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BIOTRANSFORMATION OF ISOEUGENOL AND EUGENOL BY CULTURED CELLS OF *EUCALYPTUS PERRINIANA**

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Key Word Index—*Eucalyptus perriniana*; Myrtaceae; jar fermentor culture; isoeugenol; eugenol; eugenyl β-glucoside; eugenyl β-rutinoside; eugenyl β-gentiobioside; glucosylation; biotransformation.

Abstract—Three new biotransformation products, eugenyl β-rutinoside, and isoeugenyl β-gentiobioside and β-rutinoside, together with eugenyl β-glucoside and β-gentiobioside, and isoeugenyl β-glucoside, were isolated from jar fermentor culture of *Eucalyptus perriniana* following administration of eugenol and isoeugenol, respectively. This is the first report of rhamnosylation in a biotransformation catalysed by cultured cells of *E. perriniana*.

INTRODUCTION

Biotransformations using cultured plant cells have been the subject of increasing attention [1–3]. As glycosylation is a characteristic biotransformation reaction in plant cells, they may have industrial application as biocatalysts. Many glycosylation reactions of aromatic compounds using cultured plant cells have been reported. The majority of these reactions are monoglycosylations. Glycosylation of two or more sugars is rare.

Eugenol (**1**) is the main component of clove oil and isoeugenol (**2**) occurs in many essential oils, usually with **1**. In dentistry, **1** is used as an antiseptic or a disinfectant. Chemically **2**, an isomerization product of **1** formed by heating the sodium or potassium salts of **1**, is used as the starting material in the manufacture of vanillin.

Previously, we reported that cultured cells of *Eucalyptus perriniana* are able to biotransform the monoterpenes, (−)-menthol [4] and (+)-menthol [5], the diterpene, steviol [6], and the triterpene, 18β-glycyrrhetinic acid [7], into their glucosylated and hydroxylated products. We now report on the isolation and structure elucidation of the biotransformation products of **1** and **2** produced by cultured cells of *E. perriniana* in a jar fermentor.

RESULTS AND DISCUSSION

Eucalyptus perriniana cell suspension was inoculated into a jar fermentor containing 8 l BA1 medium and cultured. The cells were harvested and extracted as described in the Experimental.

The butanol fraction of the cells to which isoeugenol (**2**) was fed contained product **3** (82.2 mg), and mixtures of products **4**+**5** (28.4 mg) and **6**+**7** (964.6 mg). The butanol fraction from the cells fed with eugenol (**1**) contained products **8** (5.0 mg), **9** (8.0 mg) and **10** (858.0 mg). After addition of material recovered from other fractions (see Experimental), the biotransformation yields were as follows: **3**, 3.6%; **4**+**5**, 0.5%; **6**+**7**, 8.2% from **2**. Products **8**, 1.6%; **9**, 0.3%; **10**, 12.9% from **1**, respectively. Products **3**–**10** were not detected on TLC analysis of the shake flask culture of *E. perriniana* to which no substrate was fed and of mixtures of the substrates and the medium that were shaken for 17 days.

The isoeugenol used in this experiment was a *trans*- and *cis*-mixture, and the ratio (*trans*-/*cis*-) was determined as 20:1 by ^{1}H NMR analysis. Product **3** was assigned a M_r of 326 (FAB-MS). In the ^{13}C NMR spectrum (Table 1), 16 carbon signals were observed. Ten carbon signals were assignable to the *trans*-isoeugenol moiety and the remainder to β-glucose. Additionally, in the ^{1}H NMR spectrum, the coupling constant between H-7 and H-8 (J = 15.5 Hz) established that the configuration of the double bond is *trans*. Thus **3** is *trans*-isoeugenyl *O*-β-D-glucopyranoside.

Products **4** and **5** were isolated as a mixture and assigned a M_r of 472 (FAB-MS). The ratio of **4**/**5** was determined as 3:1 by means of the integration data of the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, the sugar parts of the carbon signals of **4** and **5** were identical with each other except for C-1′. Those of the isoeugenol parts were slightly different. The isoeugenol part of **4** was comparable with those of **3**, so that the aglycone part of **4** is *trans*-isoeugenol. In the ^{1}H NMR spectrum, the coupling constant between H-7 and H-8 in the minor product **5** was J = 11.5 Hz, so that the configuration of double bond of **5** was determined as *cis*. To determine the sugar part of **4** and **5**, the major acetylated compound (**4a**) was isolated by HPLC after acetylation. In the ^{1}H NMR spectrum of **4a**, the coupling constants between H-1′ and H-5′ were

*Part 78 in the series 'Studies on Plant Tissue Culture'. For Part 77 see ref. [6].

