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Synthesis and chiral HPLC analysis of the dibenzyltetrahydrofuran lignans, larreatricins, 8′-*epi*-larreatricins, 3,3′-didemethoxyverrucosins and *meso*-3,3′-didemethoxynectandrin B in the creosote bush (*Larrea tridentata*): evidence for regiospecific control of coupling

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The creosote bush (*Larrea tridentata*) lignans are linked *via* 8–8′ bonds, with the simplest apparently being *E*-*p*-anol derived. Of the latter, four of the six theoretically possible diastereoisomers were isolated, namely (−)-larreatricin, (−)-8′-*epi*-larreatricin, *meso*-3,3′-didemethoxynectandrin B and the new compounds, (+)- and (−)-3,3′-didemethoxyverrucosins. Following synthesis of each in either racemic or *meso* form, and chiral HPLC separation of the antipodes of the racemates, it was established that naturally occurring (−)-larreatricin and (−)-8′-*epi*-larreatricin were present in 92 and 98% enantiomeric excess, respectively, whereas 3,3′-didemethoxyverrucosin was essentially racemic and 3,3′-didemethoxynectandrin B was in the *meso*-form. The evidence suggests that formation of these lignans occurs under regiospecific, rather than stereoselective, coupling control. This contrasts with laccase-catalyzed "random" coupling of *E*-*p*-anol *in vitro* which generates the corresponding racemic 8–8′, 8–3′ and 8–*O*–4′ linked dimeric moieties.

Introduction

The creosote bush, *Larrea tridentata* (Seese & Moçiño ex DC.) Coville, (Zygophyllaceae) contains a large variety of 8–8′ lignans in either dibenzyltetrahydrofuran (**1**–**4**),[1] dibenzylbutane (**7**–**12**)[2,3] or aryltetrahydronaphthalene (**13**–**16**)[1,4] skeletal forms (Fig. 1). Of these, the most abundant lignan, is nordihydroguaiaretic acid (NDGA, **8**), a powerful antioxidant. Its properties include uses as antihyperglycemic[5] and skin anti-aging agents,[6] as well as a polymer (*e.g.*, rubber) stabilizer and as an antioxidant in perfumery oil and photographic formulations.[7,8] While creosote bush extracts have long been used in traditional medicine by the Native Peoples of North and South America,[9,10] more detailed studies have recently revealed potent antiviral properties of its lignans,[11–13] *e.g.*, 3′-*O*-methylnordihydroguaiaretic acid (**9**) inhibits human immunodeficiency virus (HIV) type 1 transcription and replication[11] as well as human papillomavirus type 16 gene expression.[13]

Based solely on structural considerations, the biochemical entry into this class of lignans can be provisionally envisaged to occur *via* 8–8′ coupling of *E*-*p*-anol (**17**) to give the corresponding 8–8′ linked adducts, with subsequent modifications (*e.g.* regiospecific aromatic hydroxylations) to afford the various skeletal types. In the present study, it was found that the creosote bush accumulates four simple 8–8′ linked diastereomers, trivially named larreatricin (**1**), 8′-*epi*-larreatricin (**3**), 3,3′-didemethoxyverrucosin (**5**), and 3,3′-didemethoxynectandrin B (**6**). Each diastereomer was synthesized in racemic (or *meso*) form, with each racemate resolved by chiral HPLC separation. In this way, the enantiomeric purity of each of the four creosote bush lignans **1**, **3**, **5** and **6** was determined, as a forerunner to defining the factors controlling 8–8′ coupling during *L. tridentata* lignan biosynthesis.

Results and discussion

Detailed chemical analysis of the methanol extract from creosote bush leaf tissue gave, in addition to other known 8–8′ linked lignans, a chromatographic fraction that contained the four lignans **1**, **3**, **5** and **6**, all presumed to be *E*-*p*-anol **17** derived. This included the previously isolated creosote bush lignans, larreatricin (**1**)[1] and 8′-*epi*-larreatricin (**3**),[1] and the *meso*-3,3′-didemethoxynectandrin B (**6**) originally found in *Krameria interior*[14] and *K. parvifolia*,[15] as well as the hitherto unknown lignan, 3,3′-didemethoxyverrucosin (**5**). While coupling of *E*-*p*-anol (**17**) can theoretically give rise to six possible diastereomers, the other two diastereomers, **19** from *Saururus cernuus*[16] and **20** obtained by chemical synthesis,[17] were not detected. Nor were any of the fractions examined found to contain the possible 8–3′ **22** and/or 8–*O*-4′ **23** linked adducts, or derivatives thereof, in agreement with previous reports.[1–4] By contrast, *in vitro* treatment of *E*-*p*-anol **17**, using a laccase preparation from *Trametes versicolor*, affords the various 8–8′, 8–3′ and 8–*O*-4′ linked products in a *ca*. 34 : 42 : 24 ratio (see Scheme 1 and Experimental section). This suggests that, at the very least, regiochemical control of coupling occurs during *L. tridentata* lignan biosynthesis, *i.e.* affording only the 8–8′ linked products.

The previously unknown lignan, 3,3′-didemethoxyverrucosin (**5**), was identified as follows: compound **5** gave a molecular ion (EIMS) at *m*/*z* 284, whereas ESI-FTMS (negative ion mode) had a [*M* − H] of 283.1320 and a [*M* + Cl] of 319.1100 indicating a molecular formula of $C_{18}H_{20}O_3$. The IR spectrum had a hydroxy group absorption (ν_{max} 3260 cm^{-1}), which was demonstrated to be of phenolic character *via* a bathochromic shift in the UV-spectrum [λ_{max} 228 nm (log ε 4.72), 246 (4.85), 276 (4.10)]. The ^{1}H NMR spectrum displayed two three proton doublets at 0.61 (*J* 6.9 Hz) and 1.00 (*J* 6.3 Hz) corresponding to the two methyl groups at carbons C9′ and C9, respectively, as well as two multiplets at 1.67 and 2.21 due to the methine protons at C8′ and C8. In addition, the two doublets at 4.29 (*J* 9 Hz) and 5.06 (*J* 8.7 Hz) were from the oxymethine protons at C7 and C7′. From these data, it was provisionally concluded that the stereochemistry between H7 and H8 was *trans*, and that H7′ and H8′ was *cis*, as in the case of larreatricin (**1**). The remaining resonances were due to two A_2B_2 sets of aromatic resonances (6.83, 6.86, 7.23 and 7.35 ppm). The ^{13}C NMR spectrum confirmed this interpretation with resonances at 114.8,

