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BIOMIMETIC SYNTHESES OF NEUROTROPHIC AMERICANOL A AND ISOAMERICANOL A BY HORSERADISH PEROXIDASE (HRP) CATALYZED OXIDATIVE COUPLING †

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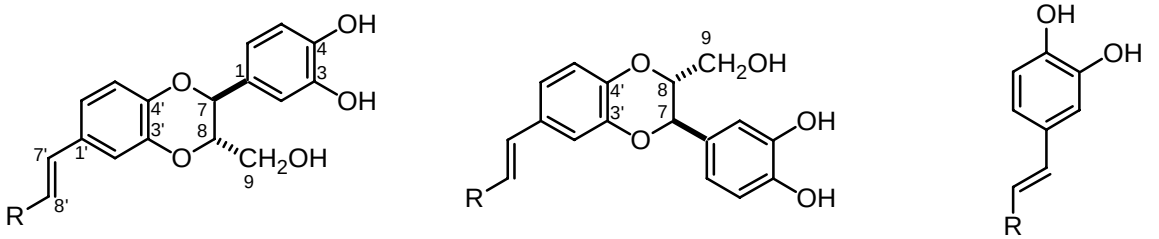
Abstract – Horseradish peroxidase (HRP) catalyzed oxidative coupling of caffeic acid has yielded novel dimeric compounds (**8**) and (**9**) having a 1,4-benzodioxane ring, which have been in turn converted to americanol A (**1**) and isoamericanol A (**2**) in a few steps respectively. Additionally, HRP has coupled with 3,4-dihydroxycinnamyl alcohol giving rise directly **1** and **2** in high yield.

Americanol A (**1**), isoamericanol A (**2**), americanin A (**3**) and isoamericanin A (**4**), belonging to unique neo-lignans having a 1,4-benzodioxane ring in their molecules, occur exclusively in the seeds of *Phytolacca americana* L.[1-3] Among them, americanol A and isoamericanol A exhibit interesting



1 R = CH_2OH
3 R = CHO

2 R = CH_2OH
4 R = CHO

5 R = COOH
6 R = CHO
7 R = CH_2OH

Figure 1 Americanol A (**1**), Isoamericanol A (**2**), Americanin A (**3**), Isoamericanin A (**4**) and 3,4-Dihydroxycinnamyl derivatives (**5-7**)

†Dedicated to Professor James P. Kutney on the occasion of his 70th birthday.

neurotrophic property, i.e., the promotion of neurite outgrowth and the enhancement of choline acetyltransferase (ChAT) activity in the primary cultures of fetal rat cerebral hemisphere.[1] Caffeic acid (**5**), and its reduced forms (**6**) and (**7**) regarded as biogenetic precursors of these particular lignans, however, had no neurotrophic property,[1] which implied that a dimeric structure having a 1,4-dioxane ring could be essential for their interesting neurotrophic activity.

Americanin A and isoamericanin A were already synthesized by employing the stepwise ether formation for the construction of the benzodioxane ring.[4] Recently, Yang *et al*. reported the enantioselective and regioselective synthesis of the 1,4-benzodioxane lignans by applying similar ether formation procedure to the chiral epoxide derived from ferulic acid.[5] Moreover, the biomimetic approach to the formation of the 1,4-benzodioxane ring was demonstrated in the synthesis of silbin, a flavonolignan, by silver oxide-mediated[6] and horseradish peroxidase (HRP)[7] catalyzed oxidative couplings between dihydroquecetin and coniferyl alcohol. It is tactically attractive that some oxidative coupling ways of caffeic acid and its reduced derivatives may lead to americanol A and isoamericanol A. The literature surveillance of Ag_2O, $FeCl_3$ and other oxidative reagents–mediated coupling of caffeic acid[8-10] in addition of our results,[11] however, had given no 1,4-benzodioxane compound.

Oxidative enzyme HRP is well known to catalyze oxidative phenol coupling of aromatic substrates to give C-O and/or C-C coupled products.[12] Thus, we have decided to utilize HRP for the construction of 1,4-benzodioxane ring. Our preliminary result has been already reported concerning the convenient syntheses of **1** and **2** by HRP catalyzed oxidative coupling of caffeic acid.[13] In this paper, we account for the full detail of HRP catalyzed oxidative coupling of caffeic acid and 3,4-dihydroxycinnamyl alcohol as well as of biomimetic syntheses of neurotrophic americanol (**1**) and isoamericanol A (**2**).

