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Towards the biosynthesis of the aromatic products of the Mediterranean mollusc *Scaphander lignarius*: isolation and synthesis of analogues of lignarenones

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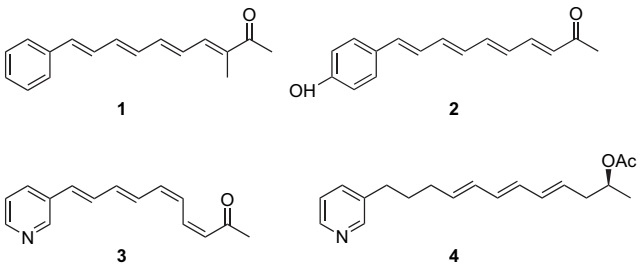
Abstract—Secondary metabolites of the Mediterranean mollusc *Scaphander lignarius* from different collection sites have been investigated, proving the constant presence of a number of minor metabolites correlated to the already known lignarenones. Complete characterization of the new metabolites has been supported by enantioselective synthesis. The data are consistent with the origin of this unique class of ω-phenyl-octanoids from a common polyketide pathway.

1. Introduction

A few species of marine molluscs of the order Cephalaspidea (Gastropoda, Opisthobranchia) produce a structurally homogenous group of oxygenated aryl-alkenyl compounds (e.g., **1**–**4**), which in some cases, have been proven to function as alarm pheromones in intra-specific communication.[1] The biosynthesis of these molecules is basically unknown, even if they all are notional products of a PKS-like pathway involving branched aryl residues as starter units and acetate, or metabolic equivalents, for the elongation steps.[2] A first piece of evidence in support to this biogenesis comes from our recent finding that the 3-alkylpyridine motif of haminols (e.g., **4**) derives from sequential condensation of malonyl-Co-A onto nicotinic acid.[3] *Scaphander lignarius*, a deep water cephalaspidean mollusc, contains an unusual group of branched phenyloctanoids named lignarenones (**5** and **6**) differing in the double bond configuration at the trienic chain.[4] In order to approach the biosynthesis of lignarenones, we have re-collected the organisms from different sites and analyzed the metabolite content as a function of the geographic variability. In this manuscript, we report the result of this study that shows the unvarying presence of the previously described lignarenones **5** and **6**, together with minor ω-phenyl alkenyl 2-hydroxy and 2-keto derivatives **7**–**11**. All these compounds are ascribable to a single biosynthetic pathway of which the aldehyde **12**, also isolated and characterized from the mollusc extracts, could be a potential intermediate. The absolute stereochemistry of the chiral metabolites **8** and **10** has been proven by enantioselective synthesis.



2. Results and discussion

In addition to lignarenones A and B (**5** and **6**), lipophilic extracts of *S. lignarius* from different sites of Italy and Spain (see Section 4), revealed the presence of a mixture of minor compounds (**7**–**11**). In particular, specimens collected along the southern coasts of Italy gave variable mixtures of **5** and **6**, as major components, together with a new derivative (**7**) here named lignarenone C. Except for the presence of the ethyl group (δ 2.46 and 1.14) α to the carbonyl moiety, this last

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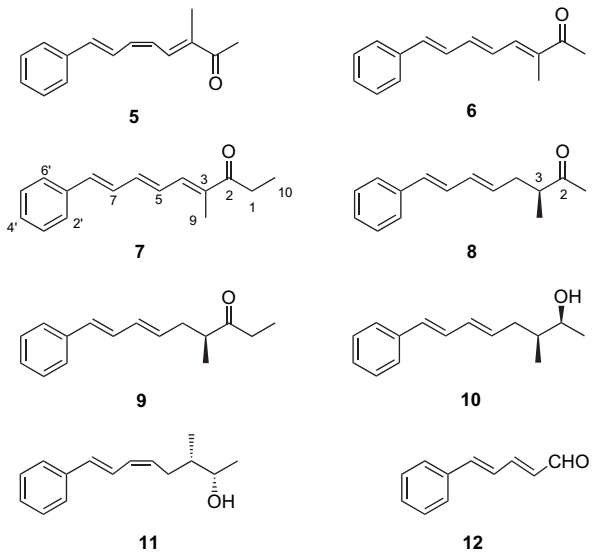
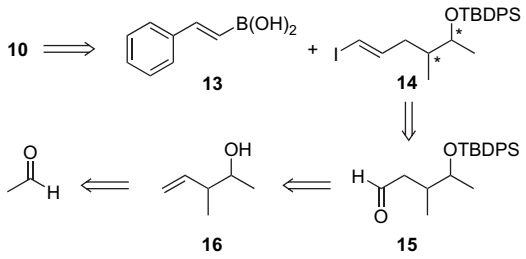


compound showed spectroscopic data identical to those of lignarenone B (**6**),[4] with an extended conjugation (λ_{max}= 348 nm) involving a linear triene system (see Section 4). The molluscs collected along the Italian coasts also contained 3,4-dihydroderivatives of **6** and **7**, here named dihydrolignarenone B (**8**) and dihydrolignarenone C (**9**), together with very minor amounts of the alcohol **10**.

Compounds **8** and **9** shared the presence of NMR signals of one (*E*,*E*)-1,3-butadiene group (δ 5.73, dt, H-5; δ 6.22, dd, H-6; δ 6.48, d, H-8; δ 6.72, dd, H-7) and one methyl doublet (δ 1.12, H_3-9), but as for the unsaturated homologues **6** and **7**, they differed in the terminal alkyl residues, a methyl group for **8** (δ 2.17, s) and an ethyl group for **9** (δ 1.05, t, J=7.2 Hz; δ 2.48, m). The ^{1}H NMR spectrum of the alcohol **10**, on the contrary, was characterized by two methyl doublets at δ 1.19 and 0.93, together with one hydroxymethine signal at δ 3.78. These data were suggestive of the presence of one hydroxy group at C-2 of the lignarenone skeleton. The definitive demonstration of the structure was achieved only after extraction of *S. lignarius* from the eastern coast of Spain. In fact, these molluscs, in addition to lignarenones A (**5**) and B (**6**), which represented 90% of the pheromone mixture, contained dihydrolignarenone B (**8**) and a significant amount of a 9:1 mixture of the *E*/*Z* isomeric alcohols **10** and **11**. In the COSY spectrum, both these compounds, named dihydrolignarenol B and dihydrolignarenol A, respectively, revealed a methyl doublet at about δ 1.20 (see Section 4) coupled to a downshifted signal at about δ 3.80. The other ^{1}H NMR signals were consistent with the depicted structures, showing major differences only due to the coupling constant between H-5 and H-6 (15.6 Hz in **10** and 10.6 Hz in **11**). The presence of the hydroxyl group at C-2 was also consistent with the APCI$^+$ MS molecular ion (*m*/*z* 217) of both products and with the resonance of the alcoholic carbon at 70.8 ppm in the ^{13}C NMR spectrum of the major isomer **10**. Furthermore, oxidation of this latter compound with tetra-*n*-propylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO)[5] gave quantitatively dihydrolignarenone B (**8**). As shown in Figure 1, the CD spectra of the oxidation product of **10** and natural **8** were identical, thus proving that the two molecules share the same configuration at C-3.