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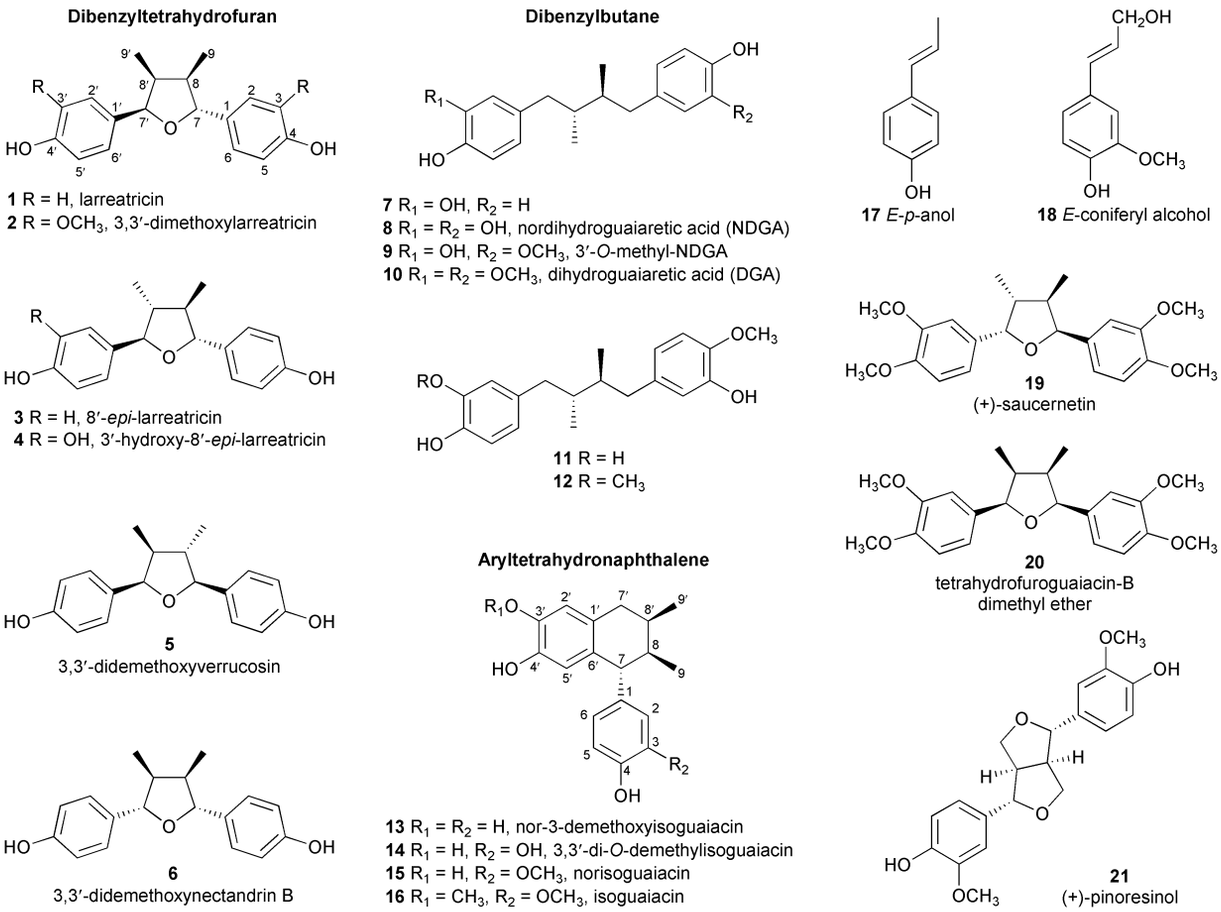
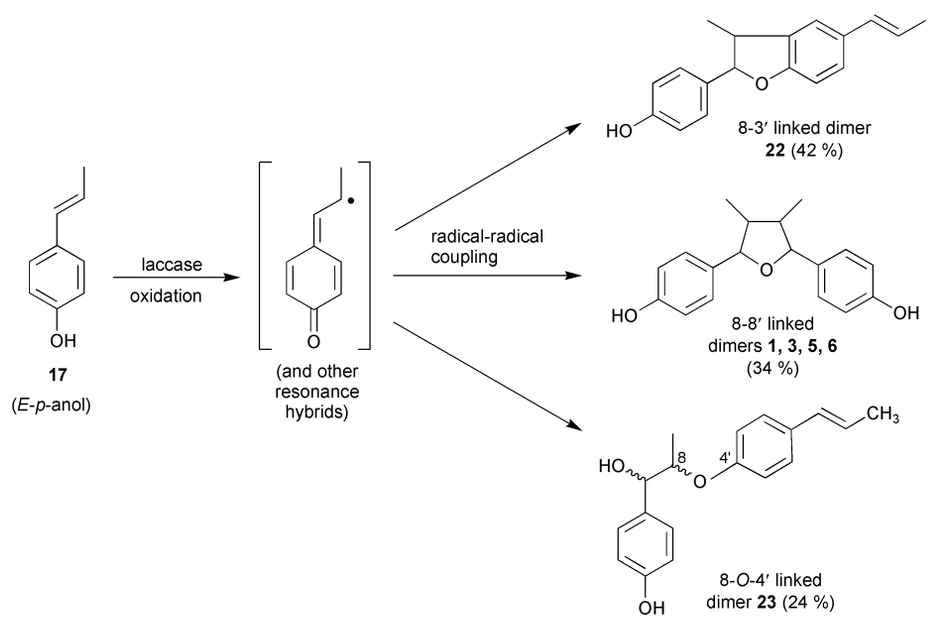


Fig. 1 Allylphenol-derived lignans found in *L. tridentata* and related substances.



Scheme 1 *Trametes versicolor* catalysed oxidation of *E-p*-anol (**17**) to afford the lignans **1**, **3**, **5**, **6**, **22**, and **23**.

115.2, 128.1, 128.4, 132.3, 132.6, 156.6 and 157.1 ppm corresponding to the two 4-hydroxyphenyl moieties, two methyl carbons C9′ and C9 at 14.6 and 15.3 ppm, two methine carbons C8′ and C8 at 46.1 and 49.2 ppm, and two oxymethine carbons at 82.7 and 87.3 ppm (C7′ and C7) for the tetrahydrofuran moiety (see Table 1, with compounds **1**, **3** and **6** included for comparison). All proton and carbon assignments for **5** were further confirmed by 2D NMR (COSY, HMBC, HMQC) experiments, with interproton couplings being established through a homonuclear COSY spectrum in Me_2CO-d_6. The homonuclear COSY spectrum for **5** indicated coupling between the methyl signal at 0.61 ppm (9′-3H) and the methine resonance at 1.67 ppm (8′-H), which itself was coupled with the oxymethine signal at 4.29 ppm (7-H). In a similar way, the methyl resonance at 1.00 ppm (9-3H) was coupled with the methine signal at 2.21 ppm (8-H), this in turn being coupled to the oxymethine resonance at 5.06 ppm (7′-H). Accordingly, compound (**5**) was the verrucosin[18–22] analogue, 3,3′-didemethoxyverrucosin.