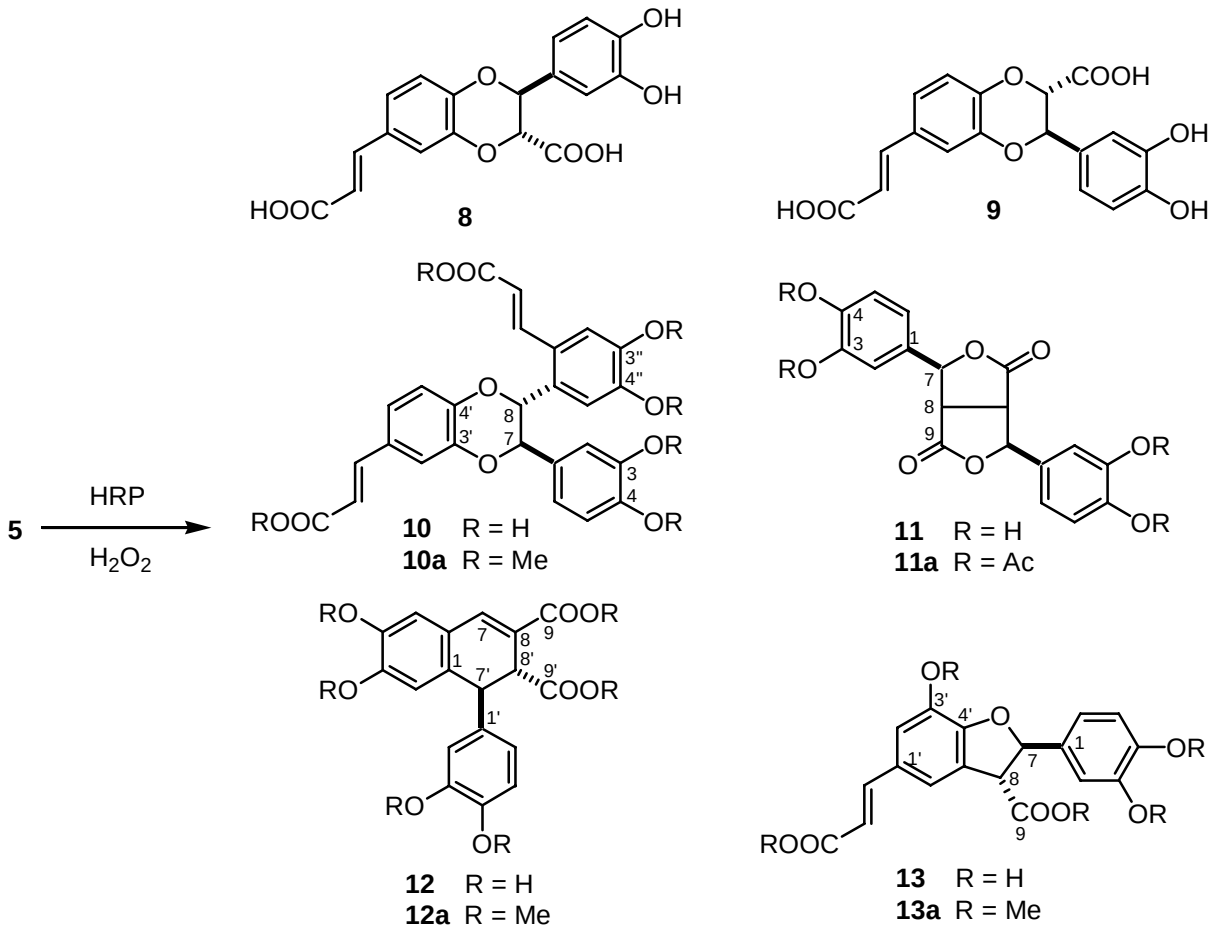
Oxidative Coupling of Caffeic acid by HRP

Caffeic acid (**5**) was incubated with HRP in phosphate buffer (0.1 M, pH 6.0) containing 18 % 1,4-dioxane in presence of H_2O_2 at 20˚C. After being stirred for 2 h, the reaction was quenched with 1M $NaHSO_3$. This solution was acidified to pH 3.0 with 1M $KHSO_4$ and extracted with ethyl acetate. Purification of the mixture by HPLC gave desired coupling compounds (**8)**, (**9**) and (**10**), and the previously known compounds (**11**), (**12**) and (**13**)[8] along with 30 % recovered starting material. (Table 1)

Table 1 Oxidative coupling of caffeic acid by HRP.

Compound	Retention Time (min)	Yield (%)[a]
5	10.2	27.7
11	10.8	14.3
12	12.4	3.8
13	13.6	2.5
8	16.2	9.3
9	19.6	7.5
10	23.0	6.4

[a] Determined by HPLC



Scheme 1 Products Obtained by HRP Catalyzed Oxidative Coupling of Caffeic Acid (**5**)

Compounds (**8**) and (**9**) had the same molecular formula ($C_{18}H_{14}O_8$), suggesting the dimeric structure of **5**. The ^{1}H NMR and ^{13}C NMR spectral data of **8** and **9** were found to be very similar to each other and showed signals due to one *E* olefin and six aromatic protons, and newly appeared oxymethines (**8**: δ_H 4.86 and 5.16, δ_C 77.1 and 77.7; **9**: δ_H 5.00 and 5.37, δ_C 76.3, 76.6), respectively. These data suggested that **8** and **9** were regioisomers on the 1,4-benzodioxane ring. In order to determine the substituents at the C-7 and C-8 positions in **8** and **9**, heteronuclear multi-bond correlation (HMBC) experiments were carried out. Compound (**8**) showed the distinct cross peaks between the H-7 signal at δ_H 4.84 and the C-4' signal at δ_C 145.6 and between the H-7 signal at δ_H 5.16 and the C-3' signal at δ_C 144.6, whereas the HMBC correlations of **9** were observed between the H-7 signal at δ_H 5.37 and the C-3' signal at δ_C 144.8 and between the H-8 signal at δ_H 5.00 and the C-4' signal at δ_C 145.8. The coupling constant between the H-7 and H-8 signal showed 5.1 Hz for **8** and 3.3 Hz for **9**, respectively, which could not distingwish *trans* and *cis* orientation on the C-7 and C-8 position. The *trans* orientation for H-7 and H-8 was elucidated on the basis of NOE between H-8 and H-2, and finally confirmed by converting **8** and **9** to **1** and **2**, respectively. Thus, **8** and **9** were established as dimeric structures corresponding to americanol (**1**) and isoamericanol (**2**), respectively.

The ^{1}H NMR spectrum of **10** showed the signals due to two *E* olefins [δ_H . 5.85 (1H, d, *J* = 15.7 Hz), 6.35 (1H, d, *J* = 15.7 Hz), 7.61 (2H, d, *J* = 15.7 Hz)], two oxymethine protons [δ_H 4.77 (1H, d, *J* = 8.1 Hz), 5.18 (1H, d, *J* = 8.1 Hz)], two ABX aromatic protons and two singlet aromatic protons. The ^{13}C NMR data of **10** displayed 26 carbons included two carboxyl groups. Methylation of **10** with trimethylsilyldiazomethane ($TMSCHN_2$) gave hexamethylate (**10a**) [δ 3.72 (3H, s), 3.74 (3H, s), 3.81 (6H, s), 3.87 (3H, s), 3.97 (3H, s); *m/z* 576.1977 for $C_{32}H_{32}O_{10}$]. These data indicated that **10** was another 1,4-benzodioxane which was formed by oxidative coupling of three caffeic acid, followed by decarboxylation. In order to confirm the linkage manner of three caffeic acids and the substitution pattern on the 1,4-benzodioxane ring, HMBC experiments of **10a** were carried out and thus the related correlations were shown in Figure 2. The *trans* orientation for H-7 and H-8 on the 1,4-dioxane ring was evident from the $J_{7,8}$ value (8.1 Hz) for **10**. Therefore, compound (**10**) was elucidated to a trimer of caffeic acid with a loss of one carboxylic acid as shown in Scheme 1.