In order to determine the absolute stereochemistry of dihydrolignarenones and dihydrolignarenols we designed an enantioselective synthesis based on Suzuki coupling between *trans*-phenylvinylboronic acid (**13**) and the chiral vinyl iodide **14** obtainable in three steps from **16** (Scheme 1). All possible diasteroisomers of this last compound are accessible from the synthetic procedure proposed by Brown and Bhat[6] through the reaction of ethanal with chiral crotyldialkylborane prepared from (*Z* or *E*) 2-butene and (+ or −) β-methoxydiisopinocampheylborane (Ipc_2BOMe).



Scheme 1. Retrosynthesis of lignarenones in *S. lignarius*.

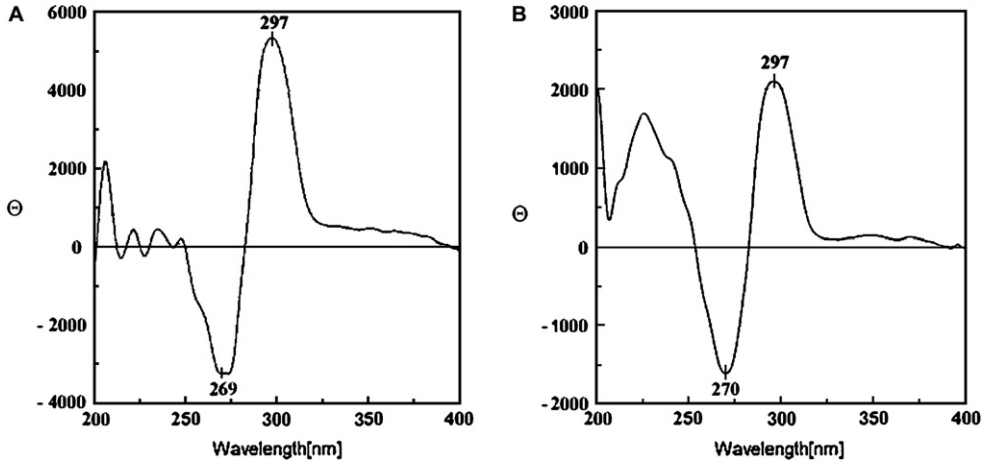
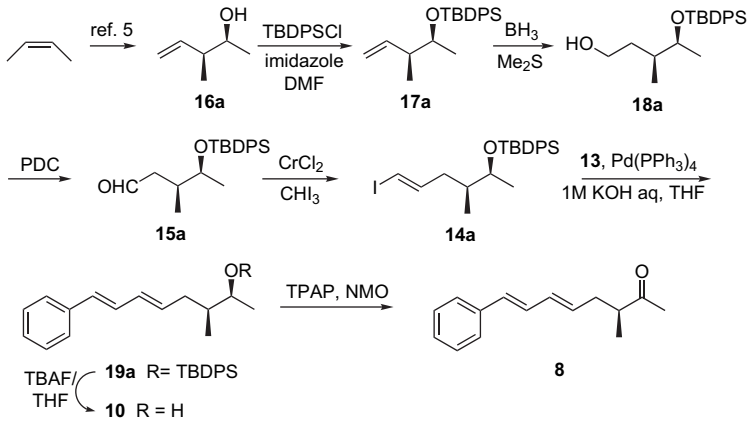


Figure 1. CD spectra of: (A) dihydrolignarenone B (**8**) and (B) the oxidation product of dihydrolignarenol B (**10**). Spectra were recorded in MeOH at concentration of 10^{-5} mg/L.



Scheme 2. Enantioselective synthesis of *syn* (2*S*,3*S*)-dihydrolignarenone B (**10**).

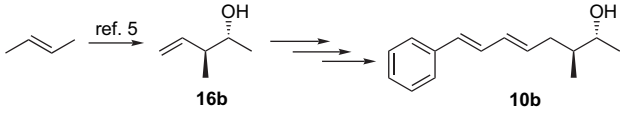
Table 1. NMR chemical shifts of natural and synthetic samples of dihydrolignarenol B (**10**)[a]

Position	Natural compound (**10**)		2*S*,3*S* Synthetic		2*R*,3*S* Synthetic	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1	0.93	14.0	0.93	14.0	0.92	15.0
2	3.78	70.8	3.78	70.8	3.68	71.5
3	1.60[b]	40.0	1.61	40.1	1.63	40.5
4	2.02 and 2.32	36.4	2.02 and 2.33	36.5	2.04 and 2.36	36.3
5	5.82	134.0	5.82	134.0	5.83	134.0

Data were recorded in $CDCl_3$.
[a] The remaining signals are identical in the three samples.
[b] Partially overlapped by the DHO signal.

For the synthesis of 2*S*,3*S* stereoisomer (Scheme 2), asymmetric crotylation of (*Z*)-2-butene with (+)-Ipc$_2$BOMe gave a chiral crotyldiisopinocampheylborane that, after reaction with ethanal at −78 °C, afforded the expected homoallylic alcohol with *S*,*S* stereochemistry (**16a**, 72% yield, 98% de, 92% ee). After protection of the hydroxyl group, the terminal alkene **17a** was hydroborated by $BH_3 \cdot Me_2S$ to provide first the primary alcohol (**18a**) and, after Cr(VI) oxidation, the aldehyde **15a** (50% yield, three steps). Conversion of aldehyde to vinyl iodide **14a** occurred via a Takai reaction[7] in 57% yield. Finally, Suzuki coupling of iodide **14a** and *trans*-phenylvinylboronic acid (**13**) afforded compound **19a** whose deprotection gave a dihydrolignarenol showing that ^{1}H and ^{13}C NMR spectra were identical to that of the natural product **10** (see Section 4). The subsequent oxidation at C-3 of this compound with tetra-*n*-propylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) afforded in good yield a keto derivative showing that NMR and CD spectra were identical to the natural dihydrolignarenone B (**8**) (synthetic θ_{297}=1764.6, θ_{268}=−97.3, *c* 12 μg/mL; natural θ_{297}=2143.3, θ_{267}=126.4, *c* 10 μg/mL).