In an analogous manner, compound **6** gave a molecular ion (EIMS) of *m*/*z* 284, whereas ESI-FTMS had a [*M* + Cl] of 319.1104, indicative of a molecular formula of $C_{18}H_{20}O_3$. The

Table 1 ^{13}C NMR spectral data of lignans **1**, **3**, **5** and **6**[a]

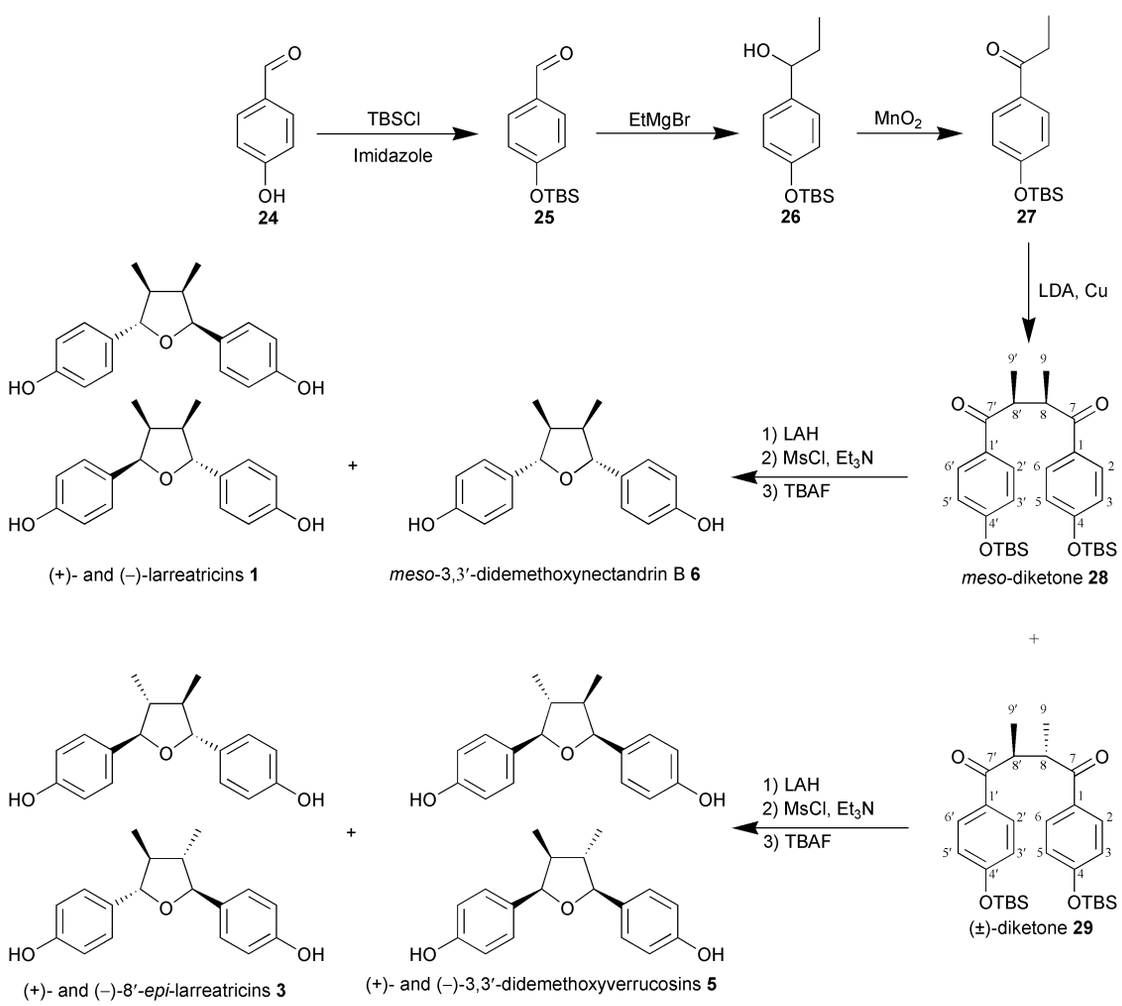
Carbon	1	3	5	6
7-C	86.1	88.7	87.3	87.3
8-C	48.4	51.9	49.2	45.1
8′-C	44.0	51.9	46.1	45.1
7′-C	85.2	88.7	82.7	87.3
1-C	135.5	134.0	132.6	133.7
2-C	128.3	128.3	128.4	127.8
3-C	115.8	115.8	115.2	115.2
4-C	157.6	157.6	157.1	156.9
5-C	115.8	115.8	115.2	115.2
6-C	128.3	128.3	128.4	127.8
1′-C	132.8	134.0	132.3	133.7
2′-C	127.9	128.3	128.1	127.8
3′-C	115.5	115.8	114.8	115.2
4′-C	157.6	157.6	156.6	156.9
5′-C	115.5	115.8	114.8	115.2
6′-C	127.9	128.3	128.1	127.8
9-C	12.1	13.9	15.3	12.5
9′-C	9.7	13.9	14.6	12.5

[a] All spectra were run in Me_2CO-d_6.

IR spectrum also revealed the presence of an hydroxy group absorption (ν_{max} 3370 cm^{-1}) as with **5**. The ^{1}H NMR and ^{13}C NMR spectra of **6** were consistent with that of *meso*-3,3′-didemethoxynectandrin B,[14] and thus was further confirmed by homonuclear COSY spectral analysis. The latter indicated coupling between the methyl signals at 0.98 ppm (9-3H and 9′-3H) and the methine group resonances at 2.22 ppm (8-H and 8′-H), which themselves were coupled to the oxymethine signal at 4.38 ppm (7-H and 7′-H). From the foregoing data and the molecular formula, it was concluded that compound **6** had a structure with a plane of symmetry as represented by the nectandrin B[18,23] analogue, 3,3′-didemethoxynectandrin B.

In previous work,[1] both larreatricin (**1**) and 8′-*epi*-larreatricin (**3**) had been isolated from the creosote bush (*L. tridentata*), but with no report of optical activity. However, since the four metabolites **1**, **3**, **5** and **6** can potentially serve as pathway intermediates to other creosote bush lignans (*e.g. via* subsequent hydroxylations, *O*-methylations, reductive transformations and cyclizations, *etc.*), it was deemed instructive to determine the optical purities of **1**, **3** and **5**. This was in part because in related studies of lignan biosynthesis in *Forsythia intermedia*, *E*-coniferyl alcohol (**18**) had been demonstrated to undergo stereoselective coupling to give only (+)-pinoresinol (**21**), this representing the entry step to that class of monolignol-derived lignans.[24–27] This therefore raised questions as to whether comparable transformations were occurring during *L. tridentata* lignan biosynthesis.