Table 1. ^{13}C NMR spectral data of products **3**–**10** [in pyridine-d_5 at 75 (**3**–**5**) or 100 MHz (**6**–**10**)]

C	3	4	5	6	7	8	9	10
1	147.3	147.4	146.9	147.4	147.4	146.4	146.5	146.4
2	150.4	150.4	150.4	150.4	150.4	150.2	150.3	150.2
3	110.6	110.5	114.0	110.8	114.5	113.7	113.7	113.6
4	133.0	133.2	132.6	133.2	133.6	134.3	134.7	134.5
5	119.5	119.6	122.4	119.8	122.5	121.3	121.4	121.6
6	116.7	117.4	117.0	117.7	117.2	116.7	117.6	117.4
7	131.4	131.4	130.3	131.4	130.2	40.0	39.9	40.0
8	124.3	124.2	125.8	124.1	125.6	138.3	138.3	138.3
9	18.6	18.6	14.9	18.3	14.7	115.6	115.5	115.6
OMe	56.1	56.1	56.1	56.2	56.2	56.0	56.0	56.0
1′	102.4	102.4	102.9	102.9	102.8	102.5	103.1	102.8
2′	75.0	75.0	75.0	74.9	74.9	75.0	74.9	74.8
3′	78.7	78.8	78.8	78.4[a]	78.4[a]	78.6	78.6	78.4[a]
4′	71.4	71.8	71.8	71.6	71.6	71.3	71.6	71.6
5′	79.0	77.5	77.5	77.7	77.6	78.9	77.3	77.7
6′	62.5	68.2	68.2	67.0	67.0	62.4	68.0	69.7
1″		102.7	102.7	105.4	105.4		102.5	105.4
2″		72.4	72.4	75.3	75.3		72.3	75.3
3″		73.0	73.0	78.4[a]	78.4[a]		72.8	78.4[a]
4″		74.2	74.2	71.3	71.3		74.1	71.1
5″		70.1	70.1	78.3[a]	78.3[a]		69.9	78.3[a]
6″		18.8	18.8	62.8	62.8		18.6	62.7

[a]Assignments may be reversed in each vertical column.

relatively large (J = 8.0–10.0 Hz), and those between H-3″ and H-5″ were J = 10.0 and 9.5 Hz. On the other hand $J_{H\text{-}1'',\,H\text{-}2''}$ and $J_{H\text{-}2'',\,H\text{-}3''}$ were 1.5 Hz and 3.5 Hz, respectively. These data suggested that the configurations of H-1′ to H-5′ and H-3″ to H-5″ are all axial and that H-2″ is equatorial. The configuration of H-1″ was not clear. In other words, it appeared that the first sugar is β-glucose and the second sugar is α- or β-rhamnose. The ^{13}C NMR data for the glucose parts of **4** and **5** were compared with the corresponding data for **3**. C-5′ was 1.5 ppm upfield of the same signal in **3** and C-6′ was 5.7 ppm downfield, so that the second rhamnose is connected at C-6′ of the inner glucose residue. The carbon data of rhamnose were compared with those of methyl-α-L- and methyl β-L-rhamnopyranosides [8]. They agreed well with those of methyl α-L-rhamnopyranoside, so that the sugar part was considered to be β-rutinose. In addition, the carbon data of the sugar part in both **4** and **5** were in good agreement with those of *p*-β-rutinosyloxystyrene [9]. It was concluded that the structures of **4** and **5** are *trans*- and *cis*-isoeugenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (*trans*- and *cis*-isoeugenyl β-rutinoside), respectively.

Products **6** and **7** were isolated as a mixture and assigned a M_r of 488. The ratio of **6** and **7** was determined to be 3:1, i.e. the same as **4** and **5**. In the ^{13}C NMR spectra, the isoeugenol part of **6** and **7** was comparable with that of **4** and **5**, respectively, so that the configuration of the double bond in **6** and **7** was *trans* and *cis*, respectively. The sugar parts were comparable with the data of (−)-menthol β-gentiobioside and their derivatives [4]. From the above results, the structures of **6** and **7** were determined as *trans*- and *cis*-isoeugenyl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (*trans*- and *cis*-isoeugenyl β-gentiobioside), respectively.

