was obtained. Under these conditions, the two diastereoisomers were isolated in 47% yield (Table 1, entry 2). Finally, the reaction was run with 2.6 mol % of (*R*,*R*)-(−)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (**14**) and $O_2$ as the oxidant (Figure 4). In agreement with the results of Matsumoto and

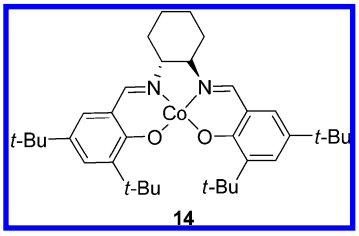



**Figure 4.** Structure of **14**.

Kuroda,[6] carpanone (**1a**) was not formed exclusively. Instead, we isolated 79% of a 9:1 mixture of **1a** and **1c** (Table 1, entry 3). From these experiments it is evident that the oxidative dimerization of pure (*E*)-7 results in the formation of a mixture of two diastereoisomeric benzoxanthenones, namely **1a** and **1c**. This indicates that the oxidative dimerization of (*E*)-7 is not completely diastereoselective as has been assumed previously by several authors.[4,7,9,10]

Next, the laccase-catalyzed reaction of pure (*E*)-7 was studied. Three laccases were used as catalysts, namely commercially available laccases from *T. versicolor*,[41] and *A. bisporus*[42] as well as a recombinant laccase from *T. versicolor*.[43] The white-rot fungus *T. versicolor* produces several laccases which can be assigned to four groups of isoenzymes *α*, *β*, *γ*, and *δ*.[44] Commercially available laccases of *T. versicolor* represent mixtures of these isoenzymes. It has been shown that Lcc*β* is the most stable one of these isoenzymes. In addition, Lcc*β* displays a high activity toward a number of substrates.[43] Because of the low solubility of (*E*)-7 in sodium acetate buffer (0.1 M, pH 5.0), the reactions were carried out in the presence of cosolvents such as acetone or methanol. When (*E*)-7 was reacted with 28 U laccase and $O_2$ as the oxidant in a 9:1 mixture (v/v) of sodium acetate buffer and acetone, 36% of a 9:1 mixture ($^1$H NMR) of **1a** and **1c** were isolated after 2 h (Table 2, entry 1). The reason for the low yield can be attributed to incomplete consumption of the starting material (TLC, $^1$H NMR spectrum of the crude product, isolation). By increasing the reaction time from 2 to 6 h, the yield could be improved to 60%. Again, a 9:1 mixture ($^1$H NMR) of **1a** and **1c** was formed (Table 2, entry 3). A further slight improvement of the yield to 62% could be achieved by extending the reaction time to 24 h (Table 2, entry 4). In addition, the influence of increasing the amount of acetone from 10 to 18 vol % in the solvent mixture was studied. With 18 vol % of acetone, (*E*)-7 was consumed completely after 2 h and 68% of a 9:1-mixture ($^1$H NMR) of **1a** and **1c** were isolated (Table 2, entry 5). Similar results were obtained when the reaction was run with a laccase from *A. bisporus* and a recombinant Lcc*β*-laccase, respectively. With the commercially available laccase from *A. bisporus* as the catalyst 65% of a 9:1 mixture ($^1$H NMR) of **1a** and **1c** were obtained (Table 2, entry 6). The reaction with the Lcc*β* laccase from *T. versicolor* as the catalyst produced 53% of a mixture of **1a** and **1c** (Table 2, entry 7). Replacement of acetone with MeOH resulted in considerably lower yields of the 9:1 mixture ($^1$H NMR) of **1a** and **1c** (Table 2, entries 8 and 9). This is probably due to lower solubility of (*E*)-7 in this solvent mixture. Separation of the two stereoisomers **1a** and **1c** was achieved by HPLC (see the Supporting Information). Structure elucidation (vide infra) clearly established that **1a** is carpanone. As in **1a**, the orientation of the two methyl groups at C-8 and at


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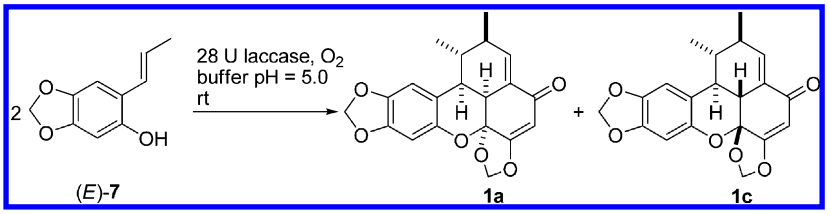
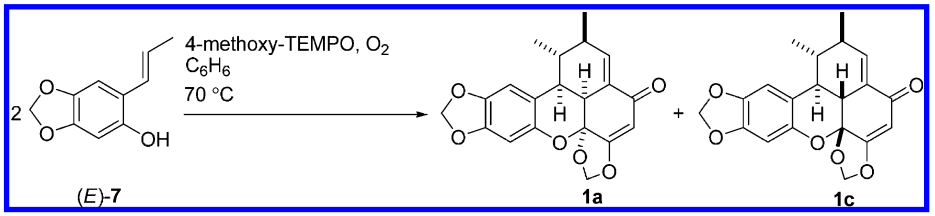
**Table 2. Optimization of the Laccase-Catalyzed Oxidative Dimerization of (*E*)-7[a]**




| entry | laccase | cosolvent (vol %) | time (h) | yield **1a** + **1c**[b] (%) | **1a**:**1c**[c] |
|---|---|---|---|---|---|
| 1 | *Tv*[d] | acetone (10) | 2 | 36 | 9:1 |
| 2 | *Ab*[e] | acetone (10) | 2 | 22 | 9:1 |
| 3 | *Tv*[a] | acetone (10) | 6 | 60 | 9:1 |
| 4 | *Tv*[d] | acetone (10) | 24 | 62 | 9:1 |
| 5 | *Tv*[d] | acetone (18) | 2 | 68 | 9:1 |
| 6 | *Ab*[e] | acetone (18) | 2 | 65 | 9:1 |
| 7 | *Lccβ*[f] | acetone (18) | 2 | 53 | 9:1 |
| 8 | *Tv*[d] | MeOH (18) | 2 | 31 | 9:1 |
| 9 | *Ab*[e] | MeOH (18) | 2 | 32 | 9:1 |

[a]1 mmol of (*E*)-7 was reacted. [b]Yields refer to yields after flash chromatography. [c]The ratio **1a**:**1c** was determined by $^1$H NMR spectroscopy after flash chromatography. [d]Commercially available laccase from *T. versicolor*. The enzyme activity was determined using ABTS as the substrate.[30] [e]Commercially available laccase from *A. bisporus*. The enzyme activity was determined using ABTS as the substrate.[30] [f]Recombinant laccase Lcc*β* from *T. versicolor*. The enzyme activity was determined using ABTS as the substrate.[30]

**Table 3. Oxidative Dimerization of (*E*)-7 Using 4-Methoxy-TEMPO as the Catalyst[a]**

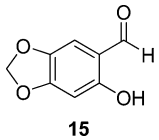



| entry | 4-methoxy-TEMPO (mol %) | time (h) | yield **1a** + **1c** (%)[b] | **1a**:**1c**[c] |
|---|---|---|---|---|
| 1 | 2.7 | 12 | 56 | 9:1 |
| 2 | 27 | 4 | 79 | 9:1 |

[a]1 mmol of (*E*)-7 was reacted. [b]Yields refer to yields after flash chromatography. [c]The ratio **1a**:**1c** was determined by $^1$H NMR spectroscopy after flash chromatography.

C-8′ in the carpanone diastereoisomer **1c** is *trans*. However, the two diastereoisomers differ in the linkage of the dihydropyran and the cyclohexene rings (ring C and ring D) at C-7′ and C-2. In contrast to carpanone (**1a**) the linkage between the two rings in **1c** is *trans*. A control experiment clearly demonstrated that **1a** is stable under the reaction conditions of the laccase-catalyzed oxidative dimerization; i.e., no conversion of **1a** into **1c** was observed. Despite of a number of attempts[8a,9] the enantioselective synthesis of carpanone has not been achieved so far. To study the enantioselectivity of the laccase-catalyzed dimerization of **7** the separation of the enantiomers of **1a** was studied by HPLC using a chiral phase. It was found that Chiralpak IB as a chiral phase is suitable for the separation of the enantiomers of **1a** as well as that of **1c**. Determination of the enantioselectivity of the laccase-catalyzed reaction of (*E*)-7 revealed that **1a** as well as **1c** were formed in racemic form; i.e., the oxidative dimerization proceeds without enantioselectivity. This holds true for all three laccases under study as well as for the reaction with **14**. In summary, all laccase-catalyzed dimerizations of (*E*)-7 proceed with exclusive formation of the two diastereoisomers **1a** and **1c** in a 9:1 ratio (Table 2). The chemical yield, however, depends on the laccase employed, the type and the amount of the cosolvent, and the reaction time.

Subsequently, it was studied whether the oxidative dimerization of (*E*)-7 can also be catalyzed by nitroxides. When (*E*)-7 was treated with 2.7 mol % of 4-methoxy-TEMPO at 70 °C, 56% of a 9:1 mixture ($^1$H NMR) of **1a** and **1c** was isolated after 12 h. In addition to **1a** and **1c**, approximately 18% of phenol-3,4-methylenedioxy-6-carbaldehyde (**15**) ($^1$H NMR of the crude product), which can arise through oxidative cleavage of the C,C-double bond in (*E*)-7, was formed (Figure 5).



**15**

**Figure 5.** Structure of **15**.

Further experiments revealed that the yield of **1a**,**c** can be controlled by the amount of 4-methoxy-TEMPO. By increasing the amount of 4-methoxy-TEMPO from 2.7 to 27 mol %, the yield of the 9:1 mixture ($^1$H NMR) of **1a** and **1c** could be improved to 79%. The diastereoselectivity of the dimerization, however, could not be influenced by the amount of the nitroxide.

To summarize, the oxidative dimerization of (*E*)-7 as well as the mixture of (*E*)- and (Z)-7 can not only be achieved by


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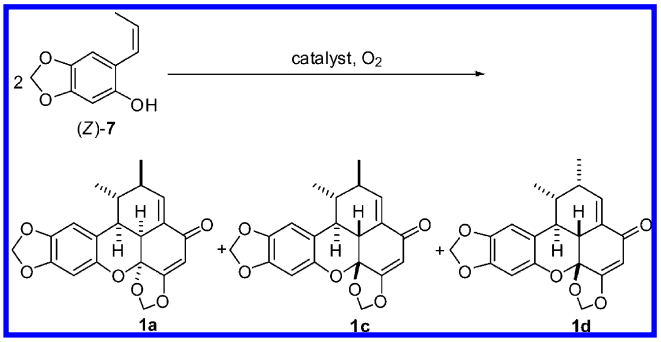
**Table 4. Oxidative Dimerization of (*Z*)-7**[a]




| entry | catalyst | time (h) | solvent | yield **1a** + **1c** + **1d**[b] (%) | **1a**:**1c**:**1d**[c] |
|---|---|---|---|---|---|
| 1 | 28 U laccase[d] | 2 | acetate buffer pH = 5.0 | 40 | 5:1:4 |
| 2 | 2.6 mol % **14** | 4 | $CH_2Cl_2$ | 43 | 5:1:4 |

[a]1 mmol of (*Z*)-7 was reacted. [b]Yields refer to yields after flash chromatography. [c]The ratio **1a**:**1c**:**1d** was determined by $^1$H NMR spectroscopy after flash chromatography. [d]Commercially available laccase from *T. versicolor*.