In order to gauge whether stereoselective or regiospecific coupling was occurring *in vivo*, it was next necessary to synthesize lignans **1**, **3** and **5** in racemic forms, as well as the *meso* form **6**, and then to develop chiral HPLC methods for the separation of the (+)- and (−)- antipodes, *i.e.* in order to develop methodologies suitable for establishing the enantiomeric purities of the isolated naturally occurring lignans. A synthetic procedure (Scheme 2) was thus devised, this being based in part on the Stevenson protocol for synthesis of (±)-deoxyschizandrin.[28] This involved initial protection of 4-hydroxybenzaldehyde **24** with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole to give **25**, followed by a Grignard reaction using EtMgBr to generate 4-*O*-*tert*-butyldimethylsilyloxyphenyl-1-propanol (**26**), which was subsequently oxidized with



Scheme 2 Synthesis of lignans **1**, **3**, **5**, and **6**.

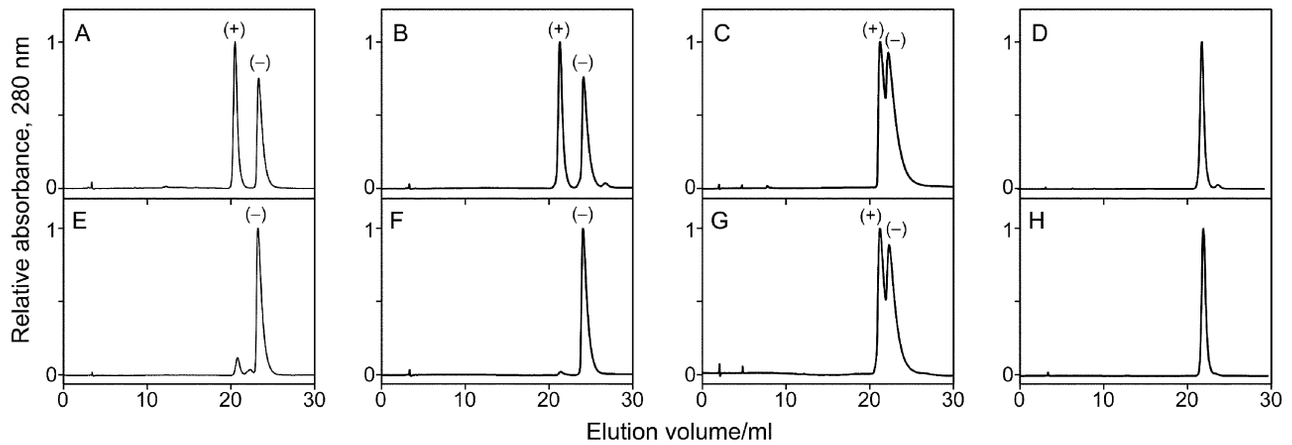


Fig. 2 Chiral HPLC analysis of larreatricin (**1**), 8′-*epi*-larreatricin **3** and 3,3′-didemethoxyverrucosin (**5**) and *meso*-3,3′-didemethoxynectandrin B (**6**). Upper panels: chiral HPLC separation of synthetic (±) stereoisomers **1**, **3** and **5** (A, B and C). Lower panels: chiral HPLC analysis of naturally occurring *L. tridentata* lignans **1**, **3** and **5** (E, F and G). Panel D and H show elution profiles of synthetic and naturally occurring *meso*-3,3′-didemethoxynectandrin B (**6**), respectively.

MnO_2 to yield the synthon 4-*O*-*tert*-butyldimethylsilyloxypropiophenone (**27**). The latter was dimerized with LDA, copper(I) trifluoromethanesulfonate in 1 : 1 equivalents to afford a mixture of the corresponding (±) racemic **29** and *meso* **28** diketones in an overall yield of 34%, with both being separated using silica gel chromatography. Subsequent individual reductions of diketones **28** and **29** with lithium aluminium hydride, followed by cyclization of the corresponding mesylated derivatives and deprotection with tetrabutylammonium fluoride (TBAF) afforded the lignans, (±)-larreatricins (**1**), (±)-8′-*epi*-larreatricins (**3**), (±)-3,3′-didemethoxyverrucosins (**5**) and *meso*-3,3′-didemethoxynectandrin B (**6**), all of which were spectroscopically identical in every respect (*i.e.*, by ^{1}H and ^{13}C NMR, UV and EI-MS analyses) to those of the isolated natural products.

Chiral HPLC separation methods were then developed for baseline separation of the antipodes of the synthetic racemates (±)-larreatricins (**1**) and (±)-8′-*epi*-larreatricins (**3**) using a Chirobiotic V column eluted with EtOH–hexanes (12 : 88), whereas (±)-3,3′-didemethoxyverrucosins (**5**) were only partially separable using the same column and only when eluted with 2-propanol–hexanes (12 : 88) (Fig. 2); the *meso* species 3,3′-didemethoxynectandrin B (**6**) was of course not resolvable, but its chromatogram is included for comparison purposes. Next, chiral HPLC analysis of the four isolated diastereomers from *L. tridentata* revealed that larreatricin (**1**) and 8′-*epi*-larreatricin (**3**) were present primarily as the (−)-antipode (in *ca.* 92% and 98% ee, respectively) with $[a]_D^{20} = -19.3$ (*c* 0.0145, MeOH) and −39.9 (*c* 0.01098, MeOH), respectively, whereas 3,3′-didemethoxyverrucosin (**5**) was in near racemic form, and compound **6** was *meso*.

It was also considered that compound **6** was possibly being generated *via* epimerization of larreatricin **1** under either the extraction or chromatographic conditions employed. However, treatment of **1** under either extraction (hexanes, MeOH) or chromatographic conditions (silica gel with $CHCl_3$–acetone and reversed phase HPLC with CH_3CN–3% HOAc in H_2O as eluant) gave essentially no epimeric product even after 7 days incubation at room temperature (data not shown). Thus, it is concluded that compounds **1**, **3**, **5** and **6** are all naturally occurring.

Taken together, these data reveal several important findings: firstly, for both the simplest lignans (**1**, **3**, **5** and **6**) as well as the others **7**–**16**, only 8–8′ coupling was noted. Second, no 8–3′ and 8–*O*-4′ lignans were detected in extracts from the creosote bush, this also indirectly being in support of overall control of coupling at the 8–8′ positions. Thirdly, the presence of four diastereomers, namely the *meso*-form **6**, (±)-3,3′-didemethoxyverrucosin B (**5**) in near racemic form, as well as larreatricin (**1**) and 8′-*epi*-larreatricin (**3**) in large enantiomeric excess, is strongly suggestive of rigorous regiospecific control of coupling at the 8–8′ position. Further work is, however, needed to establish if the enantiomeric excess of (−)-larreatricin (**1**) and (−)-8′-*epi*-larreatricin (**3**) is due instead to stereoselective coupling, or to differential rates of metabolism of the corresponding (+)-antipodes of (**1**) and (**3**) into other lignan products.