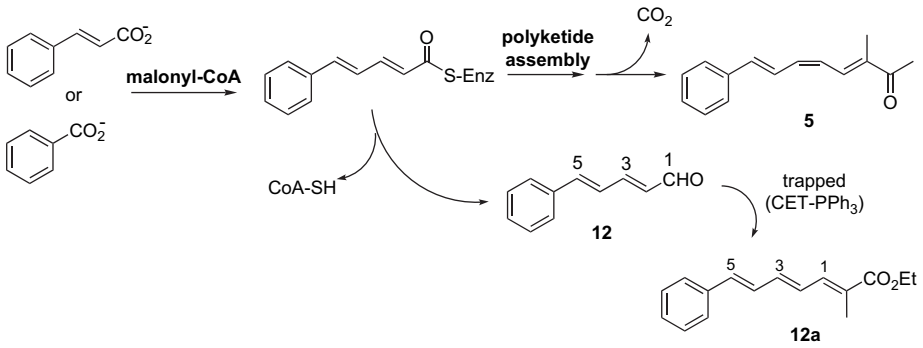
This proved the 3*S* configuration for the chiral centre not only of dihydrolignarenone B (**8**) but also of dihydrolignarenone C (**9**), as the two compounds had very similar polarimetric values ($[\alpha]_D^{23}$ +8.8 for synthetic **8**; $[\alpha]_D^{23}$ +11.4 for natural **8**; $[\alpha]_D^{23}$ +7.4 for **9**). On the other hand, considering the strong similarities of the ^{1}H and ^{13}C NMR spectra of natural and synthetic products, the above synthesis was also suggestive of 2*S*,3*S* stereochemistry for dihydrolignarenol B (**10**). This hypothesis was rigorously proven by synthesis of the *anti* isomer (**10b**), (2*R*,3*S*) 8-phenyl-2-methyl-octa-5,7-dien-2-ol, from (*E*)-2-butene (Scheme 3). In fact, the chemical shifts of a few NMR signals of this last compound were diagnostically different to conclude that the natural product could not have the same configuration of the synthetic 2*R*,3*S* material (Table 1), thus establishing unambiguously the configuration of dihydrolignarenol B (**10**) as 2*S*,3*S*.



Scheme 3. Enantioselective synthesis of *anti* (2*R*,3*S*)-dihydrolignarenone B (**10b**). The synthetic steps are identical to those described in Scheme 2.

3. Conclusions

Extracts of *S. lignarius* from Mediterranean sites contain a unique family of aryl-alkenyl compounds embracing the known lignarenones **5** and **6** and the unprecedented analogues **7**–**11**. In comparison with lignarenones A and B (**5** and **6**) that constitute about 14±6% of the lipophilic extract in every group of animals studied here, the structures of the minor metabolites (on the whole, 2.1±1.1% of the lipophilic extract) differ for one of these three major aspects: (a) presence of an alkyl chain of nine carbon atoms (**7** and **9**), (b) reduction of the carbonyl group (**10** and **11**) and (c) absence of C-3–C-4 double bond (**8** and **11**). As outlined in Scheme 4, all these changes are consistent with the origin of the products of *S. lignarius* from a single PKS pathway based on



Scheme 4. Proposed biogenesis of lignarenones via polyketide pathway.

benzoic or cinnamic acid as a starter unit and elongation rounds of malonyl-coenzyme-A. The metabolic connection of *Scaphander* metabolites is supported by the stereochemical correlations between dihydrolignarenones (**8** and **9**) and dihydrolignarenols (**10** and **11**), whereas the finding of 5-phenyl-2*E*,4*E*-pentadienal (**12**), a potential intermediate derivative of the emerging molecule, in the extracts of the molluscs appears as an indirect evidence in support of the processive mechanism (Scheme 4). The aromatic aldehyde (**12**) was characterized as ethyl ester derivative 7-phenyl-2*E*,4*E*,6*E*-heptatrienoic acid (**12a**) by a trapping approach involving treatment of aldehyde-containing fractions with carbethoxyethylidene-triphenylphosphorane (CET-TPP).[8]

Despite minor variation in the metabolic content from the three collection sites, the molluscs examined in this study show an uniform composition of the aromatic metabolites that in addition to the arguments of Andersen and Faulkner[9] may imply the origin of lignarenones in the molluscan tissues. This hypothesis needs experimental confirmation but it would be consistent with the recent studies on opisthobranch biosynthesis, which indicates the ability of these marine organisms to produce de novo ecological mediators through PKS-like pathways.[2] Feeding experiments are currently in progress to confirm the proposed biogenesis.

4. Experimental section

4.1. General

SiO_2 rotatory TLC was carried out on Chromatotron® (Harrison Research, Palo Alto, California). MS analyses were carried out with Micromass Qtof micro equipped with APCI (RP-18 column), MeOH/H_2O 87:13, flow 0.7 mL/min. NMR spectra were recorded on Bruker AMX 300 (300 MHz) and Avance DRX 400 (400 MHz). Chemical shifts are referenced to residual C_6D_5H (δ 7.16 and 128.0 ppm were taken as reference) or residual $CHCl_3$ (δ 7.26 and 77.0 ppm were taken as reference). Enantiomeric excesses of alcohols **16a** and **16b** were determined by capillary GC analysis of (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid ester derivatives, using a methylsilicon column, 30 m×0.25 mm (100 °C isotherm; t_R=101.35 and 104.20 min, respectively, for the major and minor of the two MTPA ester diastereomers derived from **16a** and t_R=98.25 and 101.70 min, respectively, for the major and minor of the two MTPA ester diastereomers derived from **16b**). Microanalyses were performed on a ThermoFinnigan Elemental Analyser EA 1112. GC–MS spectra were recorded on a GC Trace 2000 SERIES connected to Finnigan Thermoquest GLQ Plus 2000 spectrometer with an ion trap detector. Optical rotations were measured with a JASCO DIP-1000 polarimeter. All the moisture or air sensitive reactions were carried out in anhydrous solvents and flame-dried glassware under dry nitrogen or argon. Reactions were monitored by TLC, using silica gel 0.25 mm plates (Merck). Yields refer to isolated pure products.

4.2. Collection, extraction and fractionation

S. lignarius was collected from Malgrat (22 specimens) and Blanes (82 specimens) (Southeast of Spain), Gulf of Naples (Southwest of Italy) (600 specimens) and Crotone (South east of Italy) (170 specimens) by dredging (around 80 m of depth). Samples were kept frozen at −20 °C till the analyses. Released mucus was recovered by a Pasteur pipette and frozen at −20 °C immediately after dredging. The frozen animals and mucus were extracted with acetone (1.5 mL/animal and 3 mL, respectively). After removing the organic solvent, the aqueous residues were diluted with fresh water (0.4 mL/animal) and partitioned with Et_2O (0.8 mL/animal). The ether extracts were then fractionated by LH-20 (MeOH) and the products of interest were further purified by SiO_2 gel column (polarity-increasing gradient of petroleum ether/diethyl ether) followed by SiO_2 rotatory TLC (*n*-hexane/ethyl acetate 80:20) to give **5**–**12**. Final purification of the products was achieved by normal phase HPLC (μ-Porasil) using *n*-hexane/EtOAc (85:15) as an isocratic eluant (flow 3.5 mL/min, Detector UV 256 nm).