$PdCl_2$ and Co complex **14** but can also be catalyzed by laccases and 4-methoxy-TEMPO using $O_2$ as the oxidant. It is remarkable to note that irrespective of the catalyst and the oxidant used, in all cases the carpanone diastereoisomers **1a** and **1c** were formed in a 9:1 ratio. The advantage of the laccase-catalyzed reactions is that (a) there is no need for the use of a transition-metal catalyst or the use of nitroxides like 4-methoxy-TEMPO, (b) the reactions can be run in aqueous solvent systems, and (c) $O_2$ can be used as the oxidant.

It is assumed that the reaction of (*E*)-7 proceeds as a domino phenol oxidation/*anti*-*β*,*β*-radical coupling/intramolecular hetero-Diels–Alder reaction (Scheme 4). In the first step, (*E*)-7 undergoes one-electron oxidation to the resonance-stabilized radical **16**. Subsequently, dimerization of **16** takes place in the sense of a diastereoselective *anti*-*β*,*β*-coupling with formation of intermediate **9**. In the last step, an intramolecular hetero-Diels–Alder reaction with inverse electron demand occurs, and the products **1a** and **1c** are formed. When the cycloaddition proceeds via the *endo*-*E*-*syn* transition state **17**, the cycloadduct **1a** with a *cis*-linkage of the dihydropyran and the cyclohexene rings is formed. If, however, the cycloaddition goes through the *exo*-*E*-*anti* transition state **18** the formation of cycloadduct **1c** with a *trans*-linkage of dihydropyran and cyclohexene rings takes place. The preferred formation of **1a** is probably due to the fact that the *endo*-*E*-*syn* transition state **17** is sterically less demanding than the *exo*-*E*-*anti* transition state **18**. It should be mentioned that alternative pathways for the oxidative dimerization of 2-propenylsesamol (**7**) have been proposed. In case of the Pd(II)-catalyzed/mediated process, the *β*,*β*-coupled intermediate **9** may also be formed via a carbopalladative reaction based on a two-electron process. Originally, this pathway has been suggested by Chapman et al.[4] Recently, this idea has been picked up again by Poli et al.[10,47] For the oxidative dimerization of **7** with $PhI(OAc)_2$ as the oxidant a third mechanistic alternative has been proposed.[11,48]

Subsequently, the oxidative dimerization of (*Z*)-7 was studied. The study focused on experiments with laccase/$O_2$ and Co complex **14**/$O_2$. It was found that the laccase-catalyzed oxidation of (*Z*)-7, which was carried out under the same reaction conditions than the reaction of (*E*)-7, gave 40% of a mixture of **1a**, **1c**, and **1d** in a ratio of 5:1:4 ($^1$H NMR) (Table 4, entry 1). Almost identical results were observed when the oxidation of (*Z*)-7 was performed with 2.6 mol % of (*R*,*R*)-(−)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (**14**) as the catalyst (Table 4, entry 2). It is noteworthy that in both cases the three carpanone diastereoisomers **1a**, **1c**, and **1d** were formed in a 5:1:4 ratio. These results also support the view that irrespective of the catalyst used the same reaction mechanism applies. The three diastereoisomers could be separated by HPLC (see the Supporting Information). In this way, it was also possible to obtain sufficient amounts of **1d** for structure elucidation. In contrast to **1a** and **1c**, the methyl groups at C-8 and C-8′ in **1d** are *cis*-oriented. The structure elucidation also revealed that the dihydropyran and the cyclohexene rings in **1d** are *trans*-fused as they are in **1c**.

It is proposed that regardless of the geometry of the olefinic double bond of **7** the radical **16** is formed which in turn undergoes dimerization and finally an intermolecular hetero-Diels–Alder reaction. The dimerization can happen in two ways: either as an *anti*-*β*,*β*-coupling or as a *syn*-*β*,*β*-coupling. It is assumed that the transformation of (*Z*)-7 into **1a** and **1c** follows the same path than the reaction of (*E*)-7 (Scheme 4). This means that in the first step **9** is formed by an *anti*-*β*,*β*-coupling of two radicals **16** which react either via the *endo*-*E*-*syn* transition state **17** to **1a** or via the *exo*-*E*-*anti* transition state **18** to **1c**. The formation of **1d**, however, requires **20** as an intermediate which can result from a *syn*-*β*,*β*-coupling of two radicals **16** (Scheme 5). Subsequently, the *syn*-*β*,*β*-structure **20** undergoes an intermolecular hetero-Diels–Alder reaction through the *exo*-*E*-*anti* transition state **21** to yield **1d**. The formation of **1e** that would result from the corresponding *endo*-*E*-*syn* transition state **22** was not observed.

It is remarkable that starting with (*E*)-7 the Diels–Alder products **1a** and **1c** are formed exclusively. With this substrate not even a trace of **1d** could be detected. Product **1d** is formed only when (*Z*)-7 was used as the substrate. The different results obtained using (*Z*)-7 and (*E*)-7 are unexpected as both starting materials are assumed to form radical **16** as a common intermediate and so far no satisfactory explanation for the different behavior of the two isomers can be offered.

Finally, some control experiments were performed to study the effect of the laccase on the outcome of the reaction in greater detail. For this purpose, pure (*E*)-7 was stirred in acetate buffer (pH = 5.0)/acetone = 82:18 at room temperature under $O_2$ in the absence of any catalyst for 2 h. It came as a big surprise when apart from 56% of unreacted (*E*)-7 and 3% of a carpanone-containing fraction 21% of an unknown compound (*m*/*z* 356) were isolated (see the Supporting Information).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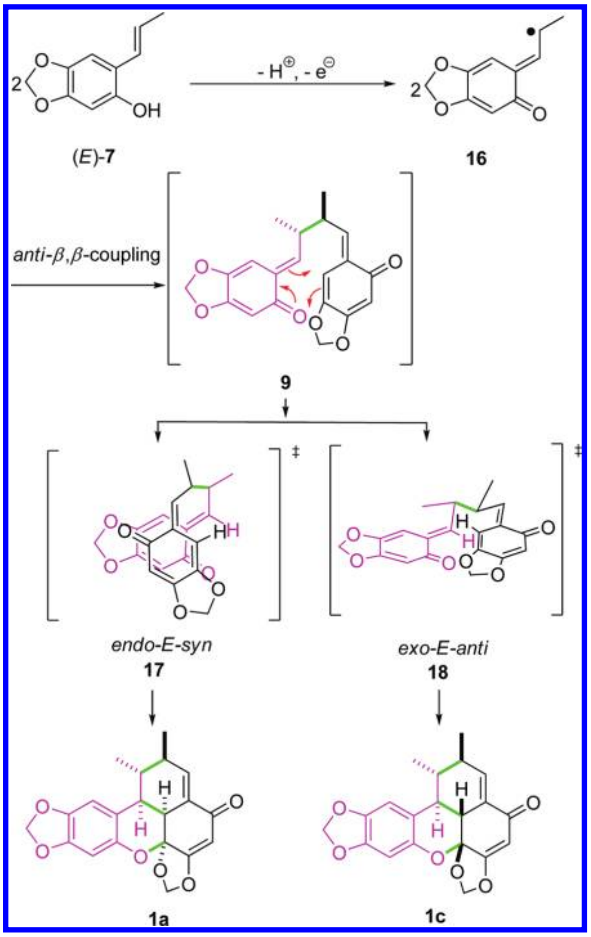
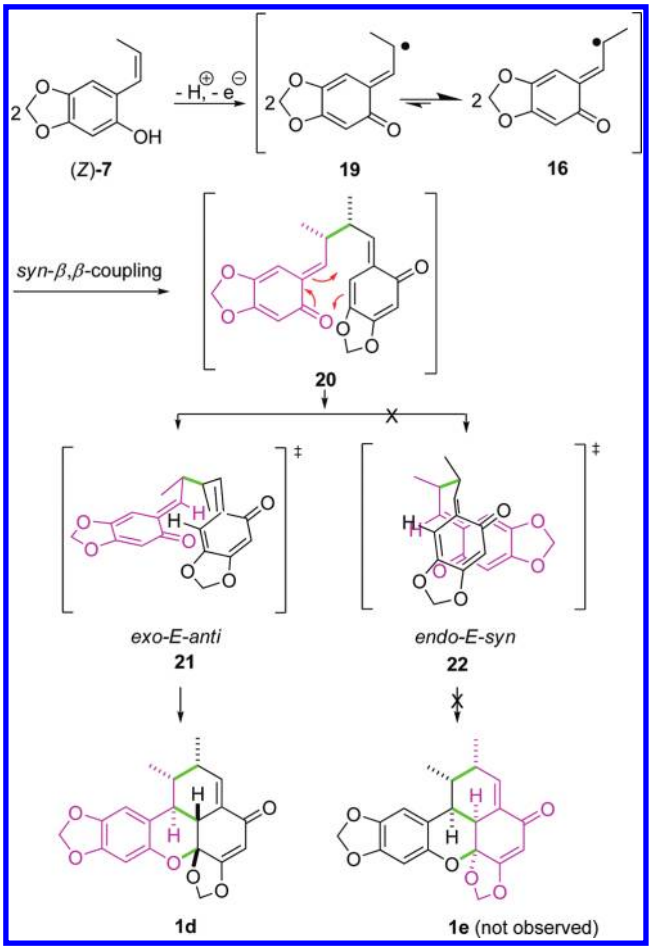
**Scheme 4. Proposed Reaction Mechanism for the Formation of 1a and 1c by Laccase-Catalyzed Domino Phenol Oxidation/*Anti*-β,β-Radical Coupling/Intramolecular Hetero-Diels–Alder Reaction of (*E*)-7**

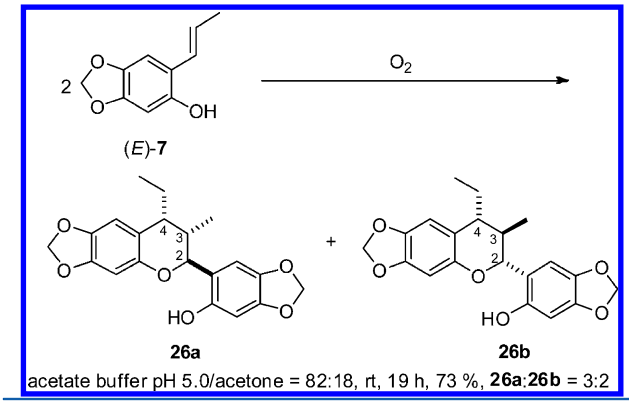



**Scheme 5. Proposed Reaction Mechanism for the Formation of 1d by Laccase-Catalyzed Domino Phenol Oxidation/*syn*-β,β-Radical Coupling/Intramolecular Hetero-Diels–Alder Reaction of (*Z*)-7**




Because of this surprising result, the reaction of (*E*)-7 with $O_2$ was studied in greater detail (Scheme 6). When the reaction of (*E*)-7 with $O_2$ in acetate buffer (pH = 5.0/acetone = 82:18) at room temperature was run for 19 h the yield of the unknown product increased to 73%. The structure elucidation revealed that the new compound was a 3:2 mixture of the two diastereoisomeric benzopyrans **26a,b**, which can be regarded as the products of an intermolecular/hetero Diels–Alder reaction. The benzopyrans **26a** and **26b** could be completely separated by column chromatography and were characterized. In both diastereoisomers, the substituents at C-2 and C-3 are *trans*-oriented. Diastereoisomers **26a** and **26b** differ only in the *cis*- and *trans*-arrangement of the substituents at C-3 and C-4 of the dihydrochromene ring. It was also found that both **26a** and **26b** were formed as racemates. That was established by determination of the optical rotation of **26a** and by separation of the enantiomers of the acetate of **26b** using HPLC on a chiral phase (see the Supporting Information). A detailed analysis revealed that traces of **26a,b** are already formed during preparation of the mixture of (*E*)- and (*Z*)-7 by base-catalyzed isomerization of 1-propenylsesamol (**11**). For the preparation of pure (*E*)- and (*Z*)-7, the Diels–Alder products **26a,b** were completely removed by column chromatography. In this way, a contamination of (*E*)-7 and (*Z*)-7 with **26a,b** could be excluded.