The possible pathway for the metabolism of isoeugenol by cultured cells of *E. perriniana* is presented in Fig. 1. It is interesting that only the *trans*-isomer was isolated as a glucoside, and that the ratios of the *trans*- and *cis*-isomers of rutinosides and gentiobiosides were 3:1 in spite of the fact that the ratio of *trans*- and *cis*-isomers of the substrate was 20:1. From these results, it was thought that the glucoside of the *cis*-isomer is the preferred substrate for the addition of the second sugar or that the *cis*-isomer is more toxic for plant cells than the *trans*-isomer.

Product **8** was assigned a M_r of 326 (FAB-MS). In the ^{13}C NMR spectrum, 16 carbon signals were observed. Ten carbon signals were assignable to the eugenol moiety, the remaining six signals were comparable to the sugar part of **3**. Thus **8** is eugenyl *O*-β-D-glucopyranoside.

Products **9** and **10** were assigned M_rs of 472 and 488, respectively (FAB-MS). In the ^{13}C NMR spectrum, carbon signals of the aglycone part in both **9** and **10** were comparable to those of **8**, and the signals of the sugar parts in **9** and **10** were comparable to those of **4** and **6**, respectively. It was concluded that the structures of **9** and **10** are eugenyl *O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (eugenyl β-rutinoside) and eugenyl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (eugenyl β-gentiobioside), respectively. The biotransformation pathway of eugenol by cultured cells of *E. perriniana* is presented in Fig. 2.

Eugenyl glycosides were more widely distributed than isoeugenyl glycosides. Compound **8** has been isolated from many plants, for example, *Chamaecyparies obtusa* [10], *Melissa officinalis* [11], *Pluchea indica* [12] and

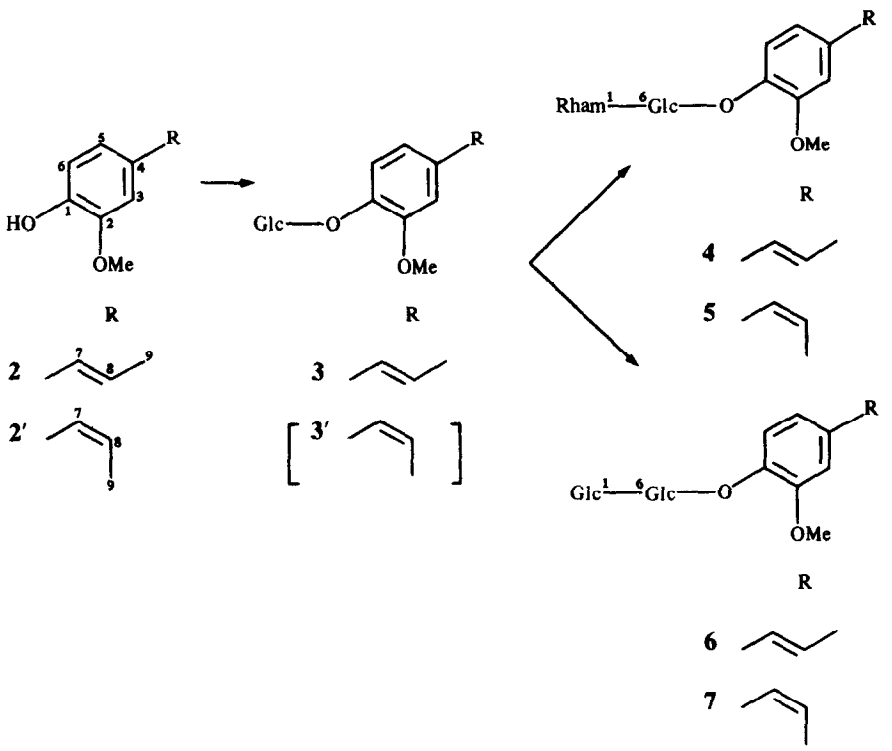

Fig. 1. Biotransformation of isoeugenol (**2**) by cultured cells of *E. perriniana*. *cis* and *trans* ratio of the substrate (**2**/**2'**) was 20:1. Compound **3'** was not isolated.