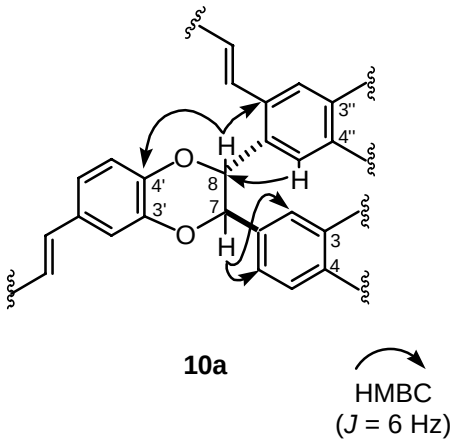
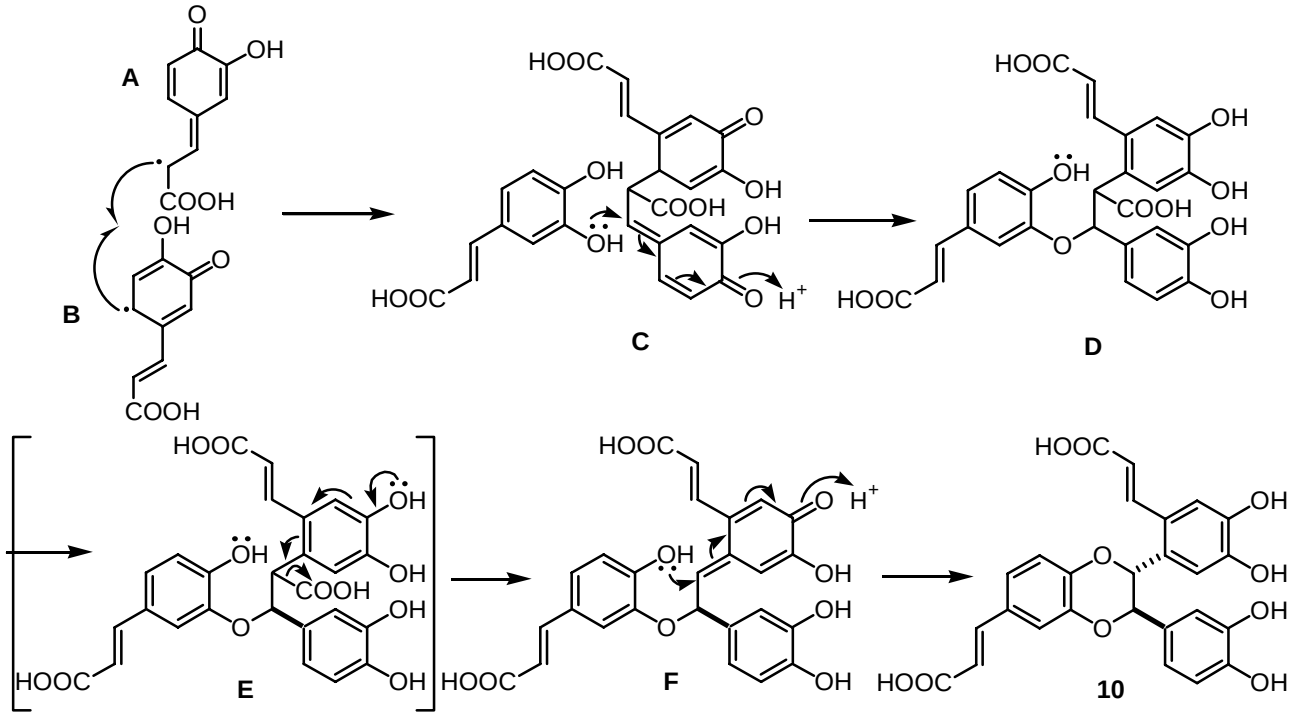