**Scheme 6. Intermolecular Oxidative Dimerization of (*E*)-7**




It is assumed that **26a,b** are formed by a domino oxidation/intermolecular hetero-Diels–Alder reaction (Scheme 7).[45] In contrast to the laccase-catalyzed oxidation of (*E*)-7 with $O_2$ to the radical **16**, the oxidation of (*E*)-7 with $O_2$ in the absence of a laccase results in the formation of the *o*-quinone methide **23** which undergoes an intermolecular inverse-electron-demand hetero-Diels–Alder reaction with (*E*)-7 as the dienophile to produce the benzopyrans **26a,b**. The cycloadduct **26a** is formed via *exo* transition state **24** while **26b** results from the corresponding *endo* transition state **25**.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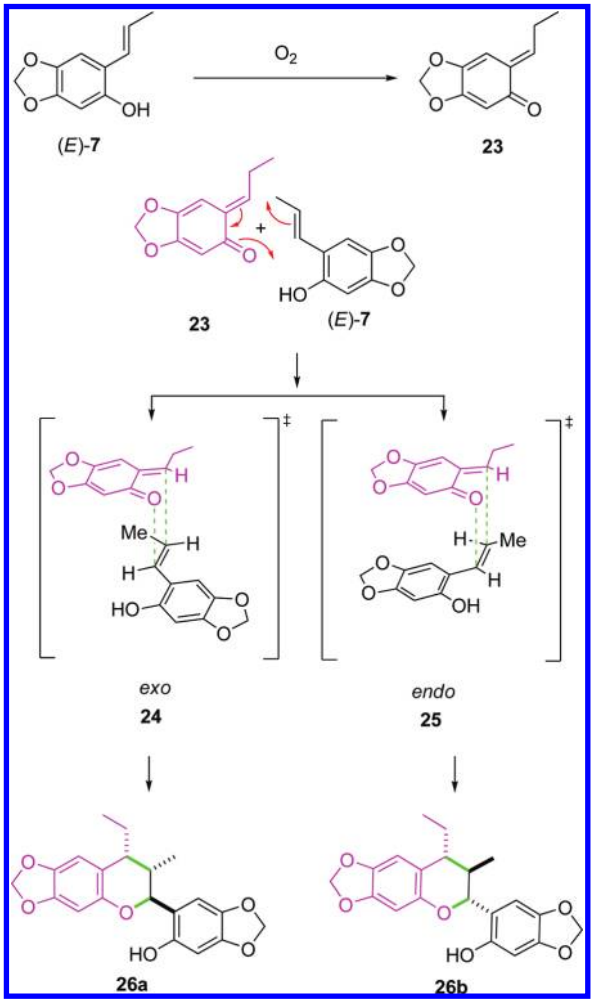
**Scheme 7. Proposed Reaction Mechanism for the Formation of 26a and 26b by Domino Oxidation/Intermolecular Hetero-Diels–Alder Reaction of (*E*)-7**




**Structure Elucidation.** Using optimized HPLC conditions (see the Supporting Information) the separation of the three diastereoisomers **1a**, **1c**, and **1d** could be achieved. Overall, the $^{1}$H and $^{13}$C NMR spectra showed high similarity except for the signals of 7′-H, 8′-H, 8-H, and 2-H of the cyclohexene ring in compounds **1a**, **1c**, and **1d**.

The major compound was identified as carpanone (**1a**) on the basis of a comparison of its fully assigned $^{1}$H and $^{13}$C NMR data with literature data of carpanone (**1a**).[4,10] Since repeated HPLC afforded only a few milligrams of each of the compounds **1c** and **1d** single crystals could not be obtained. This is why for the determination of the relative stereochemistry of **1c** and **1d** an alternative approach comprising analysis of experimental $J_{H,H}$ values and ROESY data in combination with calculated coupling constants and dihedral angles obtained by ab initio DFT calculations was applied. Thus, all possible diastereoisomers of carpanone (**1a**) which can be differentiated by NMR (16 diastereoisomers due to five stereogenic centers) were subsequently optimized at the AM1 level followed by the RHF/3-21G level and finally by the B3LYP/6-31G(d) level of theory within the Gaussian 03 package (for references see the Supporting Information). In the next step the $^{1}$H,$^{1}$H NMR coupling constants of those optimized geometries showing the best fit to the NMR data of **1a** (most probable **1aA**, **1aB**), **1c** and **1d** were recomputed at the B3LYP/6-31+G(d,p) level of theory and compared with the experimental NMR data sets. For evaluation purposes, we first applied our methodology to the determination of the already known relative stereochemistry of **1a** because the medium-sized coupling constants $^{3}J$(7′-H, 8′-H) = 2.6 Hz, $^{3}J$(2-H, 7′-H) = 7.3 Hz, and $^{3}J$(8-H, 8′-H) ∼ 1 Hz (see Table 5) did not allow an unambiguous assignment

**Table 5. Important Experimental $^{1}$H NMR Coupling Constants for 1a, 1c, and 1d**

| entry | 1 | $^{3}J_{2,7'}$ (Hz) | $^{3}J_{7',8'}$ (Hz) | $^{3}J_{8,8'}$ (Hz) | $^{3}J_{7,8}$ (Hz) |
|---|---|---|---|---|---|
| 1 | **a** | 7.3 | 2.6 | ∼1.0 | 5.2 |
| 2 | **c** | 10.4 | 11.7 | 3.7 | 6.4 |
| 3 | **d** | 10.7 | 10.0 | 9.7 | 3.8 |

of the relative configuration at C-2, C-8, C-7′, and C-8′. However, strong ROESY correlations between 7′-H and 2-H, 2-H and 9′-$CH_3$, 7′-H and 8′-H, and 7′-H and 9′-$CH_3$ indicate a *cis*-orientation of 2-H, 7′-H, and 9′-$CH_3$ as well as a *trans*-located proton 8′-H with respect to the reference plane of the molecule. Because of the complex multiplet pattern (caused by additional long-range couplings) of 8-H and 8′-H the vicinal coupling constants could not easily be deduced. Along with a weak ROESY correlation between 9-$CH_3$ and 9′-$CH_3$ two possible configurations at C-8 can be discussed (Figure 6). Selective

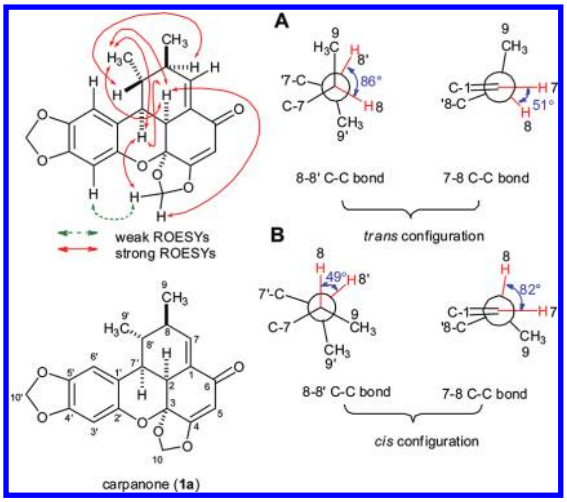



**Figure 6.** Stereochemistry and numbering of carpanone (**1a**). (A) Dihedral angles between 8-H and 8′-H (left) and 7-H and 8-H (right) in *trans*-orientation of the methyl groups. (B) Dihedral angles concerning 8-H and 8′-H (left) and 7-H and 8-H (right) in *cis*-orientation of the methyl groups.

homoband decoupling of the resonances of both methyl groups at C-9 and C-9′ (see the Supporting Information) revealed a small coupling of ∼1 Hz, which accounts for a dihedral angle of ∼90° between 8-H and 8′-H according to the Karplus relationship. Moreover, an experimental coupling constant of $^{3}J$(7-H, 8-H) = 5.2 Hz which fits quite nicely with a dihedral angle of ∼50° (calcd 51° and 5.6 Hz) establishes substructure **A** in carpanone (**1a**). On the contrary, one would expect coupling constants of $^{3}J$(8-H, 8′-H) ∼ 5 Hz (calculated +5.2 Hz and 49°) and $^{3}J$(7-H, 8-H) ∼1−2 Hz (calculated 3.2 Hz and 82°) in the case of substructure **B**. The ROESY correlations of 10-H with 2-H and 10-H with 7′-H indicate the position of the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3,4-methylenedioxycyclohexenone ring below the reference plane of the molecule **1a**.

Contrary to **1a**, the $^1$H NMR spectra of **1c** and **1d** showed partially overlapped and/or complex multiplet patterns for 7′-H, 2-H, 8′-H, and 8-H of the cyclohexene rings (see the Supporting Information) preventing stereochemical assignments based on coupling constants. Therefore, selective homoband decoupling methods as well as computational $^1$H spin pattern analysis (SpinWorks 3.1)[46] were applied to extract individual coupling constants for assignment purposes (see the Supporting Information). An observed coupling constant of 11.7 Hz between 7′-H and 8′-H in diastereoisomer **1c** compared to 2.6 Hz in **1a** (Table 5) and significant $^{13}$C chemical shifts differences of C-2 (**1a**: $\delta$ 35.2 ppm/**1c**: $\delta$ 44.3 ppm), C-7′(**1a**: $\delta$ 33.5 ppm/**1c**: $\delta$ 46.1 ppm), C-8′ (**1a**: $\delta$ 35.5 ppm/**1c**: $\delta$ 37.6 ppm), and C-8 (**1a**: $\delta$ 36.2 ppm/**1c**: $\delta$ 40.6 ppm) (Table 6) reflect important evidence for an altered relative

**Table 6. Chemical Shift Differences of 1a, 1c, and 1d in $^{13}$C NMR Spectra**

| entry | 1 | C-7′ (ppm) | C-8′ (ppm) | C-2 (ppm) | C-8 (ppm) |
|---|---|---|---|---|---|
| 1 | **a** | 33.5 | 35.5 | 35.2 | 36.2 |
| 2 | **c** | 46.1 | 37.6 | 44.3 | 40.6 |
| 3 | **d** | 46.9 | 32.0 | 44.0 | 33.6 |

stereochemistry in **1c**. Another large coupling constant between 7′-H and 2-H (10.4 Hz) along with a strong ROESY between 2-H and 8′-H indicates an alternating *trans*-diaxial orientation of these protons (2-H, 7′-H and 7′-H, 8′-H; Figure 7). An additional

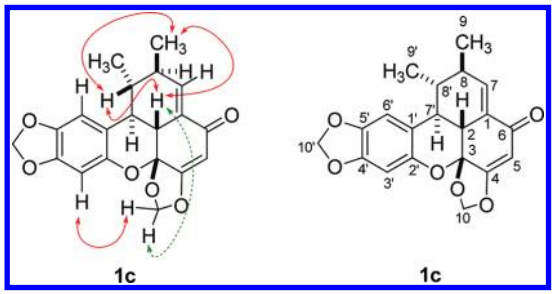


**Figure 7.** Chemical structures and numbering of **1c**. Important ROESY correlations (green arrow, weak ROESY correlations; red arrow, strong ROESY correlations).