Conclusions

Chemical synthesis of the four diastereomers, (±)-larreatricins (**1**), (±)-8′-*epi*-larreatricins (**3**), (±)-3,3′-didemethoxyverrucosins (**5**) and *meso*-3,3′-didemethoxynectandrin B (**6**) enabled resolution of the (+)- and (−)-forms of **1**, **3** and **5** by chiral HPLC. This in turn permitted determination of the enantiomeric purities of the four lignan diastereomers **1**, **3**, **5** and **6**: larreatricin (**1**) and 8′-*epi*-larreatricin (**3**) were present essentially only as the (−)-antipode, whereas 3,3′-didemethoxyverrucosin (**5**) was racemic and 3,3′-didemethoxynectandrin B was *meso*. Subsequent studies are now needed to establish if the presumed (*E*-*p*-anol **17** derived) allylphenol coupling in *L. tridentata* is only regiospecific thereby affording all four isomers, or alternatively if **1** and **3** are formed through stereoselective coupling. In any case, these findings thus differ substantially from the previous work on monolignol (*E*-coniferyl alcohol (**18**)) coupling, which afforded only the (+)-antipode of pinoresinol (**21**) *via* stereoselective coupling.[24,25] Further studies are currently underway to define the enzymology of *L. tridentata* lignan biosynthesis.

Experimental

General methods

Solvents and chemicals were either reagent or HPLC grade unless otherwise specified, with chemical reactions being carried out under anhydrous conditions in a N_2 atmosphere using dry freshly distilled solvents. Thin-layer chromatography utilized 0.25 mm E. Merck silica gel plates (60 PF_{254}) with both UV and 10% H_2SO_4 – heat being employed for visualization. Optical rotations were measured with a JASCO DIP-181 digital polarimeter at 20 °C (Hg-D line, 546 nm) using a quartz cell of 5 cm length and 1 cm^3 volume, and values are given in 10^{-1} deg cm^2 g^{-1}. UV and IR spectra were recorded on a Lambda 6 UV-visible spectrophotometer (Perkin-Elmer) and a Nicolet MX-1 interferometer, respectively. ^{1}H-NMR, ^{13}C-NMR and homonuclear COSY spectra (300 MHz) were recorded on a Varian Mercury 300 spectrometer, with chemical shifts given in

δ ppm relative to tetramethylsilane and J values in Hz. EI-MS spectra were obtained using a Waters Integrity LC/MS System, whereas ESI-FTMS analyses were carried out on a Bruker-Magnex BioAPEX 30es cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. HPLC employed an Alliance 2690 HPLC (Waters, Milford, MA, USA) using either reversed-phase (Waters, Novapak C_{18}, 150 × 3.9 mm inner diameter and SymmetryShield™ RP_{18}, 150 × 3.9 mm inner diameter) or chiral (Advanced Separation Technologies Inc. (Whippany, NJ), Chirobiotic V, 250 × 4.6 mm inner diameter) columns. [Precise elution conditions are described below for individual experiments.] Eluent detection was carried out at 280 nm, as well as by using an in-line laser polarimetric detector (PDR-Chiral, Advanced Laser Polarimeter, cell volume 56 mm^3, length 5.17 cm) connected in series to determine the enantiomeric compositions of various lignans.[27] Melting points were recorded on a Fisher-John Melting Point apparatus and were uncorrected.

Creosote bush (*L. tridentata*) plants were either mature (more than 1 year old) or seedlings (2–3 months old) grown from seed, with all being maintained in Washington State University greenhouse facilities until required. A voucher specimen has been deposited at the Washington State University herbarium (WSU120502).

Isolation of larreatricin (1), 8′-*epi*-larreatricin (3), 3,3′-didemethoxyverrucosin (5), and 3,3′-didemethoxynectandrin B (6) from *L. tridentata*

Freeze dried *L. tridentata* twigs (13.3 g) were successively extracted with hexanes (3 × 100 cm^3) and then MeOH (3 × 100 cm^3) for 12 h per extraction at room temperature with constant stirring. The MeOH solubles were combined and evaporated to dryness *in vacuo*, with the resulting residue (4 g) reconstituted in a minimal amount of MeOH, and applied to a silica gel column (5 × 40 cm) eluted with a gradient of chloroform–acetone (20 : 1 to 2 : 1). Fractions containing the lignans **1**, **3**, **5** and **6** were combined and concentrated to *ca*. 2 cm^3 and individually purified by HPLC, using a SymmetryShield™ RP_{18} column eluted with CH_3CN–3% HOAc in H_2O (3 : 7) at a flow rate of 1 ml min^{-1} to afford larreatricin (**1**) (4.5 mg), 8′-*epi*-larreatricin (**3**) (2.1 mg), 3,3′-didemethoxyverrucosin (**5**) (1.9 mg) and 3,3′-didemethoxynectandrin B (**6**) (2.0 mg). Larreatricin (**1**), 8′-*epi*-larreatricin (**3**) and *meso*-3,3′-didemethoxynectandrin B (**6**) were identified by comparison of the ^1H and ^{13}C NMR, UV, IR and EI-mass spectral data to those in the literature.[1,14]

Larreatricin (**1**): $[\alpha]_D^{20}$ −19.3 (*c* 0.0145, MeOH) and 8′-*epi*-larreatricin (**3**): $[\alpha]_D^{20}$ −39.9 (*c* 0.01098, MeOH).

3,3′-Didemethoxyverrucosin (**5**): colourless oil, λ_{max}(MeOH)/nm 228 (log ε 4.67), 275 (3.76); λ_{max}(MeOH + NaOH)/nm 228 (log ε 4.72), 246 (4.85), 276 (4.10); ν_{max} (KBr)/cm^{-1} 3260; δ_H(300 MHz; Me_2CO-d_6; Me_4Si) 0.61 (3 H, d, *J* 6.9, 9′-3H), 1.00 (3 H, d, *J* 6.3, 9-3H), 1.67 (1 H, m, 8′-H), 2.21 (1 H, m, 8-H), 4.29 (1 H, d, *J* 9.0, 7-H), 5.06 (1 H, d, *J* 8.7, 7′-H), 6.83 (2 H, d, *J* 8.4, 3′-H, 5′-H), 6.86 (2 H, d, *J* 8.4, 3-H, 5-H), 7.23 (2 H, d, *J* 8.4, 2′-H, 6′-H), 7.35 (2 H, d, *J* 8.4, 2-H, 6-H), 8.27 (1H, br s, OH), 8.35 (1H, br s, OH); for δ_C(300 MHz; Me_2CO-d_6; Me_4Si) see Table 1; *m*/*z* (EI): 284 (M^+, 4%), 162 (100), 147 (99), 133 (20), 121 (19), 107 (25), 94 (14), 77 (16); *m*/*z* (ESI-FTMS) 283.1320; (*M* − H. $C_{18}H_{20}O_3$ − H requires 283.1339), 319.1100 (*M* + Cl. $C_{18}H_{20}O_3$ + Cl requires 319.1106).