Figure 2 HMBC Correlations of **10a**

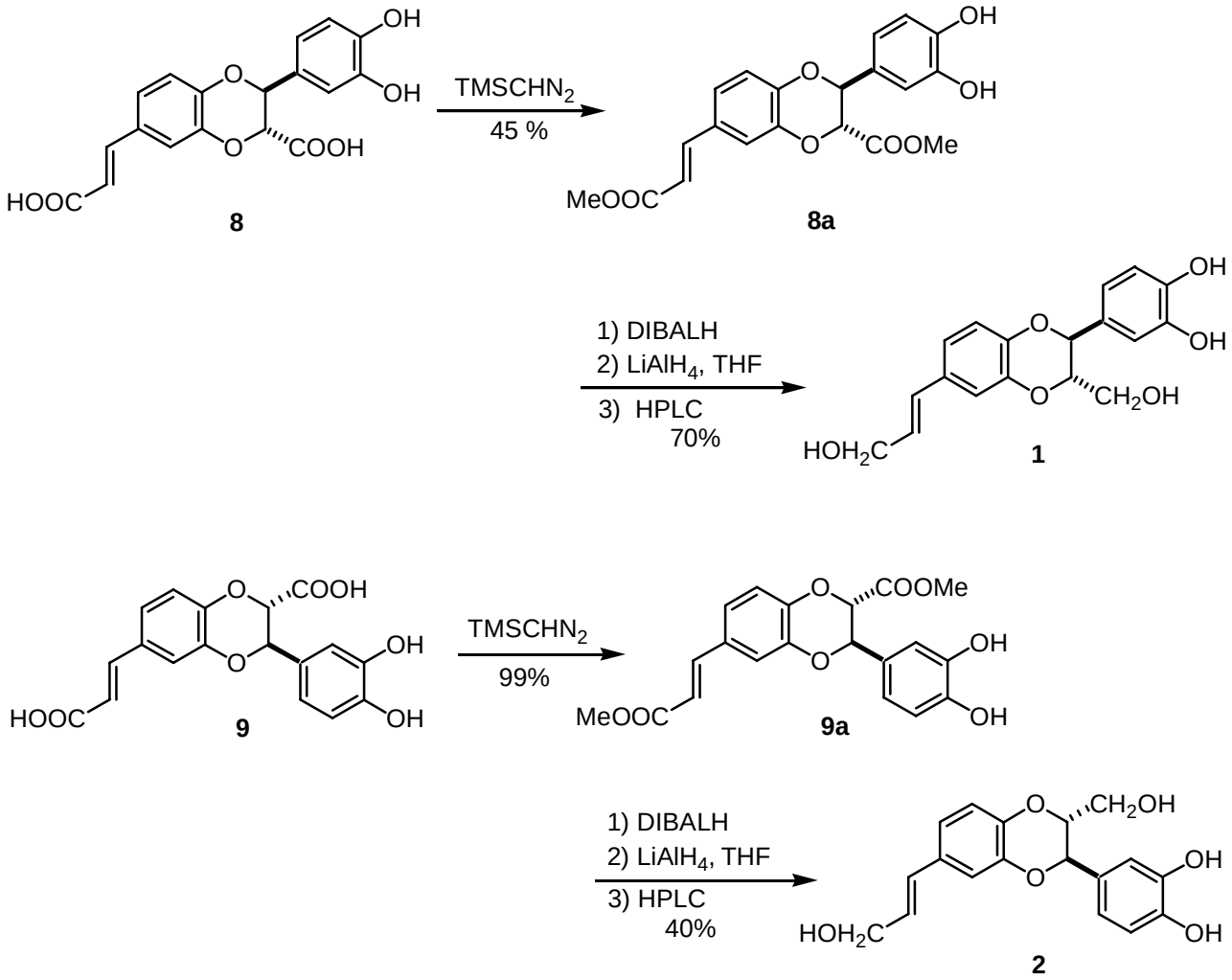
Mechanism for the formation of **10** is proposed as shown in Scheme 2. Radicals (**A**) and (**B**) generated from caffeic acid by HRP catalyzed oxidation dimerize to give a dimer (**C**), to which another caffeic acid



Scheme 2 Postulated Mechanism for the Formation of Compound (**10**)

couple in a Michael-type addition giving rise a trimer (**D**). Subsequently, decarboxylation of the benzylic carboxylic acid in trimer (**D**) presumably occurred to form a quinonemethylide (**F**), which should follow an intramolecular addition of a phenolic hydroxyl group, thereby resulting in the formation of **10** having the 1,4-benzodioxane ring.

Next, compounds (**8**) and (**9**) could be readily transformed to **1** and **2** as follows: treatment of **8** and **9** with $TMSCHN_2$ yielded the corresponding methyl diesters (**8a**) and (**9a**), the conjugated esters of which could be reduced with DIBALH. They were again subjected to $LiAlH_4$ reduction to afford americanol (**1**) and isoamericanol A (**2**) in 30 % and 40% overall yields, respectively.



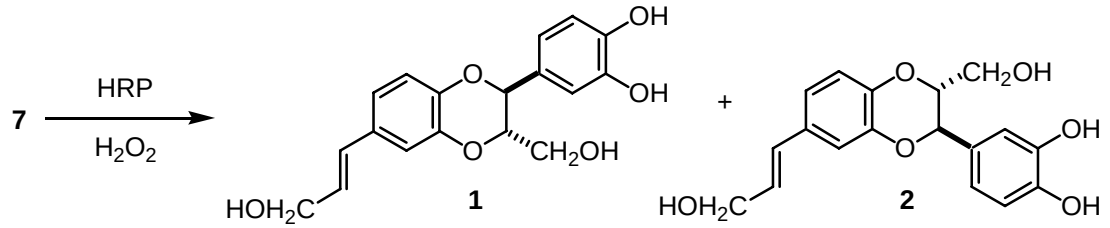
Scheme 3 Syntheses of Americanol A (**1**) and Isoamericanol A (**2**)

Oxidative Coupling of 3,4-Dihydroxy Cinnamyl Alcohol by HRP

Since we succeeded in effective generation of the 1,4-dibenzodioxane-type dimer from caffeic acid, we turned our attention to HRP catalyzed oxidation of 3,4-dihydroxycinnamyl alcohol (**7**) which was regarded as intact precursors of **1** and **2.** Hence, **7** was incubated with HRP under the same reaction

conditions as **5**. It was very pleased to report that HPLC analysis of the resultant reaction mixture indicated the formation of **1** and **2** in 82 % yield but detected no other products.

Purification of the reaction mixture by HPLC gave the major product (**2**) (60 %) and the minor product (**1**) (22 %) which were superimposed on all the spectral data and the retention time of HPLC analysis of natural isoamericanol A (**2**) and americanol A (**1**).



Scheme 4 HRP Catalyzed Oxidative Coupling of **7**

Variation of the experimental conditions such as change of pH-value (4-8) as well as use of organic co-solvents (acetonitrile, acetone and DMSO) did not effect productive ratio of the obtained dimeric compounds (**1**, **2, 8** – **13**). It is interesting to note that the products obtained by HRP catalyzed oxidative coupling of caffeic acid and 3,4-dihydroxycinnamyl alcohol show optical rotations although neither **1** nor **2** isolated from *P. americana* showed optical rotation. With great anticipation of enantioselective oxidative coupling, enantiomeric excesses (ee) of these compounds were determined, but the results were disappointed as shown in Table 2. HRP catalyzed oxidations are likely to proceed in an enantioselective fashion under suitable conditions, but HRP itself is not enzyme to strictly distinguish substrtates.[14] Hence, we should not expect as high chiral induction as other enzymatic methods such as Baker's yeast reduction of ketones[15] and Lipase-mediated hydrolysis of acetates[16] when HRP is used for oxidation of particular substrates.