ROESY between 2-H and 9-$CH_3$ as well as 8′-H and 9-$CH_3$ unequivocally arrange 2-H, 9-$CH_3$ and 8′-H above the cyclohexene ring. Finally, the weak ROESY correlation between 2-H to 10-H reveals the position of the 3,4-methylenedioxycyclohexenone ring above the reference plane of molecule **1c**. Coupling constants obtained by DFT calculations were close to experimental values (see the Supporting Information).

As for **1c**, the coupling constants of the complex multiplet patterns of **1d**, e.g., 8′-H and 8-H (Figure 8) were analyzed and extracted by spin analysis and decoupling experiments (see the Supporting Information).

Similarly to **1c** the protons 2-H, 7′-H, and 8′-H of **1d** show an alternating *trans*-axial arrangement as evidenced by vicinal coupling constants of ∼10 Hz and ROESY correlations between 2-H and 8′-H as well as 7′-H and 9′-$CH_3$ (Figure 9). Besides considerably different $^{13}$C chemical shifts values of C-8′ and C-8 (see Table 6) compared to **1c**, strong ROESYs between 2-H and 8-H as well as 9′-$CH_3$ and 9-$CH_3$ clearly establish the axial position of 8-H and the *cis*-configuration of the methyl groups 9 and 9′. As for **1c**, the weak ROESY correlation of 10-H to 2-H indicates the 3,4-methylenedioxycyclohexenone ring above the reference plane of molecule **1d**. The calculated coupling constants obtained by DFT methods were in agreement with the experimental data (see the Supporting Information).

In 2009, Poli et al.[10] reported on the $CuCl_2$-catalyzed synthesis of carpanone. They obtained an inseparable mixture of carpanone (**1a**) and a diastereoisomer **1b** whose stereostructure was elucidated by NOESY as shown in Figure 10 of their contribution. However, comparison of the whole NMR data set of isolated **1d** with the NMR spectra of the mixture of **1a** and **1b** obtained by Poli et al. ($^1$H and $^{13}$C of **1a** and **1b**, see the Supporting Information)[10] discloses that both data sets are identical suggesting that either our structural assignment of **1d** or the assignment of **1b** proposed by Poli et al.[10] has to be incorrect. Evaluation of the NOESY and $^1$H NMR spectra of the mixture of **1a** and **1b** published by Poli et al.[10] reveals that the positions of 7′-H and 9-$CH_3$ have to be corrected for the following reasons: a large coupling constant of ∼11 Hz between 2-H and 7′-H indicates a dihedral angle close to 0° or 180°. In the NOESY spectrum, a strong correlation peak between the aforementioned protons suggests a *cis*-orientation. However, detailed inspection of the cross peak gives rise to a COSY type artifact (zero-quantum coherence) due to its anti phase pattern and not to a NOESY correlation. Moreover, in the case of a *cis*-arrangement between 2-H and 7′-H one must also observe NOEs between 7′-H and 8′-H as well as between 7′-H and 8-H or 9-$CH_3$ in **1b**. Strong NOESYs between 2-H and 8′-H as well as 9-$CH_3$ and 9′-$CH_3$ along with a coupling constant of 9.7 Hz between 8-H and 8′-H (dihedral angle close to 0) clearly establish a *cis*-orientation of both methyl groups at C-9 and C-9′. Furthermore a NOE between 7′-H and 9′-$CH_3$ proved a *cis*-arrangement of 7′-H and 9′-$CH_3$. Unfortunately, NOESY correlations concerning protons 8-H and 8′-H could not be evaluated for an unambiguous assignment of stereostructure **1b** because of the partial overlap of these signals with the signals of **1a** in the investigated mixture of **1a** and **1b**. In summary, the NMR arguments presented here justify the conclusion that the minor diastereoisomer of Poli's product mixture has not structure **1b** as previously assigned, but structure **1d** (Figure 10).

## CONCLUSIONS

The laccase-catalyzed oxidative dimerization of (*E*)-propenylsesamol (**7**) which can be performed in aqueous solvent systems and under mild reaction conditions offers a new and efficient access to carpanone (**1a**). To study the influence of the stereochemistry of the olefinic double bond in **7** on the outcome of the carpanone formation, (*E*)-**7** and (*Z*)-**7** were separated and reacted in diastereoisomerically pure form using different catalysts. In contrast to the results from previous studies it was established that the reaction of pure (*E*)-**7** results in the formation of a mixture of carpanone (**1a**) and its diastereoisomer **1c**. Irrespective of the catalyst used, a 9:1 mixture of **1a** and **1c** was formed. The structure of diastereoisomer **1c**, which was unknown so far, was unambiguously elucidated by means of NMR spectroscopy, DFT calculations, and spin work analysis. Both **1a** and **1c** are the products of a domino phenol oxidation/*anti*-$\beta$,$\beta$-radical coupling/intramolecular hetero-Diels–Alder reaction. In the first step of the reaction, the oxidation of (*E*)-**7** takes place to yield radical **16**, which dimerizes diastereoselectively to give **9** as the product of an *anti*-$\beta$,$\beta$-coupling reaction. Subsequently, **9** undergoes an intramolecular hetero-Diels–Alder reaction to **1a** via the *endo-E-syn* transition state **17** and to **1c** via


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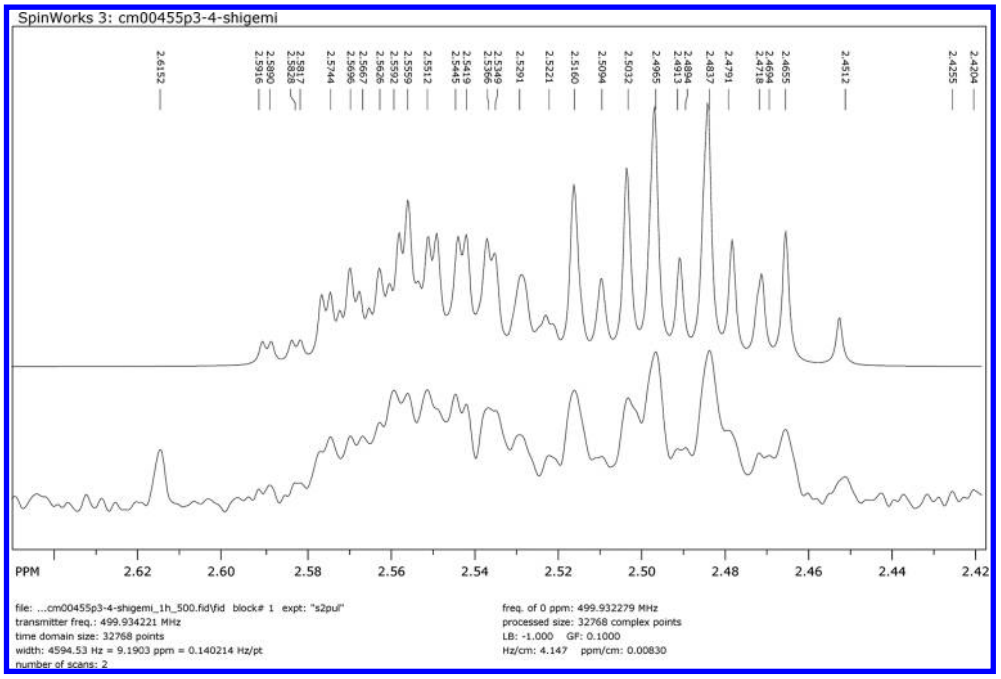


**Figure 8.** Experimental (lower) and iterated (upper) multiplet pattern of 8′-H ($\delta$ 2.50 ppm) and 8-H ($\delta$ 2.55 ppm) in **1d**.

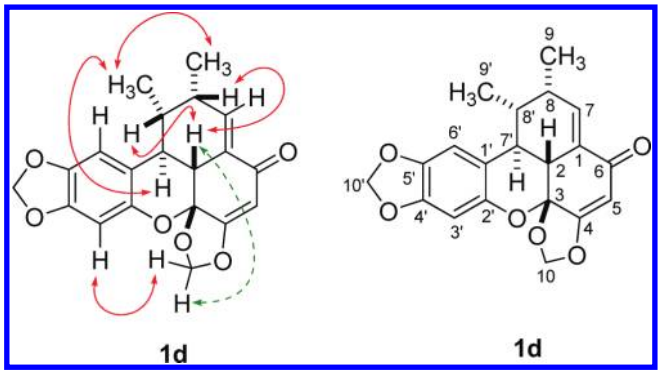


**Figure 9.** Chemical structures and numbering of **1d**. Important ROESY correlations (green arrow, weak ROESY correlations; red arrow, strong ROESY correlations).

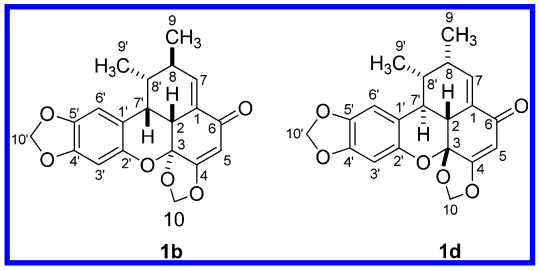


**Figure 10.** Structure of **1b** as proposed by Poli et al.,[10] and structure of **1d**.

*exo-E-anti* transition state **18**. With pure (*Z*)-7 as the substrate, carpanone (**1a**) and the two carpanone diastereoisomers **1c** and **1d** were formed in a 5:1:4 ratio. The diastereoisomers were separated by HPLC, and the structure of **1d** was elucidated by combination of NMR spectroscopy, DFT calculations, and spin work analysis. For the formation of **1d** it is assumed that the dimerization of **16** proceeds as a *syn*-$\beta,\beta$-coupling reaction and subsequent intramolecular hetero-Diels–Alder reaction of the coupling product **20** via an *exo-E-anti* transition state. So far, it remains unclear why the reactions of (*E*)- and (*Z*)-7 yield different products. The oxidation of (*E*)-7 with $O_2$ in the absence of any catalyst came as a surprise as the formation of two diastereoisomeric benzopyrans was observed. The formation of the benzopyrans can be understood in such a way that the oxidation of (*E*)-7 in the absence of any catalyst results in the formation of an *o*-quinone methide which undergoes an intermolecular hetero-Diels–Alder as a diene with (*E*)-7 as the dienophile.