4.2.1. Lignarenone C (7). Yield 5.2 mg, UV (MeOH) λ_{max}=348 nm (ε=40,500), IR (film) ν_{max}=1710 cm^{-1}, FAB^+ MS *m/z* 226 $[M]^+$, 211 $[M-CH_3]^+$, 197 $[M-Et]^+$; $APCI^+$ MS *m/z* 227.1 $[M+H]^+$; HRMS $APCI^+$ *m/z* 227.1440 $[M+H]^+$ (227.1436 calculated for $C_{16}H_{19}O^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, t, $J_{H,H}$=7.2 Hz, H_3-10), 1.96 (3H, s, H_3-9), 2.46 (2H, q, $J_{H,H}$=7.2 Hz, H_2-1), 6.71 (3H, m, H-5, H-6 and H-8), 6.93 (1H, dd, $J_{H,H}$=15.5 and 9.6 Hz, H-7), 7.13 (1H, d, $J_{H,H}$=9.2 Hz, H-4), 7.27 (1H, d, $J_{H,H}$=7.4 Hz, H-4′), 7.34 (2H, t, $J_{H,H}$=7.4 Hz, H-3′ and H-5′), 7.44 (2H, t, $J_{H,H}$=7.4 Hz, H-2′ and H-6′); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 202.1 (C-2), 139.6 (C-4), 137.7 (C-6), 136.7 (C-3), 135.9 (C-8), 135.7 (C-1′), 128.7 (C-3′, C-4′ and C-5′), 128.4 (C-5), 128.2 (C-7), 126.6 (C-2′ and C-6′), 30.5 (C-1), 11.8 (C-10), 8.8 (C-9).

4.2.2. Dihydrolignarenone B (8). Yield 3.1 mg, $[\alpha]_D^{23}$ +11.4 (*c* 0.24), UV (MeOH) λ_{max}=287 nm (ε=20,990), IR (film) ν_{max}=1710 cm^{-1}, FAB^+ MS *m/z* 214 $[M]^+$, 171 $[M-C_2H_3O]^+$, 143 $[M-C_4H_7O]^+$; $APCI^+$ MS *m/z* 215.1 $[M+H]^+$; HRMS $APCI^+$ *m/z* 215.1431 $[M+H]^+$ (215.1436 calculated for $C_{15}H_{19}O^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (3H, s, H_3-9), 2.17 (3H, s, H_3-1), 2.21 (1H, m, H-4a), 2.48 (1H, m, H-4b), 2.61 (1H, m, H-3), 5.73 (1H, m, H-5), 6.22 (1H, dd, $J_{H,H}$=15.6 and 11.2 Hz, H-6), 6.48 (1H, d, $J_{H,H}$=15.6 Hz, H-8), 6.72 (1H, dd, $J_{H,H}$=15.6 and 11.2 Hz, H-7), 7.16–7.39 (5H, m, phenyl protons); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 211.5 (C-2), 137.2 (C-1′), 132.6 (C-5), 131.5 (C-6), 131.0 (C-8), 128.7 (C-7), 128.5 (C-4′), 127.1 (C-2′ and C-6′), 126.2 (C-3′ and C-5′), 47.0 (C-3), 36.1 (C-4), 28.3 (C-1), 15.9 (C-9); ^{13}C NMR (100.6 MHz, C_6D_6) δ 209.7, 133.4, 132.1, 131.4, 131.0, 129.2, 128.8, 127.3, 46.9, 36.1, 27.9, 16.0.

4.2.3. Dihydrolignarenone C (9). Yield 2.6 mg, $[\alpha]_D^{23}$ +7.4 (*c* 0.24, MeOH), UV (MeOH) λ_{max}=289 nm (ε=21,960), IR (film) ν_{max}=1710 cm^{-1}, FAB^+ MS *m/z* 228 $[M]^+$, 199 $[M-C_2H_5]^+$, 171 $[M-C_3H_5O]^+$; $APCI^+$ MS *m/z* 229.1 $[M+H]^+$; HRMS $APCI^+$ *m/z* 229.1585 (229.1592 calculated for $C_{16}H_{21}O^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (3H, d, $J_{H,H}$=7.2 Hz, H_3-1), 1.12 (3H, d, $J_{H,H}$=7.2 Hz, H_3-9), 2.19 (1H, m, H-4a), 2.48 (3H, m, H-4b and H_2-1), 2.64 (1H, br q, $J_{H,H}$=7.1 Hz, H-3), 5.72 (1H, dt, $J_{H,H}$=15.1, 7.6 and 7.6 Hz, H-5), 6.21 (1H, dd, $J_{H,H}$=15.1 and 10.4 Hz, H-6), 6.45 (1H, d, $J_{H,H}$=15.6 Hz, H-8), 6.72 (1H, dd, $J_{H,H}$=15.6 and 10.4 Hz, H-7), 7.16–7.39 (5H, m, benzene protons).

4.2.4. Dihydrolignarenol B (10). Yield 1.1 mg, $[\alpha]_D^{23}$ −3.0 (*c* 0.05, MeOH), UV (MeOH) λ_{max}=287 nm (ε=22,900), IR (film) ν_{max}=3352 cm^{-1}; $APCI^+$ MS *m/z* 217 $[M+H]^+$, 199 $[M-H_2O+H]^+$; HRMS $APCI^+$ *m/z* 217.1601 $[M+H]^+$ (217.1592 calculated for $C_{15}H_{21}O^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, d, $J_{H,H}$=6.4 Hz, H_3-9), 1.19 (3H, d, $J_{H,H}$=6.4 Hz, H_3-1), 1.71 (1H, m, H-3a), 2.02 (1H, m, H-4a), 2.32 (1H, m, H-4b), 3.78 (1H, m, H-2), 5.82 (1H, m, H-5), 6.23 (1H, dd, $J_{H,H}$=15.6 and 11.2 Hz, H-6), 6.47 (1H, d, $J_{H,H}$=15.6 Hz, H-8), 6.77 (1H, dd, $J_{H,H}$=15.6 and 11.2 Hz, H-7), 7.20–7.38 (5H, m, benzene protons); ^1H NMR (400 MHz, C_6D_6) δ 0.84 (3H, d, $J_{H,H}$=6.8 Hz, H_3-9), 0.95 (3H, d, $J_{H,H}$=6.4 Hz, H_3-1), 1.37 (1H, br d, $J_{H,H}$=6.8 Hz, H-3), 1.88 (1H, m, H-4a), 2.24 (1H, m, H-4b), 3.51 (1H, m, H-2), 5.67 (1H, ddd, $J_{H,H}$=15.1, 7.5 and 7.3 Hz, H-5), 6.16 (1H, dd, $J_{H,H}$=15.1 and 10.4 Hz, H-6), 6.39 (1H, d, $J_{H,H}$=15.4 Hz, H-8), 6.74 (1H, dd, $J_{H,H}$=15.4 and 10.4 Hz, H-7), 7.04–7.29 (5H, m, benzene protons); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 138.5 (C-1′), 134.3 (C-5), 132.4 (C-6), 130.8 (C-8), 130.1 (C-7), 126.6 (C-4′), 70.8 (C-2), 40.4 (C-3), 36.9 (C-4), 20.6 (C-1), 14.0 (C-9).