Table 2 Enantiomeric excess of **1**-**2**, **8**-**9** and **11a**-**12a**.

Compound	% ee[b]
1	1.4
2	4.8
8	8.6
9	14.6
11a	6.4
12a	4.6

[b] Determined by HPLC with Chiral Column

In conclusion, one-step biomimetic syntheses of neurotrophic americanol A (**1**) and isoamericanol A (**2**) have been achieved by HRP catalyzed oxidative phenol coupling of 3,4-dihydroxycinnamyl alcohol. Also, we have shown that HRP has a preference for the 1,4-benzodioxane ring formation, which are not readily accessible using usual oxidative coupling methods.

EXPERIMENTAL

IR spectra were measured on a Jasco FT-IR 5300 infrared spectrophotometer. Optical rotations were measured with a Jasco DIP-1000 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-600, Unity-200 and JEOL ECP-400 instruments. The MS were recorded on a JEOL AX-500 instrument. Preparative HPLC was performed using a COSMOSIL C_{18}AR (ϕ 20 X 250 mm) column and detection at 254 nm. Enantiomeric excess (ee) determination was carried out using HPLC with a Chiralcel column CHIRALCEL OD (ϕ 4.6 X 250 mm) or CHIRALCEL OD-RH (ϕ 4.6 X 150 mm) and detection at 254 nm. CC: Silica gel (Merck, 230 ~ 400 mesh and Wakogel C-300) and Sephadex LH-20 (25 ~ 100 μm, Pharmacia). TLC: precoated silica gel 60 F_{254} (Merck, 0.25 mm) and RP-8 F_{254} (Merck, 0.25 mm). Spots were visualized by UV (254 nm) and $CeSO_4$-H_2SO_4.

Oxidative Coupling of of Caffeic Acid by Horseradish Peroxidase (HRP).

Caffeic acid (1.80 g, 10.0 mmol) was dissolved in 1,4-dioxane (30 mL) and phosphate buffer (0.1 M, pH 6.0, 130 mL) was added. HRP (10,000 unit, type II from SIGMA, USA) in buffer (5 mL) and hydrogen peroxide (0.45 %, 40 mL, 5.3 mmol) were added at rt. The reaction mixture was stirred for 2 h and then quenched with 1M $NaHSO_3$ (3 mL), acidified to pH 3.0 with 1M $KHSO_4$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue (2.21 g) was separated and purified using preparative HPLC (MeCN–H_2O–TFA = 1.0 : 2.5 : 0.3 %, 7.0 mL/min) to give **5** (180 mg), **8** (80 mg), **9** (61 mg), **10** (53 mg), **11** (124 mg), **12**(35 mg) and **13** (14 mg).

8: $[\alpha]_D^{20}$ –1.5 º (*c* 1.17, MeOH); colorless amorphous; ^{1}H NMR (400 MHz, CD_3OD) : δ 4.84 (1H, d, *J* = 5.1 Hz, H-7), 5.16 (1H, d, *J* = 5.1 Hz, H-8), 6.32 (1H, d, *J* = 15.9 Hz, H-8'), 6.74 (2H, br s, H-5 and 6), 6.83 (1H, br s, H-2), 6.96 (1H, d, *J* = 8.5 Hz, H-5'), 7.13 (1H, dd, *J* = 8.5, 1.9 Hz, H-6'), 7.17 (1H, d, *J* = 1.9 Hz, H-2'), 7.60 (1H, d, *J* = 15.9 Hz, H-7'); ^{13}C NMR (100 MHz, CD_3OD) : δ 77.1 (C-7), 77.7 (C-8), 115.3 (C-2), 116.3 (C-5), 117.6 (C-2'), 117.8 (C-8'), 118.5 (C-5'), 120.0 (C-6), 123.4 (C-6'), 128.6 (C-1), 129.9 (C-1'), 144.6 (C-3'), 145.6 (C-7'), 145.9 (C-4'), 146.5 (C-3), 147.0 (C-4), 169.6 (C-9), 170.6 (C-9'); IR (film) : 3300, 1684, 1607, 1507 cm^{-1}; EIMS *m/z* (rel. int.) : 358 (M^+, 9), 314 (68), 191 (100); HRMS (EI) calcd for $C_{18}H_{14}O_8$ 358.0688, found: 358.0668.

9: $[\alpha]_D^{20}$ –1.1 º (*c* 2.20, MeOH); colorless amorphous ; ^{1}H NMR (400 MHz, CD_3OD) : δ 5.00 (1H, d, *J* = 3.3 Hz, H-8), 5.37 (1H, d, *J* =3.3 Hz, H-7), 6.32 (1H, d, *J* = 16.1 Hz, H-8'), 6.71 (1H, s, H-5), 6.72 (1H, d, *J* = 1.8 Hz, H-6), 6.83 (1H, d, *J* = 1.8 Hz, H-2), 7.00 (1H, d, *J* = 8.4 Hz, H-5'), 7.15 (1H, dd, *J* = 8.4, 1.8 Hz, H-6'), 7.17 (1H, d, *J* = 1.8 Hz, H-2'), 7.57 (1H, d, *J* = 16.1 Hz, H-7'); ^{13}C NMR (100 MHz, $CDCl_3$) : δ 76.3 (C-7), 76.6 (C-8), 115.1 (C-2), 116.1 (C-5), 117.5 (C-2',7'), 118.4 (C-5'), 119.7 (C-6), 123.3 (C-6'), 128.1 (C-1), 130.0 (C-1'), 144.8 (C-3'), 145.8 (C-4'), 146.0 (C-8'), 146.2 (C-4), 146.7 (C-3), 170.1 (C-9), 170.6 (C-9'); IR (film) : 3185, 1688, 1607, 1507 cm^{-1}; EIMS *m/z* (rel. int.) : 358 (M^+,

10), 314 (76), 191 (100); HRMS (EI) calcd for $C_{18}H_{14}O_8$ 358.0688, found: 358.0702.