## EXPERIMENTAL SECTION

**General Remarks.** All commercially available reagents were used without further purification. Solvents used for extraction and purification were distilled prior to use. Reaction temperatures are reported as bath temperatures. Melting points were obtained on a melting point apparatus with open capillary tubes and are uncorrected. IR spectra were measured on a FT-IR spectrometer. UV/vis spectra were recorded with a spectrophotometer. Optical rotations were determined using a polarimeter. $^1$H and $^{13}$C NMR spectra were recorded at 500 (125) and 300 (75) MHz, and the NMR chemical shifts were referenced to $CDCl_3$ as solvent signals ($\delta$ = 7.26 ppm in $^1$H spectra and $\delta$ = 77.00 in $^{13}$C spectra) relative to TMS. HSQC, HMBC, NOESY, ROESY, HSQMBC, and COSY spectra were recorded on a spectrometer at 500 and 300 MHz. Coupling constants *J* (Hz) were directly taken from the spectra and are not averaged. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). 1D and 2D homonuclear NMR spectra were measured with standard pulse sequences. Low-resolution impact mass spectra were obtained at 70 eV. The intensities are reported as percentages relative to the base peak (*I* = 100%). Thin-layer chromatography (TLC) was performed on TLC silica gel 60 F254. Products were purified by column chromatography on silica gel MN 60, 0.063–0.20 mm, or by flash column chromatography on silica gel MN 60, 0.04–0.053 mm. Analytical HPLC was performed using a HPLC pump equipped with a PDA detector. Laccases from *T. versicolor* and *A. bisporus* were commercially available (EC 1.10.3.2), Lcc$\beta$: $\beta$-laccase from *T. versicolor* was heterologously expressed in *P. pastoris* X-33 cells.[43] Recombinant cells were cultured in a 7.5 L bioreactor (Infors) in basal salt medium (5 L) [$K_2SO_4$ (9.1 g), $MgSO_4{\cdot}7H_2O$ (7.5 g), KOH (6.2 g), $CaSO_4{\cdot}2H_2O$ (2.47 g), 85% $H_3PO_4$ (13.35 mL), glycerol (50 g), antifoam solution 286 (0.1 mL), biotin (0.87 mg), and $PTM_1$ (4.35 mL) trace salts per liter]. One liter of $PTM_1$ contains $CuSO_4{\cdot}5H_2O$ (6 g), NaI (0.08 g), $MnSO_4{\cdot}H_2O$ (3.0 g), $CoCl_2$ (0.5 g), $ZnCl_2$


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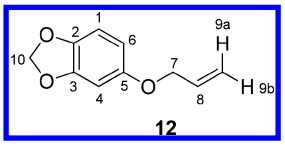
(20.0 g), $H_3BO_3$ (0.02 g), $Na_2MoO_4{\cdot}2H_2O$ (0.2 g), $FeSO_4{\cdot}7H_2O$ (65.0 g), biotin (0.2 g), and $H_2SO_4$ (5 mL). Cells were grown at 30 °C (pH 5.0, aeration 10 L/min, agitation 1000 rpm) during the glycerol phase. After depletion of glycerol, the methanol-fed batch phase was started, $CuSO_4$ (0.3 mM) was added, and the temperature was decreased to 18 °C. A dissolved $O_2$ spike controlled feeding method was applied for automatic addition of depleted methanol [20 g, with 1.2% (v/v) $PTM_1$ 0.5% (v/v)]. After 10 days of cultivation, cells were harvested (10.000 g, 20 min), and Lcc$\beta$-containing solution was concentrated by cross-flow ultrafiltration [Millipore, 10 kDa cutoff membrane (Pall)]. The activities of the enzymes were determined according to a modified procedure taken from ref 30 using a solution of ABTS [(2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] as substrate. The change in absorption was followed via UV spectroscopy ($\lambda$ = 414 nm; $\varepsilon_{414}$ = 31100 L $mol^{-1}$ $cm^{-1}$). One U is equivalent to the amount of enzyme that catalyzes the conversion of 1 $\mu$mol ABTS per minute at 24 °C and pH 5.0.

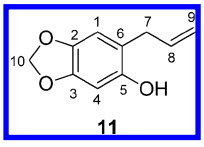
*5-Allyloxy-1,3-benzodioxole (**12**).*[39]




**12**

A solution of sesamol (**13**) (10 g, 72 mmol) in acetone (50 mL) was refluxed for 20 min over dried $K_2CO_3$ (10 g, 70.2 mmol). The mixture was cooled to room temperature, allyl bromide (10 mL, 0.11 mol) was added, and the resulting mixture was refluxed at 70 °C for 5 h under argon. The reaction mixture was cooled to room temperature, and $H_2O$ (500 mL) was added. The organic layer was removed, and the aqueous phase was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with 2 N NaOH (2 × 100 mL) and saturated NaCl solution (2 × 100 mL) and dried over $Na_2SO_4$, and the volatiles were removed under reduced pressure. The residue thus obtained was purified by column chromatography over silica gel (petroleum ether/EtOAc = 10:1) to afford 5-allyloxy-1,3-benzodioxole (**12**) as a colorless oil in 84% yield (10.7 g, 60.11 mmol): $R_f$ = 0.54 (petroleum ether/EtOAc = 10:1); IR (ATR) $\bar{\nu}$ 2886 ($OCH_2O$), 1630 (CH═$CH_2$), 1484 (arom C═C), 1178 (ArOCH), 1036 (ArOCH), 922 (C═CHH), 814 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 235 (3.63), 296 (3.66) nm; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.46 (dt, $^3J$ (7-H, 8-H) = 5.2 Hz, $^4J$ (7-H, 9a-H) = 1.5 Hz, $^4J$ (7-H, 9b-H) = 1.5 Hz, 2H, 7-H), 5.27 (dq, $^3J$ (8-H, 9b-H) = 10.5 Hz, $^2J$ (9a-H, 9b-H) = 1.5 Hz, 1H, 9b-H), 5.42 (dq, $^3J$ (8-H, 9a-H) = 17.2 Hz, $^2J$ (9a-H, 9b-H) = 1.5 Hz, 1H, 9a-H), 5.91 (s, 2H, 10-H), 6.03 (ddd, $^3J$ (7-H, 8-H) = 5.1 Hz, $^3J$ (8-H, 9b-H) = 10.3 Hz, $^3J$ (8-H, 9a-H) = 17.2 Hz, 1H, 8-H), 6.34 (dd, $^3J$ (1-H, 6-H) = 8.5 Hz, $^4J$ (4-H, 6-H) = 2.5 Hz, 1H, 6-H), 6.52 (d, $^4J$ (4-H, 6-H) = 2.5 Hz, 1H, 4-H), 6.70 (d, $^3J$ (1-H, 6-H) = 8.5 Hz, 1H, 1-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 69.8 (C-7), 98.3 (C-4), 101.1 (C-10), 106.0 (C-6), 107.9 (C-1), 117.6 (C-9), 133.4 (C-8), 141.7 (C-2), 148.2 (C-3), 154.1 (C-5); MS (EI, 70 eV) $m/z$ 178 (100) $[M]^+$, 137 (100) $[M - C_3H_5]^+$, 107 (69), 79 (8), 28 (100).

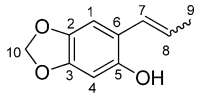
*6-Allyl-1,3-benzodioxol-5-ol (**11**).*[40]




**11**

A round-bottomed flask equipped with a reflux condenser was charged with 5-allyloxy-1,3-benzodioxole (**12**) (10 g, 56.18 mmol) and heated at 180 °C for 90 min under argon. The mixture was cooled to room temperature, and the residue obtained was purified by column chromatography over silica gel (petroleum ether/EtOAc = 10:1) to afford 6-allyl-1,3-benzodioxol-5-ol (**11**) as colorless needles in 90% yield (9.3 g, 52.24 mmol): mp 75−76 °C; $R_f$ = 0.38 (petroleum ether/EtOAc = 10:1); IR (ATR) $\bar{\nu}$ 3244 (OH), 2907 ($OCH_2O$), 1636 (CH═$CH_2$), 1457 (arom C═C), 1167 (ArOCH), 1030 (ArOCH), 909 (C═CHH), 830(arom C−H) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 235 (4.53), 302 (3.67) nm; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 3.30−3.32 (m, 2H, 7-H), 5.12−5.18 (m, 2H, 9-H), 5.92−6.01 (m, 1H, 8-H), 5.98 (s, 2H, 10-H), 6.43 (s, 1H, 4-H), 6.58 (s, 1H, 1-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 34.9 (C-7), 98.6 (C-4), 100.9 (C-10), 109.5 (C-1), 116.3 (C-9), 116.9 (C-6), 136.4 (C-8), 141.4 (C-2), 146.7 (C-3), 148.5 (C-5); MS (EI, 70 eV) $m/z$ 178 (39) $[M]^+$, 151 (8) $[M - C_2H_3]^+$, 28 (100).

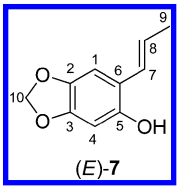
*Synthesis of 6-(Prop-1-enyl)-1,3-benzodioxol-5-ol (**7**) and Separation of (E)-**7** and (Z)-**7**.*[4]




**7**

To a solution of 6-allyl-1,3-benzodioxol-5-ol (**11**) (2.5 g, 14 mmol) in dry DMSO (25 mL) was added potassium *tert*-butoxide (1.7 g, 15 mmol), and the apparatus was flushed with argon. The reaction mixture was heated at 100 °C for 90 min under argon. The solution was cooled using an ice−water bath, water (100 mL) was added, and the mixture was acidified with 5% aq HCl (about 20 mL). The solution was extracted with EtOAc (3 × 150 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure to give 3 g of a brown liquid. The crude product was purified by column chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford (*E*)-6-(prop-1-enyl)-1,3-benzodioxol-5-ol [(*E*)-**7**] in 52% yield (1.3 g, 7.30 mmol) and (*Z*)-6-(prop-1-enyl)-1,3-benzodioxol-5-ol [(*Z*)-**7**] in 19% yield (0.475 g, 2.70 mmol).

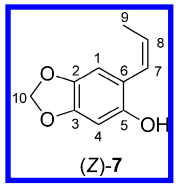
*(E)-6-(Prop-1-enyl)-1,3-benzodioxol-5-ol [(E)-**7**].*




(*E*)-**7**

mp 84−85 °C (87−88 °C);[4] $R_f$ = 0.20 (dichloromethane/cyclohexane = 5:2); IR (ATR) $\bar{\nu}$ 3319 (OH), 2898 ($OCH_2O$), 1636 (C═C), 1441 (arom C═C), 1163 (ArOCH), 1031 (ArOCH), 928 (C═CH), 833 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 258 (3.60), 326 (3.46) nm; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.88 (dd, $^3J$ (8-H, 9-H) = 6.6 Hz, $^4J$ (7-H, 9-H) = 1.7 Hz, 3H, 9-H), 4.79 (s, 1H, OH), 5.88 (s, 2H, 10-H), 6.03 (dq, $^3J$ (7-H, 8-H) = 15.7 Hz, $^3J$ (8-H, 9-H) = 6.6 Hz, 1H, 8-H), 6.39 (s, 1H, 4-H), 6.50 (dq, $^3J$ (7-H, 8-H) = 15.7 Hz, $^4J$ (7-H, 9-H) = 1.50 Hz, 1H, 7-H), 6.76 (s, 1H, 1-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 18.7 (C-9), 98.2 (C-4), 101.0 (C-10), 105.9 (C-1), 117.3 (C-6), 124.9 (C-8), 126.6 (C-7), 141.8 (C-2), 147.0 (C-3), 147.2 (C-5).