4.2.5. Dihydrolignarenol A (11). Yield 0.1 mg; $APCI^+$ MS *m/z* 217 $[M+H]^+$, 199 $[M-H_2O+H]^+$; HRMS $APCI^+$ *m/z* 217.1584 $[M+H]^+$ (217.1592 calculated for $C_{15}H_{21}O^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, d, $J_{H,H}$=6.8 Hz, H_3-9), 1.21 (3H, d, $J_{H,H}$=6.4 Hz, H_3-1), 1.38 (1H, d, $J_{H,H}$=6.7 Hz, H-3), 2.10 (1H, d, $J_{H,H}$=7.5 Hz, H-4a), 2.43 (1H, br d, $J_{H,H}$=7.5 Hz, H-4b), 3.81 (1H, m, H-2), 5.53 (1H, dt, $J_{H,H}$=10.6, 7.5 and 7.5 Hz, H-5), 6.23 (1H, t, $J_{H,H}$=10.6 Hz, H-8), 6.53 (1H, d, $J_{H,H}$=15.6 Hz, H-8), 7.07 (1H, dd, $J_{H,H}$=15.6 and 10.6 Hz, H-7) 7.20–7.42 (5H, m, phenyl protons); ^1H NMR (400 MHz, C_6D_6) δ 0.88 (3H, d, $J_{H,H}$=6.8 Hz, H_3-9), 0.94 (3H, d, $J_{H,H}$=6.4 Hz, H_3-1), 1.38 (1H, d, $J_{H,H}$=6.7 Hz, H-3), 2.10 (1H, d, $J_{H,H}$=7.5 Hz, H-4a), 2.43 (1H, br d, $J_{H,H}$=7.5 Hz, H-4b), 3.51 (1H, m, H-2), 5.48 (1H, dt, $J_{H,H}$=10.6, 7.5 and 7.5 Hz, H-5), 6.51 (1H, d, $J_{H,H}$=15.6 Hz, H-8), 6.74 (1H, dd, $J_{H,H}$=11.0 and 10.6 Hz, H-6), 7.25 (1H, dd, $J_{H,H}$=15.6 and 11.0 Hz, H-7), 7.10–7.30 (5H, m, phenyl protons).

4.2.6. Conversion of dihydrolignarenol B (10) to dihydrolignarenone B (8). To a solution of compound **10** (1.6 mg, 0.04 mmol) in dry methylene chloride (1 mL) under argon, tetrapropylammonium perruthenate (TPAP, 0.004 mmol) and *N*-methylmorpholine *N*-oxide (0.08 mmol) were added. The mixture was stirred for 2 h at room temperature then filtered through a pad of silica gel and the bed was washed with ethyl acetate (2×20 mL). The filtrate was concentrated in vacuo to give 0.7 mg of **8**. ^1H and ^{13}C NMR spectra of this product were identical to those of natural dihydrolignarenone B.

4.2.7. (*S*)-MTPA ester of alcohol 16a. To a solution of homoallylic alcohol **16a**[5] (40 mg, 0.4 mmol) and 4-(dimethylamino)pyridine (54 mg, 0.44 mmol) in THF (3 mL) was added (*R*)-MTPA–Cl (111 mg, 0.44 mmol) dropwise. The solution was stirred at room temperature overnight. Then, the solution was diluted with dichloromethane (5 mL) and washed with water (2×5 mL). The filtrate was concentrated and the residue was filtered through a short column of neutral alumina with pentane. Concentration of the filtrate provided MTPA ester (113.8 mg, 90% yield). ^1H NMR analysis integration of the vinylic protons of the two diastereomeric (*S*)-MTPA esters (δ 5.75 (m, 1H) for the (3*S*,4*S*)-**16a** isomer and 5.64 (m, 1H) for the (3*R*,4*R*)-**16a** minor isomer) confirmed the enantiomeric purity determined by GC analysis.

4.2.8. (3*S*,4*S*)-4-*tert*-Butyldiphenylsilyloxy-3-methylpent-1-ene (17a). To a solution of homoallylic alcohol **16a** (510 mg, 5.09 mmol) in dry DMF (2 mL), stirred under an argon atmosphere at room temperature, *tert*-butylchlorodiphenylsilane (TBDPSCl, 3.50 g, 12.7 mmol) and imidazole (1.73 g, 25.5 mmol) were added. The reaction mixture was stirred for 24 h and then quenched with 0.2 N aqueous HCl solution (65 mL). The resulting mixture was extracted with diethyl ether (3×20 mL), and the organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography using *n*-pentane as an eluant afforded 1.12 g (65% yield) of compound **17a** as a colourless oil: R_f (*n*-hexane) 0.2; $[\alpha]_D^{25}$ −23.2 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.72–7.65 (m, 4H), 7.45–7.33 (m, 6H), 5.93 (ddd, *J*=17.1, 10.7 and 6.7 Hz, 1H), 5.04–4.96 (m, 2H overlapped), 3.77 (m, 1H), 2.29 (m, 1H), 1.04 (s, 9H), 0.94 (d, *J*=6.1 Hz, 3H), 0.93 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 140.9, 136.0 (×2), 135.0, 134.3, 129.5 (×2), 129.4 (×2), 127.5 (×2), 127.4 (×2), 114.2, 73.0, 44.7, 27.1, 19.8, 19.4, 15.1. EIMS (70 eV) *m/z*: 338 $[M]^+$, 281, 203, 177. Anal. Calcd for $C_{22}H_{30}OSi$: C, 78.05; H, 8.93. Found: C, 78.09; H, 8.88.