10: $[\alpha]_D^{20}$ 0 º (*c* 1.10, MeOH); colorless amorphous ; ^{1}H NMR (400 MHz, CD_3OD) : δ 4.77 (1H, d, *J* = 8.1 Hz, H-7), 5.18 (1H, d, *J* = 8.1 Hz, H-8), 5.85 (1H, d, *J* = 15.7 Hz, H-8''), 6.35 (1H, d, *J* = 15.7 Hz, H-8'), 6.42 (1H, dd, *J* = 8.1, 1.8 Hz, H-6), 6.46 (1H, d, *J* = 1.8 Hz, H-2), 6.57 (1H, d, *J* = 8.1 Hz, H-5), 6.85 (1H, s, H-5''), 6.89 (1H, s, H-2''), 7.02 (1H, d, *J* = 8.4 Hz, H-5'), 7.17 (1H, dd, *J* = 8.4, 1.8 Hz, H-6'), 7.26 (1H, d, *J* = 1.8 Hz, H-2'), 7.61 (2H, d, *J* = 15.7 Hz, H-7' and H-7''); ^{13}C NMR (100 MHz, CD_3OD) : δ 78.5 (C-8), 81.8 (C-7), 112.4 (C-2''), 114.3 (C-2), 114.6 (C-5), 114.8 (C-5''), 116.1 (C-8'), 116.3 (C-2'), 116.4 (C-8''), 117.2 (C-5'), 118.9 (C-6), 121.9 (C-6'), 125.6 (C-1''), 127.1 (C-1), 127.9 (C-6''), 128.3 (C-1'), 141.4 (C-7''), 144.3 (C-3'), 144.8 (C-4 and 7'), 145.4 (C-3), 145.7 (C-3''), 146.0 (C-4'), 147.7 (C-4''), 170.5 (C-9''), 170.6 (C-9'); IR (film) : 3218, 1684, 1609, 1508 cm^{-1}.

11: $[\alpha]_D^{21}$ +3.7 º (*c* 2.30, MeOH); purple color amorphous; ^{1}H NMR (200 MHz, CD_3OD) : δ 3.84 (2H, br s, H-8 and 8'), 5.68 (2H, br s, H-7 and 7'), 6.70 (2H, dd, *J* = 8.1, 1.8 Hz, H-6 and 6'), 6.78 (2H, d, *J* = 1.8 Hz, H-2 and 2'), 6.79 (2H, d, *J* = 8.1 Hz, H-5 and 5'); ^{13}C NMR (50 MHz, CD_3OD) : δ 49.9, 84.1, 113.7, 116.7, 118.2, 131.3, 147.0, 147.4, 177.4; IR (film) : 3354, 1774, 1612 cm^{-1}.

12: $[\alpha]_D^{20}$ +11.7 º (*c* 0.80, MeOH); colorless amorphous ; ^{1}H NMR (600 MHz, CD_3OD) : δ 3.81 (1H, d, *J* = 2.5 Hz, H-8'), 4.40 (1H, d, *J* = 2.5 Hz, H-7'), 6.37 (1H, dd, *J* = 8.2, 1.9 Hz, H-6'), 6.42 (1H, d, *J* = 1.9 Hz, H-2'), 6.53 (1H, s, H-5), 6.60 (1H, d, *J* = 8.2 Hz, H-5'), 6.85 (1H, s, H-2), 7.56 (1H, s, H-7); ^{13}C NMR (150 MHz, CD_3OD) : δ 46.9 (C-7'), 48.6 (C-8'), 115.7 (C-2'), 116.2 (C-5'), 116.9 (C-2), 117.2 (C-5), 119.8 (C-6'), 123.6 (C-8), 125.2 (C-1), 131.3 (C-6), 136.5 (C-1'), 139.5 (C-7), 144.9 (C-4'), 145.6 (C-3), 146.0 (C-3'), 148.9 (C-4), 170.6 (C-9), 176.4 (C-9'); IR (film) : 3275, 1682, 1606 cm^{-1}.

13: $[\alpha]_D^{20}$ 0 º (*c* 0.75, MeOH); yellow color amorphous ; ^{1}H NMR (600 MHz, CD_3OD) : δ 4.22 (1H, d, *J* = 7.1 Hz, H-8), 5.94 (1H, d, *J* = 7.1 Hz, H-7), 6.25 (1H, d, *J* = 15.9 Hz, H-8'), 6.72 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.75 (1H, d, *J* = 8.2 Hz, H-5), 6.80 (1H, d, *J* = 1.9 Hz, H-2), 7.00 (1H, s, H-2'), 7.12 (1H, s, H-6'), 7.55 (1H, d, *J* = 15.9 Hz, H-7'); ^{13}C NMR (150 MHz, CD_3OD) : δ 57.1 (C-8), 88.7 (C-7), 113.9 (C-2), 116.3 (C-5 and 8'), 117.0 (C-2'), 117.9 (C-6'), 118.6 (C-6), 128.0 (C-5'), 129.7 (C-1'), 133.3 (C-1), 143.0 (C-3'), 146.6 (C-3 and 7'), 146.8 (C-4), 150.6 (C-4'), 170.8 (C-9'), 173.9 (C-9); IR (film) : 3238, 1699, 1610 cm^{-1}.

Methylation of 8.