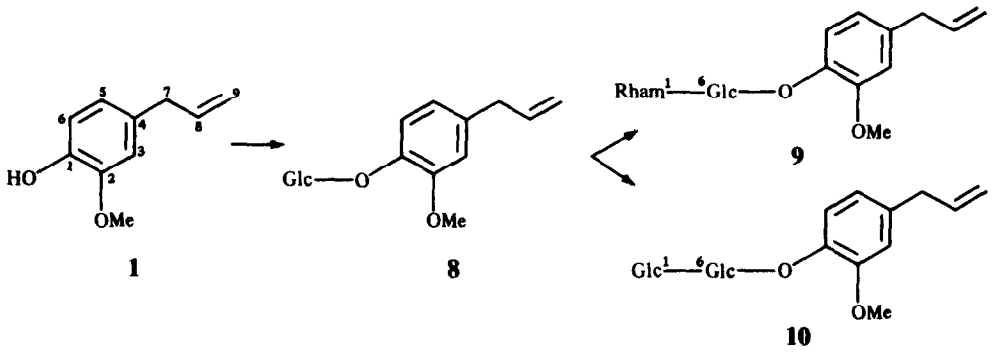

Fig. 2. Biotransformation of eugenol (**1**) by cultured cells of *E. perriniana*.

Prunus cerasus [13]. Compounds **3** and **10** have been isolated from *Lilium cordatum* [14] and Asiasari Radix [15], respectively. Other compounds, **4**–**7** and **9**, are new. The estimation of the biological activity of each of the biotransformation products is now in progress.

EXPERIMENTAL

^{1}H NMR: 300 or 400 MHz, ^{13}C NMR: 75 or 100 MHz.

Cell line. The cells used in this investigation were derived from young stems of *Eucalyptus perriniana* in 1980 and maintained on BA1 agar medium [Murashige and Skoog (MS) medium [16] supplemented with sucrose (30 g l^{-1}), agar (9 g l^{-1}) and 6-benzylaminopurine (1 mg l^{-1})], as previously reported [17].

Feeding experiment in jar fermentor. The *E. perriniana* cell suspension culture was initiated from static cultured cells in 1 l conical flasks each containing 250 ml BA1 liquid medium. The cultures were grown for 3 weeks in a reciprocal shaker (90 strokes min^{-1}) at 25° in the dark. These cell suspensions (4 flasks) were then inoculated into a 10 l jar fermentor containing 8 l BA1 medium. The culture conditions were: aeration; 0.125 vol. vol.$^{-1}$ min^{-1} (VVM), agitation; 50 rpm, culture temp. 25°. After 3 weeks culture, glucose (100 g in 400 ml H_2O) and the substrate [isoeugenol (**2**) or eugenol (**1**), 1 g in 15 ml EtOH] were administered. Additional substrate administrations (×3) were performed at 2 or 3 day intervals (a total of 4 g of substrate was administered). The cells were cultured for an additional 7 days after the last addition. The cells and the medium were separated

by filtration with suction, and the medium passed through a Diaion HP20 column. The column was washed with H_2O and then eluted with MeOH. The MeOH eluate was concd under red. pres. and the residue was partitioned between H_2O and *n*-BuOH (M-HP-fr). The cells were extracted (×2) with MeOH at room temp., and the extract concd under red. pres. The residue was partitioned between EtOAc (C-EA-fr) and H_2O, and the H_2O layer was further extracted (×2) with *n*-BuOH (C-Bu-fr).

Isolation of biotransformation products of compound **2**. The above procedure gave C-EA-fr (6.032 g), C-Bu-fr (13.981 g) and M-HP-fr (679 mg). The C-Bu-fr was chromatographed on silica gel and the biotransformation products detected by UV absorption on TLC (Merck Art. 5715) and purified by HPLC [column: Senshu Pak ODS-4301-N, solvent MeOH–H_2O (1:1)]. Products **3** (82.2 mg), **4**+**5** (28.4 mg) and **6**+**7** (964.6 mg) were obtained. Quantitative analyses of C-EA-fr and M-HP-fr were performed by using the same HPLC system. C-EA-fr: **3**, 142.8 mg; **4**+**5**, 14.3 mg; **6**+**7**, 5.6 mg; M-HP-fr: **3**, 63.4 mg; **4**+**5**, 15.4 mg; **6**+**7**, 7.8 mg.