4.2.9. (3*S*,4*S*)-4-*tert*-Butyldiphenylsilyloxy-3-methyl-pentan-1-ol (18a). A solution of compound **17a** (36 mg, 0.106 mmol) in dry hexane (2 mL) under an argon atmosphere was cooled to 0 °C and $BH_3 \cdot SMe_2$ (0.160 mL,

0.319 mmol, 2 M solution in THF) was added. The mixture was warmed to room temperature and stirred for 26 h, then treated with 3 M aqueous NaOH solution (0.88 mL, 2.64 mmol) and H_2O_2 (0.135 mL, 35% w/w aqueous solution). After stirring overnight at room temperature, the mixture was extracted with diethyl ether (3×20 mL), dried over Na_2SO_4, filtrated and concentrated in vacuo, giving a crude product, which was purified by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 100:0 to 60:40). Alcohol **18a** of 32 mg (85% yield) was obtained, as a colourless oil: R_f (30% Et_2O/*n*-pentane) 0.25; $[\alpha]_D^{25}$ −1.8 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.71–7.65 (m, 4H), 7.47–7.34 (m, 6H), 3.79 (dq, *J*=6.4 and 3.0 Hz, 1H), 3.68 (m, 1H), 3.63 (m, 1H), 2.20 (m, 1H), 1.80 (m, 1H), 1.73 (m, 1H), 1.40 (m, 1H), 1.06 (s, 9H), 0.98 (d, *J*=6.4 Hz, 3H), 0.80 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 135.9 (×2), 134.4, 133.9, 129.7 (×2), 129.6 (×2), 127.6 (×2), 127.4 (×2), 73.3, 61.9, 42.5, 35.0, 27.0, 19.2, 18.3, 16.6. *m/z*: 356 $[M]^+$, 299, 199, 139. Anal. Calcd for $C_{22}H_{32}O_2Si$: C, 74.10; H, 9.05. Found: C, 74.35; H, 8.82.

4.2.10. (3*S*,4*S*)-4-*tert*-Butyldiphenylsilyloxy-3-methylpentan-1-al (15a). To a solution of compound **18a** (59 mg, 0.166 mmol) in dry methylene chloride (2 mL) under an argon atmosphere, pyridinium dichromate (125 mg, 0.331 mmol) and activated 4 Å molecular sieves (112 mg) were added. The reaction mixture was stirred at room temperature for 3 h, diluted with diethyl ether (4 mL) and stirred again for 30 min. The resulting suspension was filtered through a pad of silica gel/$CaSO_4$ (9:1, 1.5 g) and the bed was washed with diethyl ether (3×10 mL). The filtrate was concentrated in vacuo giving 53 mg of aldehyde **15a** (90% yield) as a colourless oil: R_f (30% Et_2O/*n*-pentane) 0.70; $[\alpha]_D^{25}$ −1.1 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.71 (m, 1H), 7.70–7.62 (m, 4H), 7.47–7.34 (m, 6H), 3.82 (dq, *J*=6.3 and 3.2 Hz, 1H), 2.66 (m, 1H), 2.29–2.14 (m, 2H overlapped), 1.05 (s, 9H), 0.95 (d, *J*=6.3 Hz, 3H), 0.85 (d, *J*=6.7 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 202.6, 135.8 (×2), 134.4, 133.9, 129.7 (×2), 129.6 (×2), 127.7 (×2), 127.5 (×2), 72.2, 46.3, 35.0, 27.1, 20.5, 19.3, 15.7. *m/z*: 354 $[M]^+$, 297, 219, 199. Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.58; H, 8.42.

4.2.11. (1*E*,4*S*,5*S*)-5-*tert*-Butyldiphenylsilyloxy-1-iodo-4-methyl-hex-1-ene (14a). To a cooled solution (0 °C) of anhydrous $CrCl_2$ (110 mg, 0.897 mmol) in dry THF/dioxane 1:6 (2.4 mL) under nitrogen, a solution of aldehyde **15a** (53 mg, 0.150 mmol) and iodoform (118 mg, 0.299 mmol) in dry THF/dioxane 1:6 (1.2 mL) was added slowly. The reaction mixture was stirred at 0 °C for 24 h then quenched with saturated aqueous NH_4Cl solution (20 mL), extracted with petroleum ether (3×30 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 100:0 to 95:5) afforded 41 mg of iodoalkene **14a** (57% yield) as a colourless oil: R_f (*n*-hexane) 0.50; $[\alpha]_D^{25}$ −23.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.67–7.65 (m, 4H), 7.42–7.37 (m, 6H), 6.41 (ddd, *J*=14.2, 7.3 and 7.3 Hz, 1H), 5.88 (d, *J*=14.2 Hz, 1H), 3.79 (dq, *J*=12.5 and 6.2 Hz, 1H), 2.33 (m, 1H), 1.86 (m, 1H), 1.55 (m, 1H), 1.05 (s, 9H), 0.96 (d, *J*=6.2 Hz, 3H), 0.82 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 145.9, 135.9 (×2), 134.7, 134.1, 129.6 (×2), 129.5 (×2), 127.6 (×2), 127.4 (×2), 74.9, 72.0, 39.6, 38.7, 27.1, 19.4 (2C overlapped), 14.5. *m/z*: 478 $[M]^+$, 421, 217, 199. Anal. Calcd for $C_{23}H_{31}IOSi$: C, 57.73; H, 6.53. Found: C, 58.02; H, 6.42.

4.2.12. (1*E*,3*E*,6*S*,7*S*)-7-*tert*-Butyldiphenylsilyloxy-6-methyl-1-phenylocta-1,3-diene (19a). A mixture of vinylic iodide **14a** (41 mg, 0.085 mmol), $Pd(PPh_3)_4$ (10 mg, 0.0085 mmol) and *trans*-2-phenylvinylboronic acid (**13**, 25 mg, 0.17 mmol) in THF/KOH 2 M aqueous 4:1 (1.25 mL) was stirred under nitrogen at 50 °C for 4 h. Then the THF was removed in vacuo and the suspension was extracted with methylene chloride (3×10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash chromatography using petroleum ether as an eluant gave 26 mg of coupling product **19a** (67% yield) as a colourless oil: R_f (*n*-pentane) 0.50; $[\alpha]_D^{25}$ −64.2 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.74–7.67 (m, 4H), 7.47–7.35 (m, 8H), 7.31 (m, 2H), 7.21 (m, 1H), 6.74 (dd, *J*=15.6 and 10.5 Hz, 1H), 6.42 (d, *J*=15.6 Hz, 1H), 6.16 (dd, *J*=15.3 and 10.5 Hz, 1H), 5.74 (m, 1H), 3.84 (m, 1H), 2.47 (m, 1H), 1.96 (m, 1H), 1.62 (m, 1H), 1.09 (s, 9H), 1.00 (d, *J*=6.3 Hz, 3H), 0.87 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 137.7, 135.9 (×2), 135.1, 134.5, 134.2, 131.5 (×2), 129.8 (×2), 129.4 (×2), 129.3 (×2), 128.4, 127.4 (×2), 127.3 (×2), 126.9, 126.3, 126.0, 72.6, 40.6, 35.6, 27.0, 19.5, 19.3, 14.6. *m/z*: 454 $[M]^+$, 397, 199. Anal. Calcd for $C_{31}H_{38}OSi$: C, 81.88; H, 8.42. Found: C, 81.90; H, 8.39.