A solution of **8** (51 mg, 0.14 mmol) in MeOH (1 mL) was added to a $TMSCHN_2$ (2M in hexane, 0.18 mL, 0.36 mmol) at rt for 20 min. After being concentrated *in vacuo*, the residue was purified by HPLC (MeCN-H_2O-TFA : 2 : 1 : 0.3 %; 4.0 mL/min) to give the methyl ester (**8a**) (24 mg, 45 %).

8a: yellow color amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.67 (3H, s), 3.80 (3H, s), 4.70 (1H, d, *J* = 6.2 Hz), 5.12 (1H, d, *J* = 6.2 Hz), 6.30 (1H, d, *J* = 15.9 Hz), 6.82-6.92 (4H, m), 7.10 (1H, dd, *J* = 8.7, 1.6

Hz), 7.13 (1H, br d, *J* = 1.8 Hz), 7.59 (1H, d, *J* = 15.9 Hz); IR (film) : 3421, 1702, 1637, 1585, 1507, 1439, 1273 cm^{-1}; EIMS *m/z* (rel. int.) : 386 (M, 88), 354 (12), 326 (30), 194 (100); EIMS (EI) Calcd for $C_{20}H_{18}O_8$386.1001, found: 386.0972.

Reduction of 8a.

A solution of **8a** (13 mg, 0.03 mmol) in THF (2 mL) was added to a DIBAL (1M in toluene, 0.18 mL, 0.18 mmol) at 0˚C. After being stirred at rt for 13 h, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (15 mg) was dissolved in THF (1.2 mL) and added to a solution of suspension of $LiAlH_4$ (1.8 mg, 0.05 mmol) at 0˚C. After being stirred at 2.5 h, the reaction mixture was diluted with water, and extracted with EtOAc. The combined organic layers were washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the residue, which was purified by HPLC [COSMOSIL 5C_{18}AR-II (ϕ 10 X 250 mm); MeOH:MeCN:H_2O = 2.0 : 2.5 : 5.5; 2.0 mL/min] to afforded **1** (7 mg, 70 %), which was identical in all respects with natural americanol A.

1: mp 124-126 ºC (from EtOAc-acetone); $[\alpha]_D^{20}$ +0.6 º (*c* 2.56, MeOH).

Methylation of 9.

A solution of **9** (45 mg, 0.13 mmol) in MeOH (1 mL) was added to a $TMSCHN_2$ (2M in hexane, 0.18 mL, 0.36 mmol) at rt for 15 min. After being concentrated *in vacuo*, the residue was purified by HPLC (MeCN-H_2O-TFA : 2 : 1 : 0.3 %; 4.0 mL/min) to give the methyl ester (**9a**) (53 mg, 99 %).

9a: white color amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.58 (3H, s), 3.79 (3H, s), 4.96 (1H, br d, *J* = 3.4 Hz), 5.33 (1H, br d, *J* = 3.4 Hz), 6.28 (1H, d, *J* = 16.1 Hz), 6.79-6.84 (4H, m), 7.01 (1H, br d, *J* = 8.4 Hz), 7.12 (1H, br s), 7.57 (1H, d, *J* = 16.1 Hz); EIMS *m/z* (rel. int.) : 386 (M^+, 76), 194 (100); EIMS (HR) Calcd for $C_{20}H_{18}O_8$ 386.1001, found: 386.0975.

Reduction of 9a.

A solution of **9a** (20 mg, 0.05 mmol) in THF (3 mL) was added to DIBAL (1M in toluene, 0.23 mL, 0.23 mmol) at 0˚C. After being stirred at rt for 12 h, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (11 mg) was dissolved in THF (1.2 mL) and added to a solution of $LiAlH_4$ (1.4 mg, 0.04 mmol) in THF (1 mL) at 0˚C. After being stirred at 2.5 h, the reaction mixture was diluted with water, and extracted with EtOAc. The combined organic layers were washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the residue, which was purified by HPLC [COSMOSIL 5C_{18}AR-II (ϕ 10 X 250 mm); MeOH:MeCN:H_2O = 2.0 : 2.5 : 5.5; 2.0 mL/min] to

afforded **2** (7 mg, 40 %), which was identical in all respects with natural isoamericanol A.

2: mp 154-155 ºC (from EtOAc-acetone); $[\alpha]_D^{20}$ +3.2 º (*c* 1.50, MeOH).

Methylation of 10.

A solution of **10** (22 mg, 0.044 mmol) in MeOH (1.5 mL) was added to a $TMSCHN_2$ (2M in hexane, 0.27 mL, 0.54 mmol) at rt for overnight. After being concentrated *in vacuo*, the residue was purified by prep. TLC ($CHCl_3$-MeOH, 49:1) to give the methyl ester (**10a**) (10 mg, 39 %) as a colorless oil.

10a: ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.72 (3H, s, MeO-3), 3.74 (3H, s, MeOOC-9''), 3.81 (6H, s, MeOOC-9' and MeO-4), 3.87 (3H, s, MeO-3''), 3.97 (3H, s, MeO-4''), 4.84 (1H, d, *J* = 8.1 Hz, H-7), 5.30 (1H, d, *J* = 7.7 Hz, H-8), 5.77 (1H, d, *J* = 15.4 Hz, H-8''), 6.35 (1H, d, *J* = 16.1 Hz, H-8'), 6.49 (1H, br d, *J* = 7.7 Hz, H-6), 6.51 (1H, br s, H-2), 6.62 (1H, d, *J* = 8.1 Hz, H-5), 6.78 (1H, s, H-2''), 7.01 (1H, s, H-5''), 7.07 (1H, d, *J* = 8.0 Hz, H-5'), 7.15 (1H, dd, *J* = 8.4, 1.8 Hz, H-6'), 7.29 (1H, d, *J* = 1.8 Hz, H-2'), 7.41 (1H, d, *J* = 15.4 Hz, H-7''), 7.65 (1H, d, *J* = 16.1 Hz, H-7'); ^{13}C NMR (150 MHz, CD_3OD) : δ 51.5 (MeOOC-9''), 51.6 (MeOOC-9'), 55.7 (MeO-3), 55.8 (MeO-4), 55.9 (MeO-3''), 56.2 (MeO-4''), 76.4 (C-8), 80.7 (C-7), 108.4 (C-2''), 109.7 (C-2 and C-5''), 110.7 (C-5), 116.4 (C-8'), 116.7 (C-2'), 117.7 (C-5' and C-8''), 120.0 (C-6), 122.3 (C-6'), 126.9 (C-1''), 127.4 (C-1), 128.3 (C-6''), 128.6 (C-1'), 139.7 (C-7''), 143.9 (C-3'), 144.3 (C-7'), 145.6 (C-4'), 149.0 (C-3), 149.3 (C-3''), 149.5 (C-4), 151.0 (C-4''), 166.5 (C-9''), 167.6 (C-9'); EIMS *m/z* (rel. int.) : 576 (M^+, 50), 384 (100), 371 (30), 341 (30), 325 (63), 151 (50); EIMS (HR) Calcd for $C_{32}H_{32}O_{10}$ 576.1996, found: 576.1977.