trans-*Isoeugenyl β-glucoside* (**3**). Amorphous solid, $[\alpha]_D^{27}$ −60° (MeOH; *c* 1.0); UV λ_{max}^{MeOH} nm (log ε): 255.9 (4.18); ^{1}H NMR (pyridine-d_5): δ1.64 (3H, *dd*, *J* = 6.5, 1.5 Hz, H-9), 3.65 (3H, *s*, OMe), 4.00 (1H, *m*, H-5′), 4.26 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-6′a), 4.41 (1H, *dd*, *J* = 12.0, 2.5 Hz, H-6′b), 5.56 (1H, *d*, *J* = 7.0 Hz, H-1′), 6.10 (1H, *dq*, *J* = 15.5, 6.5 Hz, H-8), 6.26 (1H, *dd*, *J* = 15.5, 1.5 Hz, H-7), 6.85 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-5), 7.00 (1H, *d*, *J* = 2.0 Hz, H-3), 7.42 (1H, *d*, *J* = 8.0 Hz, H-6); FAB-MS *m/z* 349 $[M+Na]^+$.

trans- *and* cis-*Isoeugenyl β-rutinoside* (**4**+**5**). Amorphous solid, $[\alpha]_D^{28}$ −59° (MeOH; *c* 1.05); UV λ_{max}^{MeOH} nm (log ε): 256.4 (4.19); ^{1}H NMR (pyridine-d_5): δ1.47 [3/4H, *d*, *J* = 6.0 Hz, H-6″ (*cis*)], 1.48 [9/4H, *d*, *J* = 6.0 Hz, H-6″ (*trans*)], 1.59 [9/4H, *dd*, *J* = 6.5, 1.0 Hz, H-9 (*trans*)], 1.73 [3/4H, *dd*, *J* = 7.0, 1.5 Hz, H-9 (*cis*)], 3.58 [3/4H, *s*, OMe (*cis*)], 3.61 [9/4H, *s*, OMe (*trans*)], 5.33 (1H, *br s*, H-1″), 5.40 [3/4H, *br d*, *J* = 7.0 Hz, H-1′ (*trans*)], 5.55 [1/4H, *br d*, *J* = 7.5 Hz, H-1′ (*cis*)], 5.55 [1/4H, *dq*, *J* = 11.5, 7.0 Hz, H-8 (*cis*)], 5.96 [3/4H, *dq*, *J* = 15.5, 6.5 Hz, H-8 (*trans*)], 6.17 [3/4H, *dd*, *J* = 15.5, 1.0 Hz, H-7 (*trans*)], 6.29 [1/4H, *dd*, *J* = 11.5, 1.5 Hz, H-7 (*cis*)], 6.84 [1/4H, *d*, *J* = 1.5 Hz, H-3 (*cis*)], 6.94 [3/4H, *d*, *J* = 1.5 Hz, H-3 (*trans*)], 7.00 [1/4H, *dd*, *J* = 8.0, 1.5 Hz, H-5 (*cis*)], 7.01 [3/4H, *dd*, *J* = 8.0, 1.5 Hz, H-5 (*trans*)], 7.52 [3/4H, *d*, *J* = 8.0 Hz, H-6 (*trans*)], 7.54 [1/4H, *d*, *J* = 8.0 Hz, H-6 (*cis*)]; FAB-MS *m/z* 495 $[M+Na]^+$.