4.2.13. (2*S*,3*S*)-Dihydrolignarenol B (10). To a solution of compound **19a** (17 mg, 0.037 mmol) in dry THF (0.5 mL) under argon, TBAF (0.30 mL, 0.30 mmol, 1 M solution in THF) was added. The mixture was stirred at room temperature for 24 h then quenched by adding water (2 mL). The THF was removed in vacuo and the resulting suspension was extracted with ethyl acetate (3×3 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 100:0 to 8:2) afforded 8 mg of alcohol **10** (98% yield) as a colourless oil: R_f (30% Et_2O/petroleum ether) 0.65; $[\alpha]_D^{25}$ −1.4 (*c* 0.6, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 2H), 7.30 (m, 2H), 7.20 (m, 1H), 6.76 (dd, *J*=15.6 and 10.4 Hz, 1H), 6.45 (d, *J*=15.6 Hz, 1H), 6.23 (dd, *J*=15.1 and 10.4 Hz, 1H), 5.82 (dt, *J*=15.1 and 7.4 Hz, 1H), 3.78 (m, 1H), 2.33 (m, 1H), 2.02 (m, 1H), 1.61 (m, 1H), 1.19 (d, *J*=6.5 Hz, 3H), 0.93 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 137.5, 134.0, 132.0, 130.3, 129.1, 128.5, 127.2, 126.1, 70.8, 40.1, 36.5, 20.4, 14.0. Anal. Calcd for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 82.99; H, 9.40.

4.2.14. (3*S*)-Dihydrolignarenone B (8). To a solution of compound **10** (9 mg, 0.04 mmol) in dry methylene chloride (1 mL) under argon, tetrapropylammonium perruthenate (1.5 mg, 0.004 mmol), *N*-methylmorpholine *N*-oxide (10 mg, 0.08 mmol) and activated 4 Å molecular sieves were added. The mixture was stirred at room temperature for 90 min then filtered through a pad of silica gel and the bed was washed with ethyl acetate (3×30 mL). The filtrate was concentrated in vacuo. Purification by flash chromatography using petroleum ether/diethyl ether 8:2 as an eluant

afforded 7 mg of ketone **8** (82% yield) as a colourless oil: R_f (30% Et_2O/petroleum ether) 0.85; $[\alpha]_D^{35}$ +8.8 (*c* 0.4, $CHCl_3$); R_f (^1H and ^{13}C NMR data (400 MHz, $CDCl_3$)) were identical to those of the natural product **8**. Anal. Calcd for $C_{15}H_{18}O$: C, 84.07; H, 8.47. Found: C, 84.01; H, 8.51.

4.2.15. (3*S*,4*R*)-4-*tert*-Butyldiphenylsilyloxy-3-methylpent-1-ene (17b). A solution of homoallylic alcohol **16b** (6.5 g, 62 mmol) in dry DMF (35 mL) was treated with *tert*-butylchlorodiphenylsilane (TBDPSCl, 42.9 g, 156 mmol) and imidazole (21.1 g, 310 mmol) as described for **16a**. Purification by flash chromatography using *n*-pentane as an eluant afforded 14.4 g (68% yield) of compound **17b** as a colourless oil: R_f (*n*-hexane) 0.50; $[\alpha]_D^{25}$ −1.9 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (m, 4H), 7.45–7.33 (m, 6H), 5.74 (ddd, *J*=17.2, 10.5 and 7.4 Hz, 1H), 4.95 (m, 1H), 4.90 (m, 1H), 3.82 (m, 1H), 2.23 (m, 1H), 1.06 (s, 9H), 1.03 (d, *J*=6.9 Hz, 3H), 0.95 (d, *J*=6.3 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 141.0, 135.9 (×2, overlapped), 134.9, 134.3, 129.5 (×2), 129.4 (×2), 127.5 (×2), 127.3 (×2), 114.4, 72.6, 44.8, 27.0, 19.4, 19.3, 14.3. *m/z*: 338 $[M]^+$, 281, 203. Anal. Calcd for $C_{22}H_{30}OSi$: C, 78.05; H, 8.93. Found: C, 78.00; H, 9.12.

4.2.16. (3*S*,4*R*)-4-*tert*-Butyldiphenylsilyloxy-3-methylpentan-1-ol (18b). A solution of compound **17b** (1.4 g, 4.1 mmol) in dry hexane (40 mL) was treated with $BH_3 \cdot SMe_2$ (6.0 mL, 12 mmol, 2 M solution in THF) and then with 3 M aqueous NaOH solution (33 mL, 99 mmol) and H_2O_2 (5.1 mL, 35% w/w aqueous solution) as described for **16a**. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 95:5 to 80:20) gave 0.99 g of alcohol **18b** (69% yield) as a colourless oil: R_f (20% Et_2O/*n*-pentane) 0.38; $[\alpha]_D^{25}$ −1.0 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.69 (m, 4H), 7.46–7.34 (m, 6H), 3.77 (m, 1H), 3.58 (m, 1H), 3.49 (m, 1H), 1.64 (m, 1H), 1.52 (m, 1H), 1.46–1.33 (m, 2H overlapped), 1.06 (s, 9H), 1.00 (d, *J*=6.3 Hz, 3H), 0.94 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 135.9 (×2), 134.7, 134.1, 129.6 (×2), 129.4 (×2), 127.5 (×2), 127.4 (×2), 72.7, 60.8, 36.6, 35.4, 27.0, 19.3, 18.7, 14.4. *m/z*: 356 $[M]^+$, 299, 199, 139. Anal. Calcd for $C_{22}H_{32}O_2Si$: C, 74.10; H, 9.05. Found: C, 73.90; H, 9.03.

4.2.17. (3*S*,4*R*)-4-*tert*-Butyldiphenylsilyloxy-3-methylpentan-1-al (15b). A solution of compound **18b** (0.86 g, 2.4 mmol) in dry methylene chloride (30 mL) was treated with pyridinium dichromate (1.8 g, 4.8 mmol) and activated 4 Å molecular sieves (1.7 g) as described for **18a**. Aldehyde **15b** of 0.58 g (68% yield) was obtained as a colourless oil: R_f (30% Et_2O/*n*-pentane) 0.71; $[\alpha]_D^{25}$ +11.8 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.66 (dd, *J*=2.8 and 1.5 Hz, 1H), 7.67 (m, 4H), 7.48–7.34 (m, 6H), 3.73 (m, 1H), 2.52 (ddd, *J*=16.2, 4.5 and 1.5 Hz, 1H), 2.21 (ddd, *J*=16.2, 8.8 and 2.8 Hz, 1H), 2.12 (m, 1H), 1.05 (s, 9H), 0.98 (d, *J*=6.3 Hz, 3H), 0.96 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 202.8, 135.9 (×2), 134.5, 133.8, 129.7 (×2), 129.6 (×2), 127.6 (×2), 127.5 (×2), 72.6, 46.9, 35.4, 27.0, 19.9, 19.4, 15.9. *m/z*: 358 $[M]^+$, 301, 223, 199. Anal. Calcd for $C_{22}H_{30}O_2Si$: C, 74.53; H, 8.53. Found: C, 74.89; H, 8.26.