*(Z)-6-(Prop-1-enyl)-1,3-benzodioxol-5-ol [(Z)-**7**].*




(*Z*)-**7**

$R_f$ = 0.18 (dichloromethane/cyclohexane = 5:2); IR (ATR) $\bar{\nu}$ 3479 (OH), 2890 ($OCH_2O$), 1627 (C═C), 1439 (arom C═C), 1165 (ArOCH), 1034 (ArOCH), 932 (C═CH), 845 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 256 (3.88), 312 (3.85) nm; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.71 (dd, $^3J$ (8-H, 9-H) = 6.9 Hz, $^4J$ (7-H, 9-H) = 1.8 Hz, 3H, 9-H), 4.83 (s, 1H, OH), 5.90 (s, 2H, 10-H), 5.94 (dq, $^3J$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(7-H, 8-H) = 11.1 Hz, $^3J$ (8-H, 9-H) = 6.9 Hz, 1H, 8-H), 6.28 (dq, $^3J$ (7-H, 8-H) = 11.1 Hz, $^4J$ (7-H, 9-H) = 1.5 Hz, 1H, 7-H), 6.48 (s, 1H, 4-H), 6.56 (s, 1H, 1-H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.5 (C-9), 97.5 (C-4), 101.0 (C-10), 108.3 (C-1), 114.9 (C-6), 124.0 (C-8), 130.9 (C-7), 141.1 (C-2), 147.3 (C-3), 147.6 (C-5).

**General Procedure for the Oxidative Dimerization of (*E*)- and (*Z*)-7 Using Commercially Available Laccases as Catalysts.** Laccase (28 U) dissolved in sodium acetate buffer (0.1 M, pH 5.0, 1 mL) was added to a solution of **7** (178 mg, 1.00 mmol) in a cosolvent (2 or 4 mL) and sodium acetate buffer (0.1 M, pH 5.0, 17 mL). The resulting suspension was stirred at room temperature under $O_2$. NaCl (3 g) was added, and the aqueous phase was extracted with dichloromethane (80 mL). The phases were separated, and saturated NaCl solution (20 mL) was added to the aqueous phase. The aqueous phase was extracted with dichloromethane (80 mL). This procedure was repeated twice. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2).

*1. Oxidative Dimerization of a Mixture of (E)- and (Z)-**7** Using a Commercially Available Laccase from T. versicolor as the Catalyst.* Reaction of an 8:2 mixture of (*E*)/(*Z*)-7 (180 mg, 1.01 mmol) with laccase (2.6 mg, 28 U) according to the general procedure gave a mixture of **1a**, **1c**, and **1d** as a white solid in 46% yield (83 mg, 0.23 mmol). The ratio of **1a**:**1c**:**1d** after flash chromatography was 6:1:3 ($^1$H NMR).

*2. Oxidative Dimerization of (E)-**7** Using $PdCl_2$ as the Reagent According to Chapman et al.*[4] $PdCl_2$ (90.0 mg, 0.5 mmol) was added to a solution containing (*E*)-7 (178 mg, 1.00 mmol) and NaOAc (670 mg, 8 mmol) in MeOH (17 mL) and $H_2O$ (3 mL). The solution was stirred at 38 °C for 2 h and then allowed to settle for 1 h at room temperature. The suspension was extracted with diethyl ether (3 × 30 mL). The combined organic phases were washed with 10% NaOH solution (2 × 20 mL) and $H_2O$ (2 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a** and **1c** as a white solid in 42% yield (75 mg, 0.21 mmol). The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*3. Oxidative Dimerization of (E)-**7** Using $PdCl_2$ as the Catalyst According to Poli et al.*[10] NaOAc (98.4 mg, 1.2 mmol) and $PdCl_2$ (17.74 mg, 0.1 mmol) were added to a solution of (*E*)-7 (175 mg, 0.98 mmol) in MeOH/$H_2O$ = 1:1 (6 mL). The resulting suspension was stirred at 50 °C for 4 h under $O_2$. The reaction mixture was cooled to room temperature, and $H_2O$ (20 mL) was added. The aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic phases were dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a** and **1c** as a white solid in 47% (77%)[10] yield (81 mg, 0.23 mmol). The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*4. Oxidative Dimerization of (E)-**7** Using Co Complex **14** as Catalyst.* (*R*,*R*)-(−)-*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (**14**) (15.5 mg, 0.026 mmol) was added to a solution of (*E*)-7 (183 mg, 1.03 mmol) in dichloromethane (10 mL). The resulting suspension was stirred under $O_2$ at room temperature for 4 h. To remove the complex from the reaction mixture, the solution was filtrated, and the residue was washed with dichloromethane (10 mL). The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a** and **1c** as a white solid in 79% yield (144 mg, 0.41 mmol). The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*5. Oxidative Dimerization of (E)-**7** Using Commercially Available Laccases as Catalysts.* Reaction of (*E*)-7 (182 mg, 1.02 mmol) with *A. bisporus*/*T. versicolor* laccase (49/2.6 mg, 28 U) according to the general procedure gave a mixture of **1a** and **1c** as a white solid. The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*6. Oxidative Dimerization of (E)-**7** Using a Lcc$\beta$ Laccase from T. versicolor as a Catalyst.* Reaction of (*E*)-7 (189 mg, 1.06 mmol) with laccase (8.2 mL, 28 U) according to the general procedure gave a mixture of **1a** and **1c** as a white solid. The reaction mixture was then lyophilized. The residue was refluxed for 90 min in dichloromethane (50 mL). After filtration the solvent was removed under reduced pressure. The crude product obtained was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a** and **1c** as a white solid in 53% yield (97 mg, 0.27 mmol). The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*7. Determination of the Enantiomeric Excess of **1a**, **1c**, and **1d** by Chiral HPLC.* The oxidative dimerization of (*E*)-7 was carried out with three different laccases (commercially available laccase from *T. versicolor*, commercially available laccase from *A. bisporus*, genetically modified Lcc$\beta$ laccase from *T. versicolor*). The crude product was purified by column chromatography on $SiO_2$ to give a mixture of **1a** and **1c**. Compounds **1a** and **1c** were separated by analytical HPLC (see the Supporting Information). After HPLC separation, the solvent was removed in vacuo, and the enantiomers of **1a** were separated by chiral HPLC (CHIRALPAK IB column; 250 mm × 4.6 mm; 5 $\mu$m; *n*-hexane/ethanol = 1:1; flow rate of 1 mL/min; UV detection $\lambda$ = 280 nm) to afford the two enantiomers ($t_R$ 4.95 min and $t_R$ 5.45 min) (Figure 11).The enantiomeric excess was calculated from the peak areas (Table 7).

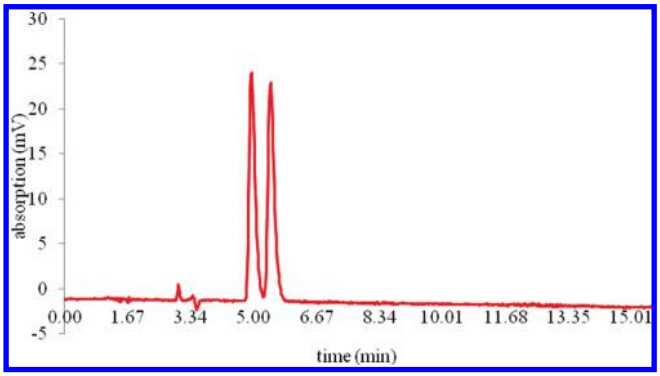



**Figure 11.** HPLC chromatogram of the separation of the carpanone enantiomers on an analytical column with a chiral stationary phase.

**Table 7. Determination of Enantiomeric Excess of the Laccase-Catalyzed Oxidations of (*E*)-7**

| entry | laccase | enantiomeric excess (**1a**) |
|---|---|---|
| 1 | commercialy available; *T. versicolor* | 0.44 |
| 2 | commercialy available; *A. bisporus* | 0.25 |
| 3 | recombinant Lcc$\beta$; *T. versicolor* | 0.43 |

The enantiomers of **1c** were separated by chiral HPLC (CHIRALPAK IB column; 250 mm × 4.6 mm; 5 $\mu$m; *n*-hexane/ethanol = 1:1; flow rate of 1 mL/min; UV detection $\lambda$ = 280 nm) to afford the two enantiomers ($t_R$ 5.07 min and $t_R$ 5.57 min) (Figure 12).The enantiomeric excess was calculated from the peak areas to ee (%) = 1.03.

The enantiomers of **1d** were separated by chiral HPLC (CHIRALPAK IB column; 250 mm × 4.6 mm; 5 $\mu$m; *n*-hexane/ethanol = 1:1; flow rate of 1 mL/min; UV detection $\lambda$ = 280 nm) to afford the two enantiomers ($t_R$ 5.50 min and $t_R$ 6.18 min) (Figure 13). The enantiomeric excess was calculated from the peak areas to ee (%) = 0.93.

*8. Oxidative Dimerization of (E)-**7** Using 4-Methoxy-TEMPO as the Reagent.* 4-Methoxy-TEMPO (50 mg, 0.27 mmol) was added to a solution of (*E*)-7 (184 mg, 1.03 mmol) in 10 mL of dry benzene. The resulting suspension was stirred under $O_2$ at 70 °C for 4 h. The suspension was concentrated under reduced pressure, and the


dx.doi.org/10.1021/jo300263k | J. Org. Chem. 2012, 77, 4528−4543

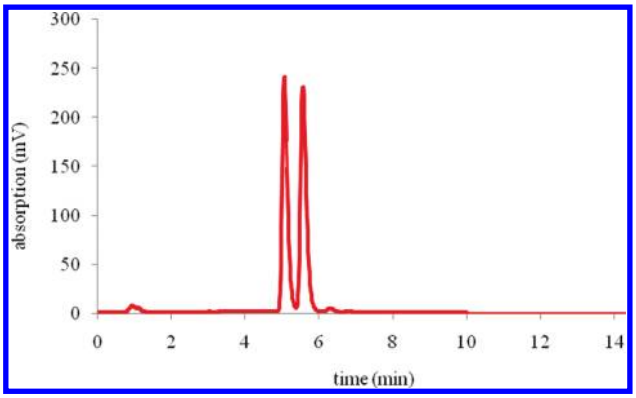



**Figure 12.** HPLC chromatogram of the separation of the enantiomers of **1c** on an analytical column with a chiral stationary phase.

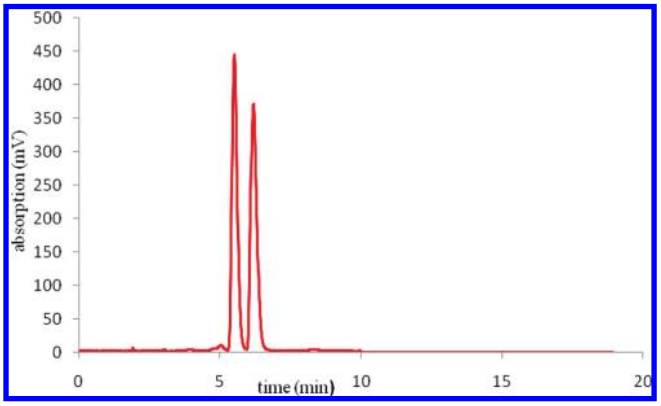



**Figure 13.** HPLC chromatogram of the separation of the enantiomers of **1d** on an analytical column with a chiral stationary phase.

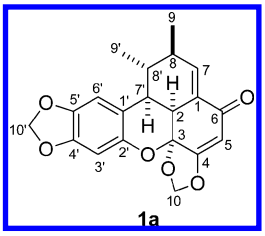
resulting residue was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a** and **1c** as a white solid in 79% yield (147 mg, 0.42 mmol). The ratio of **1a**:**1c** in the crude product was 9:1 ($^1$H NMR). The ratio of **1a**:**1c** after flash chromatography was 9:1 ($^1$H NMR).

*9. Oxidative Dimerization of (Z)-7 Using a Commercially Available Laccase from T. versicolor as the Catalyst.* Reaction of (*Z*)-7 (184 mg, 1.03 mmol) with laccase (2.6 mg, 28 U) according to the general procedure gave a mixture of **1a**, **1c**, and **1d** as a white solid in 40% yield (74 mg, 0.21 mmol). The ratio of **1a**:**1c**:**1d** after flash chromatography was 5:1:4 ($^1$H NMR). The compounds **1a**, **1c**, and **1d** were separated by analytical HPLC (see the Supporting Information).