3,3′-Didemethoxynectandrin B (**6**): colourless oil; λ_{max}(MeOH)/nm 224 (log ε 4.46), 280 (3.69); ν_{max} (KBr)/cm^{-1} 3370; δ_H(300 MHz; Me_2CO-d_6; Me_4Si) 0.98 (6 H, d, *J* 6.6, 9-3H, 9′-3H), 2.22 (2 H, m, 8-H, 8′-H), 4.38 (2 H, d, *J* 6.9, 7-H, 7′-H), 6.84 (4 H, d, *J* 8.4, 3-H, 5-H, 3′-H, 5′-H), 7.3 (4 H, d, *J* 8.4, 2-H, 6-H, 2′-H, 6′-H), 8.31 (2 H, br s, OH); for δ_C(300 MHz; Me_2CO-d_6; Me_4Si) see Table 1; *m*/*z* (EI) : 284 (M^+, 4), 162 (100), 147 (99), 133 (20), 121 (19), 107 (25), 94 (14), 77 (16); *m*/*z* (ESI-FTMS) 319.1104 (*M* + Cl. $C_{18}H_{20}O_3$ + Cl requires 319.1106).

Synthesis of (±)-larreatricins (1), *meso*-3,3′-didemethoxynectandrin B (6), (±)-8′-*epi*-larreatricins (3) and (±)-3,3′-didemethoxyverrucosins (5)

Larreatricin (**1**) and its stereoisomers (**3**, **5** and **6**) were synthesized using the strategy of Stevenson and co-workers[28] for (±)-deoxyschizandrin, with the following modifications:

4-*O*-*tert*-Butyldimethylsilyloxybenzaldehyde (25). To a solution of 4-hydroxybenzaldehyde (**24**) (1 g, 8.2 mmol) in THF (40 cm^3) under N_2 at 0 °C, was sequentially added imidazole (1.05 g, 15.4 mmol) and *tert*-butyldimethylsilyl chloride (TBSCl) (2.1 g, 14.0 mmol), with the contents being stirred for 6 h.[29] The reaction mixture was quenched with saturated NH_4Cl solution (20 cm^3) and extracted with EtOAc (3 × 50 cm^3), with the resulting organic solubles washed successively with water (2 × 25 cm^3) and brine solution (2 × 25 cm^3). The organic solubles were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, with the residue so obtained reconstituted in a minimum amount of hexanes and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 95 : 5) to afford 4-*O*-*tert*-butyldimethylsilyloxybenzaldehyde (**25**) (1.8 g, 7.6 mmol, 93%) as a colourless oil; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.25 (6 H, s, 2Me-TBS), 0.99 (9 H, s, 3Me-TBS), 6.94 (2 H, d, *J* 8.7, 3-H, 5-H), 7.79 (2 H, d, *J* 8.4, 2-H, 6-H), 9.88 (1 H, s, CHO).

4-*O*-*tert*-Butyldimethylsilyloxypropiophenone (27). To a solution of 4-*O*-*tert*-butyldimethylsilyloxybenzaldehyde (**25**) (27 g, 114 mmol) in dry diethyl ether (100 cm^3) under N_2 at 0 °C was added EtMgBr (1.0 M in THF, 108 cm^3, 108 mmol), with the whole allowed to stir for 30 min. The reaction mixture was quenched with saturated NH_4Cl solution (100 cm^3) and extracted with EtOAc (3 × 100 cm^3), with the resulting organic solubles washed successively with water (2 × 50 cm^3) and brine solution (2 × 50 cm^3). The organic solubles were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, with the residue reconstituted in a minimum amount of chloroform and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 9 : 1) to afford 4-*O*-*tert*-butyldimethylsilylphenyl-1-propanol (**26**) (24.9 g, 93.4 mmol, 81.9%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.19 (6 H, s, 2Me-TBS), 0.89 (3 H, t, *J* 7.5, CH_3), 0.98 (9 H, s, 3Me-TBS), 1.6–1.9 (2 H, m, CH_2), 2.04 (1 H, s, OH), 4.53 (1 H, t, *J* 6.6,–*CH*OH), 6.81 (2 H, d, *J* 8.4, 3-H, 5-H), 7.20 (2 H, d, *J* 8.4, 2-H, 6-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.32, 10.3, 18.2, 25.7, 31.8, 119.8, 127.0, 137.1, 154.9.

The propanol derivative (**26**) was dissolved in chloroform (100 cm^3) and added to a suspension of MnO_2 (40 g, 460 mmol) in chloroform (100 cm^3) with the resulting suspension heated (~65 °C) for 48 h. The reaction mixture was then cooled, filtered, with the chloroform solubles washed successively with water (3 × 50 cm^3) and brine solution (3 × 50 cm^3). The organic solubles were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, with the residue reconstituted in a minimum amount of chloroform and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 9 : 1) to afford 4-*O*-*tert*-butyldimethylsilyloxypropiophenone **27** (17.53 g, 66.2 mmol, 78%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.23 (6 H, s, 2Me-TBS), 0.98 (9 H, s, 3Me-TBS), 1.21 (3 H, t, *J* 7.4, CH_3), 2.95 (2 H, q, *J* 7.4, CH_2), 6.87 (2 H, d, *J* 9.0, 3-H, 5-H), 7.89 (2 H, d, *J* 9.0, 2-H, 6-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.26 (2Me-TBS), 8.5, 18.3 (C-TBS), 25.6 (3Me-TBS), 31.4, 119.8, 130.0, 130.4, 159.9, 199.4 (C=O).

(±)-(8*S*,8′*S*)-8,8′-Dimethyl-7,7′-bis(4,4′-di-*O*-*tert*-butyldimethylsilyloxyphenyl)butane-7,7′-dione (29) and *meso*-(8*R*,8′*S*)-8,8′-dimethyl-7,7′-bis(4,4′-di-*O*-*tert*-butyldimethylsilyloxyphenyl)butane-7,7′-dione (28). To a solution of diisopropyl-