4.2.18. (1*E*,4*S*,5*R*)-5-*tert*-Butyldiphenylsilyloxy-1-iodo-4-methyl-hex-1-ene (14b). Compound **15b** (2.3 g, 6.5 mmol) was treated with anhydrous $CrCl_2$ (4.8 g, 39 mmol) and iodoform (5.1 g, 13.0 mmol) in dry THF/dioxane 1:6 (17 mL) as described for **18a**. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 100:0 to 95:5) afforded 2.4 g of iodoalkene **14b** (78% yield) as a colourless oil: R_f (10% Et_2O/*n*-pentane) 0.94; $[\alpha]_D^{25}$ +9.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 4H), 7.47–7.34 (m, 6H), 6.29 (ddd, *J*=14.4, 7.5 and 7.5 Hz, 1H), 5.86 (ddd, *J*=14.4, 1.5 and 1.3 Hz, 1H), 3.73 (dq, *J*=12.5 and 6.2 Hz, 1H), 2.05 (m, 1H), 1.82 (m, 1H), 1.60 (m, 1H), 1.05 (s, 9H), 0.96 (d, *J*=6.2 Hz, 3H), 0.87 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 145.4, 135.9 (×2), 134.7, 134.1, 129.6 (×2), 129.5 (×2), 127.6 (×2), 127.4 (×2), 74.9, 72.1, 39.4, 27.0, 19.3, 18.8, 14.2. *m/z*: 478 $[M]^+$, 421, 199. Anal. Calcd for $C_{23}H_{31}IOSi$: C, 57.73; H, 6.53; I, 26.52. Found: C, 57.51; H, 6.66; I, 26.83.

4.2.19. (1*E*,3*E*,6*S*,7*R*)-7-*tert*-Butyldiphenylsilyloxy-6-methyl-1-phenylocta-1,3-diene (19b). A solution of vinylic iodide **14b** (0.22 g, 0.46 mmol) in THF/KOH 2 M aqueous 4:1 (6 mL) was treated with $Pd(PPh_3)_4$ (80 mg, 0.069 mmol) and *trans*-2-phenylvinylboronic acid (**13**, 0.20 g, 1.38 mmol) as described for **14a**. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 100:0 to 97:3) gave 0.155 g of coupling product **19b** (74% yield) as a colourless oil: R_f (1% Et_2O/*n*-pentane) 0.50; $[\alpha]_D^{25}$ +26.6 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.80–7.69 (m, 4H), 7.55–7.37 (m, 9H), 7.29 (m, 1H), 7.26 (m, 1H), 6.75 (dd, *J*=15.6 and 10.4 Hz, 1H), 6.46 (d, *J*=15.6 Hz, 1H), 6.17 (dd, *J*=15.3 and 10.4 Hz, 1H), 5.65 (ddd, *J*=15.2, 7.6 and 7.6 Hz, 1H), 3.86 (m, 1H), 2.20 (m, 1H), 1.99 (m, 1H), 1.75 (m, 1H), 1.15 (s, 9H), 1.07 (d, *J*=6.4 Hz, 3H), 1.00 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 137.7, 136.0 (×2), 135.0, 134.5, 134.4, 131.6 (×2), 129.9 (×2), 129.6 (×2), 129.5 (×2), 128.6, 127.6, 127.5 (×2), 127.1 (×2), 126.4, 126.2, 72.4, 40.5, 36.5, 27.1, 19.4, 18.5, 14.3. *m/z*: 408 $[M]^+$, 401, 202. Anal. Calcd for $C_{31}H_{38}OSi$: C, 81.88; H, 8.42. Found: C, 81.76; H, 8.68.

4.2.20. (2*R*,3*S*)-Dihydrolignarenol B (10b). A solution of compound **19b** (0.78 g, 1.7 mmol) in dry THF (25 mL) was treated with TBAF (7.0 mL, 7.0 mmol, 1 M solution in THF) as described for **19a**. Purification by flash chromatography (gradient elution with petroleum ether/diethyl ether mixtures from 9:1 to 6:4) afforded 0.32 g of alcohol **10b** (87% yield) as a colourless oil; $[\alpha]_D^{25}$ −8.0 (*c* 1.0, $CHCl_3$); ^1H and ^{13}C NMR data (400 MHz, $CDCl_3$) δ 7.39 (m, 2H), 7.31 (m, 2H), 7.21 (m, 1H), 6.78 (dd, *J*=15.6 and 10.5 Hz, 1H), 6.46 (d, *J*=15.6 Hz, 1H), 6.24 (dd, *J*=15.0 and 10.4 Hz, 1H), 5.83 (dt, *J*=15.0 and 7.4 Hz, 1H), 3.68 (m, 1H), 2.36 (m, 1H), 2.04 (m, 1H), 1.63 (m, 1H), 1.18 (d, *J*=6.3 Hz, 3H), 0.92 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 137.5, 134.0, 132.0, 130.3, 129.2, 128.6, 127.2, 126.1, 71.5, 40.5, 36.3, 19.9, 15.0. Anal. Calcd for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 83.21; H, 9.38.

4.2.21. Trapping reaction with carbethoxyethylidene (CET)-triphenylphosphorane. Compound **12** was obtained by SiO_2 chromatography (petroleum ether/diethyl ether 85:15) on extracts of animals from Malgrat (Spain). The product was then derivatized with carbethoxyethylidene

(CET)-triphenylphosphorane in 0.5 mL CH_2Cl_2 at room temperature per 2 h. The reaction mixture was dried at reduced pressure and the oily residue was purified to give 0.4 mg of **12a**. *Compound **12a***: GC–MS *m/z* 242 $[M+H]^+$, 213 $[M-Et+H]^+$, 197 $[M-OEt+H]^+$; ^1H NMR (400 MHz, C_6D_6) δ 1.05 (3H, t, $J_{H,H}$=7.2 Hz, Et), 2.01 (3H, s, Me), 4.09 (2H, q, $J_{H,H}$=7.2 Hz, Et), 6.34 (1H, dd, $J_{H,H}$=14.5 and 9.7 Hz, H-3), 6.39 (1H, dd, $J_{H,H}$=14.5 and 10.7 Hz, H-2), 6.40 (1H, d, $J_{H,H}$=15.6 Hz, H-5), 6.66 (1H, dd, $J_{H,H}$=15.6 and 10.2 Hz, H-4), 7.03–7.28 (5H, m, phenyl protons), 7.54 (1H, br d, $J_{H,H}$=10.7 Hz, H-1).

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