Acethylation of 11.

A solution of **11** (11.0 mg, 0.031 mmol) was acetylated with Ac_2O (0.5 mL) and pyridine (0.5 mL) overnight. The usual work-up afforded an acetate (**11a**) (7.5 mg, 46 %) as a colorless oil.

11a: $[\alpha]_D^{20}$ +38.1 º (*c* 0.80, MeOH); yellow color amorphous; ^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.30 (6H, s), 2.31 (6H, s), 3.61 (2H, d, *J* = 2.6 Hz), 5.91 (2H, d, *J* = 2.6 Hz), 7.18 (2H, dd, *J* = 1.8 Hz), 7.23 (2H, d, *J* = 8.4 Hz), 7.25 (2H, dd, *J* = 8.4, 1.8 Hz); IR (film) : 1769, 1213 cm^{-1}; CIMS *m/z* (rel. int.) : 527 ($M+H^+$, 40), 398 (100); HRMS (CI) Calcd for $C_{26}H_{23}O_{12}$ 527.1190, found: 527.1197.

Methylation of 12.

Compound (**12**) (5 mg, 0.016 mmol) was treated with CH_2N_2 at rt overnight. After being concentrated *in vacuo*, the residue was purified by HPLC [COSMOSIL 5C_{18}AR-II (ϕ 10 X 250 mm); MeCN–H_2O = 2 : 1; 1.5 mL/min] to give the methyl ester (**12a**) (2 mg, 24 %).

12a: $[\alpha]_D^{20}$ –1.3 º (*c* 0.20, MeOH); colorless amorphous ; ^{1}H NMR (200 MHz, acetone-d_6) : δ 3.65 (3H, s), 3.76 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.83(3H, s), 3.92(3H, s), 4.00 (1H, d, *J* = 2.6 Hz), 4.65 (1H, d,

J = 2.6 Hz), 6.43 (1H, dd, *J* = 8.4, 2.2 Hz), 6.33 (1H, d, *J* = 2.2 Hz), 6.66 (1H, s), 6.69 (1H, d, *J* = 8.4 Hz), 6.88 (1H, s), 7.68 (1H, s); EIMS *m/z* (rel. int.) : 422 (M^+, 31), 382 (100); EIMS (EI) Calcd for $C_{24}H_{26}O_8$ 422.1628, found: 422.1627.

Methylation of 13.

Compound (**13**) (8 mg, 0.021 mmol) was treated with CH_2N_2 at rt overnight. After being concentrated *in vacuo*, the residue was chromatographed by Sephadex LH-20 (MeOH-$CHCl_3$, 1:1) and HPLC [COSMOSIL 5C_{18}AR-II (ϕ 10 X 250 mm); MeCN:H_2O = 2 : 1; 1.5 mL/min] to give the methyl ester (**13a**) (2 mg, 17 %).

13a: colorless amorphous; ^{1}H NMR (200 MHz, CD_3OD) : δ 3.81 (3H, s), 3.84 (3H, s), 3.87 (3H, s), 3.88 (3H, s), 3.93 (3H, s), 4.35 (1H, d, *J* = 8.2 Hz), 6.13 (1H, d, *J* = 8.2 Hz), 6.32 (1H, d, *J* = 15.9 Hz), 6.85 (1H, d, *J* = 8.2 Hz), 6.87 (1H, s), 6.92 (1H, d, *J* = 1.8 Hz), 6.95 (1H, dd, *J* = 8.2, 1.8 Hz), 7.03 (1H, br s), 7.20 (1H, br s), 7.65 (1H, d, *J* = 15.9 Hz); EIMS *m/z* (rel. int.) : 428 (M^+, 32), 414 (39), 396 (100); HRMS (EI) Calcd for $C_{23}H_{24}O_8$ 428.1413, found: 428.1442.

Oxidative coupling of 3,4-dihydroxycinnamyl alcohol catalyzed by HRP.

3,4-Dihydroxycinnamyl alcohol (85 mg, 0.51 mmol) was dissolved in 1,4-dioxane (5 mL) and phosphate buffer (0.1 M, pH 6.0, 35 mL) was added. HRP (500 unit, type II from SIGMA, USA) in buffer (1 mL) and 3 % hydrogen peroxide (0.6 mL, 0.53 eq) were added. The reaction mixture was stirred for 30 min at rt and then quenched with 1M $NaHSO_3$ (1 mL), acidified to pH 3.0 with 1M $KHSO_4$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue (72 mg) was purified by using HPLC (MeOH:MeCN:H_2O = 2.0 : 2.5 : 5.5) to give **1** (22 %) and **2** (60 %).

1: mp 126-127 ºC; $[\alpha]_D^{22}$ +7.2 º (*c* 1.40, MeOH).

2: mp 155-157 ºC; $[\alpha]_D^{22}$ –16.8 º (*c* 0.3, MeOH).

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