amine (10.5 cm^3, 75 mmol) in dry THF (100 cm^3) under N_2 at 0 °C was added *n*-BuLi (1.6 M in hexanes, 45 cm^3, 71.9 mmol), with the suspension then cooled to −78 °C this being held for 30 min. To this was sequentially added dropwise 4-*O*-*tert*-butyldimethylsilyloxypropiophenone (**27**) (16.5 g, 62.4 mmol) in THF (50 cm^3), followed by copper(I) trifluoromethanesulfonate (25 g, 75 mmol) in acetonitrile (60 cm^3). Following stirring for 9 h, the reaction mixture was quenched with saturated NH_4Cl solution (100 cm^3) and extracted with ethyl acetate (3 × 100 cm^3), with the corresponding organic solubles washed successively with water (3 × 50 cm^3) and brine solution (3 × 50 cm^3). The organic solubles were dried (Na_2SO_4) and evaporated to dryness *in vacuo*, with the residue reconstituted in a minimum amount of chloroform and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 95 : 5) to afford (±)-(8*S*,8′*S*)-8,8′-dimethyl-7,7′-bis(4,4′-di-*O*-*tert*-butyldimethylsilyloxyphenyl)butane-7,7′-dione (**29**) (7.37 g, 14 mmol, 22.4%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.23 (12 H, s, 4Me-TBS), 0.98 (18 H, s, 6Me-TBS), 1.27 (6 H, d, *J* 6.9, 9-3H, 9′-3H), 3.90 (2 H, m, 8-H, 8′-H), 6.86 (4 H, d, *J* 8.7, 3-H, 5-H, 3′-H, 5′-H), 7.91 (4 H, d, *J* 8.7, 2-H, 6-H, 2′-H, 6′-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.2, 15.8, 18.3, 25.6, 43.2, 119.8, 129.5, 130.6, 159.9 and 202.8, and *meso*-(8*R*,8′S)-8,8′-dimethyl-7,7′-bis(4,4′-di-*O*-*tert*-butyldimethylsilyloxyphenyl)-butane-7,7′-dione (**28**) (3.47 g, 6.6 mmol, 10.6%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.25 (12 H, s, 4Me-TBS), 1.0 (18 H, s, 6Me-TBS), 1.10 (6 H, d, *J* 6.3, 9-3H, 9′-3H), 3.97 (2 H, m, 8-H, 8′-H), 6.90 (4 H, d, *J* 8.7, 3-H, 5-H, 3′-H, 5′-H), 7.99 (4 H, d, *J* 8.7, 2-H, 6-H, 2′-H, 6′-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.2, 17.7, 18.3, 25.6, 43.0, 119.9, 130.3, 130.6, 160.4, 202.4.

(±)-Larreatricins (1) and *meso*-3,3′-didemethoxynectandrin B (6). The *meso*-diketone (**28**) (650 mg, 1.23 mmol) was dissolved in dry diethyl ether (5 cm^3) under N_2 at 0 °C, and added to a suspension of lithium aluminium hydride (LAH) (150 mg, 3.95 mmol) in diethyl ether (5 cm^3). Following stirring for 2 h, excess LAH was destroyed by addition of ethyl acetate (50 cm^3) with the resulting suspension washed with water (25 cm^3). The combined organic solubles were then washed with brine solution (25 cm^3), dried (Na_2SO_4) and evaporated to dryness *in vacuo* (670 mg). The crude product was next dissolved in dry methylene chloride (20 cm^3) under N_2 at 0 °C, and added to a suspension of methanesulfonyl chloride (216 mm^3, 2.79 mmol) in triethylamine (885 mm^3, 6.35 mmol) with the contents being stirred for 1 h. The reaction mixture was quenched with saturated NH_4Cl solution (30 cm^3) and extracted with ethyl acetate (2 × 50 cm^3). The organic solubles were then washed successively with water (30 cm^3) and brine solution (30 cm^3), dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue so obtained was reconstituted in a minimum amount of hexanes and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 95 : 5) to afford the di-*O*-*tert*-butyldimethylsilyl ether derivative of **1** (168 mg, 0.33 mmol, 26.6%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.17 (12 H, s, 4Me-TBS), 0.59 (3 H, d, *J* 6.9, 9′-3H), 0.97 (3 H, d, *J* 6.9, 9-3H), 0.97 (18 H, s, 6Me-TBS), 2.41 (2 H, m, 8-H, 8′-H), 4.63 (1 H, d, *J* 9.3, 7-H), 5.44 (1 H, d, *J* 4.2, 7′-H), 6.80 (2 H, d, *J* 8.4, 3′-H, 5′-H), 6.82 (2 H, d, *J* 8.4, 3-H, 5-H), 7.18 (2 H, d, *J* 8.4, 2′-H, 6′-H), 7.24 (2 H, d, *J* 8.4, 2-H, 6-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.35, −4.33, 9.5, 11.8, 18.2, 18.3, 25.7, 43.3, 47.6, 84.7, 85.4, 119.5, 119.8, 127.0, 127.1, 133.2, 135.7, 154.2 and 154.8, and the di-*O*-*tert*-butyldimethylsilyl ether derivative of **6** (282 mg, 0.55 mmol, 44.7%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.19 (12 H, s, 4Me-TBS), 0.98 (18 H, s, 6Me-TBS), 1.0 (6 H, d, *J* 6.9, 9′-3H, 9-3H), 2.29 (2 H, m, 8-H, 8′-H), 4.44 (2 H, d, *J* 6.6, 7-H, 7′-H), 6.82 (4 H, d, *J* 8.7, 3-H, 5-H, 3′-H, 5′-H), 7.29 (4 H, d, *J* 8.7, 2-H, 6-H, 2′-H, 6′-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.31, −4.29, 12.9, 18.2, 25.7, 44.6, 87.2, 119.8, 127.4, 134.7 and 154.9.

To a solution of (±)-larreatricin-di-*O*-*tert*-butyldimethylsilyl ethers (168 mg, 0.33 mmol) in dry THF (10 cm^3) under N_2 at 0 °C was added tetrabutylammonium fluoride (1.0 M in THF, 714 mm^3, 0.71 mmol).[29] Following stirring for 1 h, the reaction mixture was quenched with saturated NH_4Cl solution (25 cm^3) and extracted with EtOAc (2 × 50 cm^3), with the resulting organic solubles washed successively with water (25 cm^3) and brine solution (25 cm^3). The organic solubles were next dried (Na_2SO_4) and evaporated to dryness *in vacuo*, with the residue reconstituted in a minimum amount of hexanes and subjected to silica gel column chromatography (eluted with hexanes–EtOAc, 3 : 2) to afford (±)-larreatricins (**1**) [(7*R*,7′*R*)-7,7′-bis(4,4′-dihydroxyphenyl)-(8*R*,8′*S*)-8,8′-dimethyltetrahydrofuran] (92 mg, 0.32 mmol, 98%) as colourless needles, mp 163–164 °C [lit.,[1] 161–163 °C (from C_6H_6 + Et_2O)]. ^{1}H and ^{13}C NMR (Table 1), UV, IR and mass spectra were identical to those reported in the literature.[1]

meso-3,3′-Didemethoxynectandrin B-di-*O*-*tert*-butyldimethylsilyl ether (282 mg, 0.55 mmol) was deprotected and purified in a similar manner as above to give *meso*-3,3′-didemethoxynectandrin B (**6**) [(7*R*,7′*S*)-7,7′-bis(4,4′-dihydroxyphenyl)-(8*R*,8′*S*)-8,8′-dimethyltetrahydrofuran] (151.6 mg, 0.53 mmol, 96.4%) as a colourless oil; δ_H(300 MHz; Me_2CO-d_6; Me_4Si) 0.99 (6 H, d, *J* 6.6 Hz, 9-3H, 9′-3H), 2.22 (2 H, m, 8-H, 8′-H), 4.37 (2 H, d, *J* 6.9, 7-H, 7′-H), 6.83 (4 H, d, *J* 8.4, 3-H, 5-H, 3′-H, 5′-H), 7.3 (4 H, d, *J* 8.4, 2-H, 6-H, 2′-H, 6′-H), 8.31 (2 H, br s, OH); for ^{13}C NMR spectral data see Table 1; UV, IR and EI-MS data were identical to those of isolated **6**.