*10. Oxidative Dimerization of (Z)-7 Using Co Complex **14** as Catalyst.* (*R*,*R*)-(−)-*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (**14**) (15.5 mg, 0.026 mmol) was added to a solution of (*Z*)-7 (181 mg, 1.02 mmol) in dichloromethane (10 mL). The resulting suspension was stirred under $O_2$ at room temperature for 4 h. To remove the complex from the reaction mixture, the solution was filtrated and the residue was washed with dichlorometane (10 mL). The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **1a**, **1c**, and **1d** as a white solid in 43% yield (78 mg, 0.22 mmol). The ratio of **1a**:**1c**:**1d** after flash chromatography was 5:1:4 ($^1$H NMR).

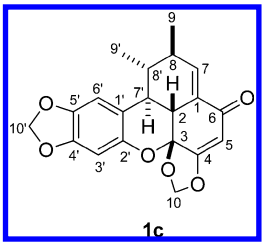
**Isomerization Experiment with Carpanone (1a).** Sodium acetate buffer (0.1 M, pH 5.0, 4 mL) was added to a solution of carpanone (**1a**) (50 mg, 0.14 mmol) in acetone (1.1 mL). A solution of laccase (8 U) from *T. versicolor* was dissolved in sodium acetate buffer (0.1 M, pH 5.0, 1 mL) and added dropwise and with stirring to the carpanone suspension. The reaction mixture was stirred under $O_2$ at room temperature for 2 h. The reaction mixture was extracted with dichloromethane (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. In the crude product only carpanone (**1a**) could be detected ($^1$H NMR). The crude product was purified by column chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford pure carpanone (**1a**) ($^1$H NMR) as a white solid in quantitative yield (50 mg, 0.14 mmol).

*(3R*)-(2β,7′β,8α,8′β)-1,7-Didehydro-1,2,3,6-tetrahydro-2′,3-epoxy-3,4:4′,5′-bis(methylenedioxy)-2,7′-cyclolignan-6-one) (**1a**).*




mp 189−190 °C (lit.[4] mp 185 °C); $R_f$ = 0.10 (dichloromethane/cyclohexane = 5:2); IR (ATR) $\bar{\nu}$ 2869 ($OCH_2O$), 1674 (CO), 1622 (C═C), 1478 (arom C═C), 1158 (ArOCH), 1032 (ArOCH), 910 (C═CH), 841 (arom CH) cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 262 (4.00), 297 (3.66) nm; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.71 (d, $^3J$ (8-H, 9-H) = 7.7 Hz, 3H, 9-H), 1.15 (d, $^3J$ (8′-H, 9′-H) = 7.0 Hz, 3H, 9′-H), 2.22 (ddq, $^3J$ (8-H, 9-H) = 7.7 Hz, $^3J$ (7-H, 8-H) = 5.2 Hz, $^3J$ (8-H, 8′-H) = 2.5 Hz, 1H, 8-H), 2.52 (br dq, $^3J$ (8′-H, 9′-H) = 7.2 Hz, $^3J$ (8-H, 8′-H) = 2.5 Hz, $^3J$ (7′-H, 8′-H) = 2.6 Hz, 1H, 8′-H), 3.19 (dt, $^3J$ (2-H, 7′-H) = 7.4 Hz, $^4J$ (2-H, 7-H) = 2.4 Hz, 1H, 2-H), 3.28 (dd, $^3J$ (7′-H, 8′-H) = 2.7 Hz, $^3J$ (7′-H, 2-H) = 7.2 Hz, 1H, 7′-H), 5.64 (s, 1H, 10-H), 5.67 (s, 1H, 10-H), 5.70 (s, 1H, 5-H), 5.90 (d, $^2J$ (10′-H, 10′-H) = 1.4 Hz, 1H, 10′-H), 5.91 (d, $^2J$ (10′-H, 10′-H) = 1.4 Hz, 1H, 10′-H), 6.33 (s, 1H, 3′-H), 6.80 (s, 1H, 6′-H), 7.02 (ddd, $^3J$ (7-H, 8-H) = 5.2 Hz, long-range $J$ = 1.0 Hz, $^4J$ (2-H, 7-H) = 2.5 Hz, 1H, 7-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 21.2 (C-9), 21.4 (C-9′), 33.5 (C-7′), 35.2 (C-2), 35.5 (C-8′), 36.2 (C-8), 98.8 (C-10), 99.3 (C-3′), 100.1 (C-3), 100.4 (C-5), 101.2 (C-10′), 107.1 (C-6′), 115.3 (C-1′), 126.3 (C-1), 142.5 (C-7), 143.1 (C-5′), 145.1 (C-2′), 146.6 (C-4′), 168.3 (C-4), 186.9 (C-6); MS (EI, 70 eV) $m/z$ 354 (100) [M]$^+$, 177 (72), 147 (60), 59 (28), 28 (40).

*(3S*)-(2α,7′β,8α,8′β)-1,7-Didehydro-1,2,3,6-tetrahydro-2′,3-epoxy-3,4:4′,5′-bis(methylenedioxy)-2,7′-cyclolignan-6-one) (**1c**).*




$R_f$ = 0.10 (dichloromethane/cyclohexane =5:2); IR (ATR) $\bar{\nu}$ 2924 ($OCH_2O$), 1675 (CO), 1610 (C═C), 1477 (arom C═C), 1150 (ArOCH), 1037 (ArOCH), 977 (C═CH), 894 (arom CH) cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 278 (2.77) nm; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.22 (d, $^3J$ (8-H, 9-H) = 7.5 Hz, 3H, 9-H), 1.30 (d, $^3J$ (8′-H, 9′-H) = 6.6 Hz, 3H, 9′-H), 2.08 (ddq, $^3J$ (8′-H, 9′-H) = 6.6 Hz, $^3J$ (7′-H, 8′-H) = 11.6 Hz, $^3J$ (8-H, 8′-H) = 3.7 Hz, 1H, 8′-H), 2.40 (ddd, $^3J$ (7′-H, 8′-H) = 11.8 Hz, $^3J$ (2-H, 7′-H) = 10.4 Hz, $^4J$ (6′-H, 7′-H) = 1.1 Hz, 1H, 7′-H), 2.42 (m, $^3J$ (8-H, 9-H) = 7.5 Hz, $^3J$ (7-H, 8-H) = 6.4 Hz, $^3J$ (8-H, 8′-H) = 3.7 Hz, 1H, 8-H), 2.90 (ddd, $^4J$ (2-H, 7-H) = 2.7 Hz, $^3J$ (2-H, 7′-H) = 10.2 Hz, long-range $J$ = 1.6 Hz, 1H, 2-H), 5.42 (s, 1H, 10-H), 5.55 (s, 1H, 10-H), 5.84 (s, 1H, 5-H), 5.95 (d, $^2J$ (10′-H, 10′-H) = 1.5 Hz, 1H, 10′-H), 5.97 (d, $^2J$ (10′-H, 10′-H) = 1.5 Hz, 1H, 10′-H), 6.60 (s, 1H, 3′-H), 6.88 (d, $^4J$ (6′-H, 7′-H) = 1.2 Hz, 1H, 6′-H), 7.11 (dd, $^3J$ (7-H, 8-H) = 6.4 Hz, $^4J$ (2-H, 7-H) = 2.7 Hz, 1H, 7-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 20.2 (C-9), 22.2 (C-9′), 37.6 (C-8′), 40.6 (C-8), 44.3 (C-2), 46.1 (C-7′), 96.0 (C-10), 101.4 (C-10′), 101.6 (C-3), 102.1 (C-3′), 104.5 (C-5), 105.2 (C-6′), 124.0 (C-1′), 132.8 (C-1), 142.8 (C-7), 144.1 (C-5′), 146.2 (C-4′), 147.2 (C-2′), 164.6 (C-4), 183.8 (C-6); MS (EI, 70 eV) $m/z$ 354 (45) [M]$^+$, 324 (22), 309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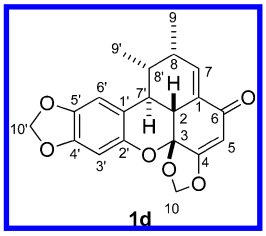
(23), 203 (31), 177 (48), 147 (100), 32 (33); HRMS (EI, $M^+$) calcd for $C_{20}H_{18}O_6$ (354.1104), found 354.1121.

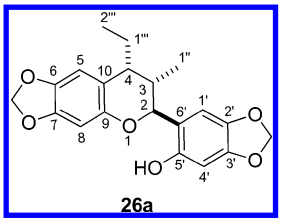
*(3S*)-(2α,7′β,8β,8′β)-1,7-Didehydro-1,2,3,6-tetrahydro-2′,3-epoxy-3,4:4′,5′-bis(methylenedioxy)-2,7′-cyclolignan-6-one (**1d**).*




$R_f$ = 0.10 (dichloromethane/cyclohexane = 5:2); IR (ATR) $\bar{\nu}$ 2924 ($OCH_2O$), 1674 (CO), 1603 (C═C), 1479 (arom C═C), 1154 (ArOCH), 1036 (ArOCH), 986 (C═CH), 894 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 280 nm (3.83); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.99 (d, $^3J$ (8′-H, 9′-H) = 6.6 Hz, 3H, 9′-H), 1.33 (d, $^3J$ (8-H, 9-H) = 7.3 Hz, 3H, 9-H), 2.05 (ddd, $^3J$ (7′-H, 8′-H) = 10.0 Hz, $^3J$ (2-H, 7′-H) = 10.6 Hz, $^4J$ (6′-H, 7′-H) = 0.9 Hz, 1H, 7′-H), 2.50 (ddq, $^3J$ (8′-H, 9′-H) = 6.6 Hz, $^3J$ (7′-H, 8′-H) = 10.0 Hz, $^3J$ (8-H, 8′-H) = 9.7 Hz, 1H, 8′-H), 2.55 (bdd, $^4J$ (2-H, 8-H) = 2.4 Hz, $^3J$ (7-H, 8-H) = 3.8 Hz, $^3J$ (8-H, 9-H) = 7.3 Hz, $^3J$ (8-H, 8′-H) = 9.7 Hz, 1H, 8-H), 2.83 (ddd, $^4J$ (2-H, 7-H) = 2.5 Hz, $^3J$ (2-H, 7′-H) = 10.9 Hz, long-range $J$ = 1.3 Hz, 1H, 2-H), 5.49 (s, 1H, 10-H), 5.64 (s, 1H, 10-H), 5.83 (s, 1H, 5-H), 5.95 (d, $^2J$ (10′-H, 10′-H) = 1.4 Hz, 1H, 10′-H), 5.96 (d, $^2J$ (10′-H, 10′-H) = 1.4 Hz, 1H, 10′-H), 6.61 (s, 1H, 3′-H), 6.78 (d, $^4J$ (6′-H, 7′-H) = 1.2 Hz, 1H, 6′-H), 6.89 ppm (bdd, $^3J$ (7-H, 8-H) = 3.8 Hz, $^4J$ (2-H, 7-H) = 2.3 Hz, 1H, 7-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 15.7 (C-9), 17.2 (C-9′), 32.0 (C-8′), 33.6 (C-8), 44.0 (C-2), 46.9 (C-7′), 96.4 (C-10), 101.4 (C-10′), 101.6 (C-3), 101.9 (C-3′), 104.0 (C-5), 104.7 (C-6′), 123.3 (C-1′), 136.3 (C-1), 142.7 (C-7), 144.3 (C-5′), 146.4 (C-4′), 146.5 (C-2′), 165.3 (C-4), 192.3 (C-6); MS (EI, 70 eV) $m/z$ 354 (40) $[M]^+$, 324 (42), 309 (40), 203 (29), 177 (49), 147 (100), 32 (68); HRMS (EI, $M^+$) calcd for $C_{20}H_{18}O_6$ (354.1104), found 354.1105.