(±)-8′-*epi*-Larreatricins (3) and (±)-3,3′-didemethoxyverrucosins (5). The (±)-diketones (**29**) (740 mg, 1.4 mmol) were converted exactly as above into the di-*O*-*tert*-butyldimethylsilyl ether derivatives of (**3**) (321 mg, 0.63 mmol, 45%) as a colourless oil, δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.21 (12 H, s, 4Me-TBS), 1.02 (18 H, s, 6Me-TBS), 1.05 (6 H, d, *J* 6.0, 9-3H, 9′-3H), 1.81 (2 H, m, 8-H, 8′-H), 4.66 (2 H, d, *J* 9.0, 7-H, 7′-H), 6.85 (4 H, d, *J* 8.4, 3-H, 5-H, 3′-H, 5′-H), 7.29 (4 H, d, *J* 8.4, 2-H, 6-H, 2′-H, 6′-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.57, −4.54, 13.5, 18.0, 25.5, 50.7, 87.9, 119.5, 127.1, 134.8 and 154.6, and the di-*O*-*tert*-butyldimethylsilyl ethers of **5** (179 mg, 0.35 mmol, 25%) as a colourless oil; δ_H(300 MHz; $CDCl_3$; Me_4Si) 0.18 (6 H, s, 2Me-TBS), 0.20 (6 H, s, 2Me-TBS), 0.61 (3 H, d, *J* 6.9, 9′-3H), 0.98 (9 H, s, 3Me-TBS), 0.99 (9 H, s, 3Me-TBS), 1.01 (3 H, d, *J* 6.6, 9-3H), 1.75 (1 H, m, 8′-H), 2.21 (1 H, m, 8-H), 4.35 (1 H, d, *J* 9.3, 7-H), 5.11 (1 H, d, *J* 8.7, 7′-H), 6.82 (2 H, d, *J* 8.4, 3′-H, 5′-H), 6.85 (2 H, d, *J* 8.4, 3-H, 5-H), 7.20 (2 H, d, *J* 8.4, 2′-H, 6′-H), 7.35 (2 H, d, *J* 8.4, 2-H, 6-H); δ_C(300 MHz; $CDCl_3$; Me_4Si) −4.34, −4.32, 14.8, 15.2, 18.2, 25.7, 45.9, 48.3, 82.7, 87.2, 119.5, 119.8, 127.7, 127.9, 133.3, 133.8, 154.4 and 155.0. These derivatives were then individually deprotected with tetrabutylammonium fluoride in THF exactly as described above to afford (±)-8′-*epi*-larreatricins (**3**) [(7*S*,7′*S*)-7,7′-bis(4,4′-dihydroxyphenyl)-(8*S*,8′*S*)-8,8′-dimethyltetrahydrofuran] (176 mg, 0.62 mmol, 98%) as colorless needles, mp 232–234 °C [lit.,[1] 230–232 °C (from Me_2CO)], ^{1}H and ^{13}C NMR (Table 1), UV, IR and mass spectra were identical to those reported in the literature;[1] and (±)-3,3′-didemethoxyverrucosins (**5**) [(7*S*,7′*R*)-7,7′-bis(4,4′-dihydroxyphenyl)-(8*S*,8′*S*)-8,8′-dimethyltetrahydrofuran] (97 mg, 0.34 mmol, 97%) as a colourless oil; δ_H(300 MHz; Me_2CO-d_6; Me_4Si) 0.61 (3 H, d, *J* 7.0, 9′-3H), 1.00 (3 H, d, *J* 6.4, 9-3H), 1.67 (1 H, m, 8′-H), 2.21 (1 H, m, 8-H), 4.29 (1 H, d, *J* 9.0, 7-H), 5.06 (1 H, d, *J* 8.7, 7′-H), 6.82 (2 H, d, *J* 8.4, 3′-H, 5′-H), 6.85 (2 H, d, *J* 8.4, 3-H, 5-H), 7.23 (2 H, d, *J* 8.4, 2′-H, 6′-H), 7.35 (2 H, d, *J* 8.4, 2-H, 6-H); for ^{13}C NMR spectral data see Table 1; UV, IR and EI-MS data were identical to those of isolated **5**.

Chiral HPLC Analyses

Synthetic (±)-larreatricins (**1**), (±)-8′-*epi*-larreatricins (**3**), (±)-3,3′-didemethoxyverrucosins (**5**) and *meso*-3,3′-didemeth-

oxynectandrin B (**6**) were individually subjected to chiral HPLC, using a Chirobiotic V column. Both (±)-larreatricins (**1**) and (±)-8′-*epi*-larreatricins (**3**) as well as *meso*-3,3′-didemethoxynectandrin B (**6**) were eluted using ethanol–hexanes (12 : 88) for resolution, whereas 2-propanol–hexanes (12 : 88) was employed for (±)-3,3′-didemethoxyverrucosins (**5**). All eluents were dually monitored, at a flow rate of 1 ml min^{-1}, using an UV detector (Waters 996 photodiode array, λ 280 nm) connected in series to an Advanced Laser Polarimeter (cell volume 56 mm^3, length 5.17 cm; PDR-Chiral, Palm Beach Gardens, FL, USA).[27]

Trametes versicolor catalyzed radical–radical coupling of *E-p*-anol (17) and identification of coupling products

Each assay consisted of [8-^{14}C] *E-p*-anol (**17**) (9 mM, 2.22 kBq), *T. versicolor* laccase II[30] (0.14 μg) and potassium phosphate buffer (50 mM, pH 7.0) in a total volume of 250 mm^3. Assays were initiated by addition of laccase, and following incubation for 8 h at 30 °C, the reaction mixture was extracted with EtOAc (2 × 500 mm^3). After centrifugation (13,800 × *g*, 5 min), the EtOAc solubles were decanted and evaporated to dryness *in vacuo*. Reconstitution of the latter in MeOH–H_2O (1 : 1, 120 mm^3) next occurred, with an aliquot (100 mm^3) being subjected to reversed phase HPLC (Nova-Pak C_{18} column) eluted as follows: flow rate of 1 ml min^{-1}; isocratic solvent system A–B (CH_3CN–3% HOAc in H_2O) (30 : 70) for the first 15 min followed by a linear A–B gradient from 30 : 70 to 90 : 10 between 15 and 40 min. Fractions containing 8–8′, 8–3′ and 8–*O*-4′ linked lignans were collected at one-minute intervals and subjected to liquid scintillation counting analysis. The 8–8′ and 8–3′ linked products were identified by comparison of their ^{1}H NMR spectral data with those of synthetic standards and literature data.[31]

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