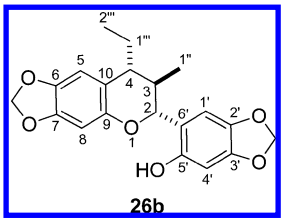
**Oxidative Cyclization of (*E*)-7 in Sodium Acetate Buffer (0.1 M, pH 5.0) in the Absence of a Catalyst.** A solution of (*E*)-7 (176 mg, 0.99 mmol) in acetone (4 mL) was added to sodium acetate buffer (0.1 M, pH 5.0, 18 mL). The resulting suspension was stirred under $O_2$ at room temperature for 19 h. NaCl (3 g) was added to the reaction mixture, and the aqueous phase was extracted with dichloromethane (80 mL). Saturated NaCl solution (20 mL) was added to the aqueous phase. The aqueous phase was extracted with dichloromethane (80 mL). This procedure was repeated twice. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue thus obtained was purified by column chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford a mixture of **26a** and **26b** as a colorless oil in 73% yield (128 mg 0.36 mmol). The ratio of **26a**:**26b** in the crude product was 3:2 ($^1$H NMR).

*(2S*,3S*,4R*)-1,3-Dioxol[6,7-f]-4-ethyl-3-methyl-2-[(1,3-benzodioxol-5-ol)-yl]-3,4-dihydro-2H-benzopyrane (**26a**).*



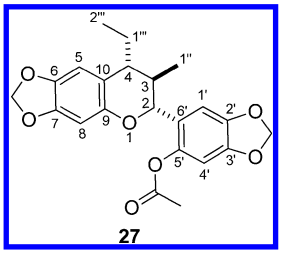

mp 42−43 °C; $R_f$ = 0.2 (dichloromethane/cyclohexane = 5:2); $[\alpha]^{20}_D$ = 0 (*c* 2.35, $CHCl_3$); IR (ATR) $\bar{\nu}$ 3428 (OH), 2876 ($OCH_2O$), 1479 (arom C═C), 1158 (ArOCH), 1149 (arom OH), 1035 (ArOCH), 857 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 236 (2.86), 303 (3.04) nm; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.86 (d, $^3J$ (1″-H, 3-H) = 7.1 Hz, 3H, 1″-H), 1.03 (t, $^3J$ (1‴-H, 2‴-H) = 7.5 Hz, 3H, 2‴-H), 1.51 (ddq, $^3J$ (1‴a-H, 2‴-H) = 7.4 Hz, $^2J$ (1‴a-H, 1‴b-H) = 13.4 Hz, $^3J$ (4-H, 1‴a-H) = 8.6 Hz, 1H, 1‴a-H), 1.77 (ddq, $^3J$ (4-H, 1‴b-H) = 4.8 Hz, $^3J$ (1‴b-H, 2‴-H) = 7.4 Hz, $^2J$ (1‴a-H, 1‴b-H) = 13.5 Hz, 1H, 1‴b-H), 2.47 (ddq, $^3J$ (2-H, 3-H) = 9.8 Hz, $^3J$ (3-H, 1″-H) = 6.9 Hz, $^3J$ (3-H, 4-H) = 4.5 Hz, 1H, 3-H), 2.54 (ddd, $^3J$ (4-H, 3-H) = 4.6 Hz, $^3J$ (4-H, 1‴a-H) = 8.5 Hz, $^3J$ (4-H, 1‴b-H) = 4.6 Hz, 1H, 4-H), 4.98 (d, $^3J$ (2-H, 3-H) = 10.0 Hz, 1H, 2-H), 5.89 (s, 2H, $OCH_2O$), 5.90 (s, 2H, $OCH_2'O$), 6.17 (s, 1H, OH), 6.44 (s, 2H, 8-H and 4′-H), 6.55 (s, 1H, 1′-H), 6.56 (s, 1H, 5-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 12.7 (C-2‴), 14.7 (1″-C), 23.8 (C-1‴), 34.4 (C-3), 40.9 (C-4), 79.4 (C-2), 98.4 (C-8), 99.4 (C-4′), 100.9 ($OCH_2O$), 101.2 ($OCH_2'O$), 107.8 (C-1′), 108.6 (C-5), 116.8 (C-6′), 118.5 (C-10), 141.1 (C-2′), 141.8 (C-6), 146.7 (C-7), 147.2 (C-9), 147.9 (C-5′), 149.8 (C-3′); MS (EI, 70 eV) $m/z$ 356 (36) $[M]^+$, 178 (100), 151 (24), 72 (20), 59 (32); HRMS (EI, $M^+$) calcd for $C_{20}H_{20}O_6$ (356.1260), found 356.1261.

*(2R*,3R*,4R*)-1,3-Dioxol[6,7-f]-4-ethyl-3-methyl-2-[(1,3-benzodioxol-5-ol)-yl]-3,4-dihydro-2H-benzopyrane (**26b**).*




$R_f$ = 0.2 (dichloromethane/cyclohexane = 5:2); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.73 (t, $^3J$ (1‴-H, 2‴-H) = 7.5 Hz, 3H, 2‴-H), 0.85 (d, $^3J$ (1″-H, 3-H) = 7.1 Hz, 3H, 1″-H), 1.79−1.91 (m, 2H, 1‴-H), 2.22 (ddq, $^3J$ (2-H, 3-H) = 9.8 Hz, $^3J$ (3-H, 1″-H) = 6.9 Hz, $^3J$ (4-H, 3-H) = 10.0 Hz, 1H, 3-H), 2.65 (ddd, $^3J$ (4-H, 3-H) = 10.0 Hz, $^3J$ (4-H, 1‴a-H) = 9.1 Hz, $^3J$ (4-H, 1‴b-H) = 4.2 Hz, 1H, 4-H), 4.46 (d, $^3J$ (2-H, 3-H) = 10.0 Hz, 1H, 2-H), 5.89 (d, $^2J$ = 1.2 Hz, 1H, $OCH_2O$), 5.90 (d, $^2J$ = 1.2 Hz, 1H, $OCH_2O$), 5.91 (d, 1H, $^2J$ = 1.2 Hz, 1H, $OCH_2'O$), 5.92 (d, 1H, $^2J$ = 1.2 Hz, 1H, $OCH_2'O$), 6.44 (s, 1H, 8-H), 6.50 (s, 1H, 4′-H), 6.51 (s, 1H, 1′-H), 6.62 (1H, s, OH), 6.66 (s, 1H, 5-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 8.3 (C-2‴), 16.8 (C-1″), 24.5 (C-1‴), 34.6 (C-3), 42.5 (C-4), 84.9 (C-2), 99.0 (C-8), 99.6 (C-4′), 101.0 ($OCH_2O$), 101.2 ($OCH_2'O$), 106.6 (C-5), 108.3 (C-1′), 116.0 (C-6′), 118.2 (C-10), 140.9 (C-2′), 143.0 (C-6), 146.0 (C-7), 148.1 (C-3′), 149.6 (C-9), 150.0 (C-5′); MS (EI, 70 eV) $m/z$ 356 (22) $[M]^+$, 178 (100), 151 (13), 28 (20).

*(2R*,3R*,4R*)-1,3-Dioxol[6,7-f]-4-ethyl-3-methyl-2-[(1,3-benzodioxol-5-acetyl)yl]-3,4-dihydro-2H-benzopyrane (**27**).*




To a solution of **26b** (50 mg, 0.14 mmol) in pyridine (1 mL) were added acetic acid anhydride (200 μL, 2.1 mmol) and DMAP (2 mg, 0.016 mmol), and the reaction mixture was stirred at room temperature for 4 h. Then, 5% HCl (4 mL) was added, the aqueous layer was extracted with dichloromethane (3 × 2 mL), and the combined organic layers were evaporated under reduced pressure. The crude product was purified by column chromatography over silica gel (dichloromethane/cyclohexane = 5:2) to afford **27** in 20% yield (12 mg, 0.03 mmol): $R_f$ = 0.20 (dichloromethane/cyclohexane = 5:2); IR (ATR) $\bar{\nu}$ 29626 ($OCH_2O$), 1759 (CO), 1628 (C═C), 1479 (arom C═C), 1257 (CO), 1151 (ArOCH), 1035 (ArOCH), 797 (arom CH) $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (lg $\varepsilon$) 237 (3.07), 295 (3.09) nm; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.76 (t, $^3J$ (1‴-H, 2‴-H) = 7.3 Hz, 3H, 2‴-H), 0.79 (d, $^3J$ (1″-H, 3-H) = 6.6 Hz, 3H, 1″-H), 1.86 (m, $^3J$ (4-H, 1‴-H) = 7.6 Hz, $^3J$ (4-H, 1‴-H) = 4.3 Hz, 2H, 1‴-H), 2.07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(ddq, $^3J$ (2-H, 3-H) = 10.1 Hz, $^3J$ (3-H, 1″-H) = 6.6 Hz, $^3J$ (4-H, 3-H) = 10.1 Hz, 1H, 3-H), 2.21 (s, 3H, $COCH_3$), 2.66 (ddd, $^3J$ (4-H, 3-H) = 9.6 Hz, $^3J$ (4-H, 1‴-H) = 8.5 Hz, $^3J$ (4-H, 1‴-H) = 4.0 Hz, 1H, 4-H), 4.56 (d, $^3J$ (2-H, 3-H) = 9.7 Hz, 1H, 2-H), 5.87 (d, $^2J$ (a-H, b-H) = 1.5 Hz, 1H, $OCH_2O$), 5.89 (d, 1H, $^2J$ (a-H, b-H) = 1.5 Hz, $OCH_2O$), 6.00 (s, 2H, $OCH_2'O$), 6.38 (s, 1H, 8-H), 6.61 (s, 1H, 4′-H), 6.66 (s, 1H, 5-H), 6.91 (s, 1H, 1′-H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 8.7 (C-2‴), 15.9 (C-1″), 20.7 ($CH_3$), 24.0 (C-1‴), 35.4 (C-3), 42.5 (C-4), 78.3 (C-2), 98.8 (C-8), 100.9 ($OCH_2O$), 101.9 ($OCH_2'O$), 104.1 (C-4′), 106.4 (C-5), 107.0 (C-1′), 117.2 (C-10), 125.0 (C-6′), 142.1 (C-6), 142.8 (C-5′), 145.9 (C-2′), 146.0 (C-7), 147.6 (C-3′), 150.9 (C-9), 169.4 (CO); MS (EI, 70 eV) $m/z$ 356 (36) $[M]^+$, 178 (100), 151 (24), 72 (20), 59 (32); HRMS (EI, $M^+$) calcd for $C_{22}H_{22}O_7$ (398.1365) found 398.1368.

## ■ ASSOCIATED CONTENT

### Supporting Information

Copies of the NMR spectra for all compounds, DFT calculations, Cartesians, spin work analysis, HPLC separation methods, and HPLC chromatograms. This information is available free of charge via the Internet at http://pubs.acs.org.

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