trans- *and* cis-*Isoeugenyl β-gentiobioside* (**6**+**7**). Amorphous solid, $[\alpha]_D^{27}$ −76° (MeOH; *c* 0.55); UV λ_{max}^{MeOH} nm (log ε): 255.6 (4.19); ^{1}H NMR (pyridine-d_5). δ1.73 [9/4H, *dd*, *J* = 6.5, 1.5 Hz, H-9 (*trans*)], 1.83 [3/4H, *dd*, *J* = 7.0, 2.0 Hz, H-9 (*cis*)], 3.74 [3/4H, *s*, OMe (*cis*)], 3.76 [9/4H, *s*, OMe (*trans*)], 3.82 (1H, *ddd*, *J* = 9.0, 5.0, 2.5 Hz, H-5″), 4.00 (1H, *dd*, *J* = 8.5, 8.0 Hz, H-2″), 4.30 (1H, *dd*, *J* = 11.5, 5.5 Hz, H-6″b), 4.31 (1H, *dd*, *J* = 11.5, 6.0 Hz, H-6′b), 4.42 (1H, *dd*, *J* = 11.5, 2.5 Hz, H-6″a), 4.74 (1H, *br d*, *J* = 11.5 Hz, H-6′a), 5.02 (1H, *d*, *J* = 8.0 Hz, H-1″), 5.50 [3/4H, *d*, *J* = 7.5 Hz, H-1′ (*trans*)], 5.52 [1/4H, *d*, *J* = 7.5 Hz, H-1′ (*cis*)], 5.68 [1/4H, *dq*, *J* = 11.5, 7.0 Hz, H-8 (*cis*)], 6.08 [3/4H, *dq*, *J* = 15.5, 6.5 Hz, H-8 (*trans*)], 6.32 [3/4H, *dd*, *J* = 15.5, 1.5 Hz, H-7 (*trans*)], 6.42 [1/4H, *dd*, *J* = 11.5, 2.0 Hz, H-7 (*cis*)], 6.89 [1/4H, *d*, *J* = 2.0 Hz, H-3 (*cis*)], 7.06 [3/4H, *d*, *J* = 2.0 Hz, H-3 (*trans*)], 7.07 [1/4H, *dd*, *J* = 8.0, 2.0 Hz, H-5 (*cis*)], 7.09 [3/4H, *dd*, *J* = 8.0, 2.0 Hz, H-5 (*trans*)], 7.68 [3/4H, *d*, *J* = 8.0 Hz, H-6 (*trans*)], 7.70 [1/4H, *d*, *J* = 8.0 Hz, H-6 (*cis*)]; FAB-MS *m/z* 511 $[M+Na]^+$.

Acetylation of compound **4**. The mixture of compounds **4** and **5** was acetylated with Ac_2O and pyridine. The reaction mixture was evapd and purified by HPLC [C_{18}; MeOH–H_2O (9:1)] to obtain acetate **4a**.

trans-*Isoeugenyl β-rutinoside hexaacetate* (**4a**). Amorphous solid, ^{1}H NMR ($CDCl_3$): δ1.21 (3H, *d*, *J* = 6.0 Hz, H-6″), 1.64, 1.67 (each 3H, *s*, Ac), 1.70 (3H, *dd*, *J* = 6.5, 1.5 Hz, H-9), 1.73, 1.76, 1.78, 1.85 (each 3H, *s*, Ac), 3.34 (1H, *ddd*, *J* = 10.0, 7.0, 3.0 Hz, H-5′), 3.42 (3H, *s*, OMe), 3.48 (1H, *dd*, *J* = 11.5, 7.0 Hz, H-6′a), 3.58 (1H, *dd*, *J* = 11.5, 3.0 Hz, H-6′b), 3.96 (1H, *dq*, J = 9.5, 6.0 Hz, H-5″), 4.76 (1H, *d*, *J* = 1.5 Hz, H-1″), 4.88 (1H, *d*, *J* = 8.0 Hz, H-1′), 5.05 (1H, *dd*, *J* = 10.0, 9.5 Hz, H-4′), 5.46 (1H, *dd*, *J* = 9.5, 9.5 Hz, H-3′), 5.48 (1H, *dd*, *J* = 10.0, 9.5 Hz, H-4″), 5.57 (1H, *dd*, *J* = 9.5, 8.0 Hz, H-2′), 5.67 (1H, *dd*, *J* = 10.0, 3.5 Hz, H-3″), 5.69 (1H, *dd*, *J* = 3.5, 1.5 Hz, H-2″), 6.08 (1H, *dq*, *J* = 15.5, 6.5 Hz, H-8), 6.33 (1H, *dd*, *J* = 15.5, 1.5 Hz, H-7), 6.80 (1H, *br s*, H-3), 7.08–7.24 (2H, *m*, H-5 and H-6).

Isolation of biotransformation products of eugenol. The fractionation procedure described above gave C-EA-fr (4.392 g), C-Bu-fr and M-HP-fr (616 mg). The C-Bu-fr was passed through a Diaion HP20 column and eluted with 50% MeOH [C-Bu-HP(50)-fr] and MeOH. The MeOH eluate was chromatographed on silica gel and further purified by HPLC, to give products **8** (7.0 mg), **9** (8.0 mg) and **10** (858.0 mg). Quantitative analyses of other fractions was also performed. C-EA-fr: **8**, 74.6 mg; **9**, 0.9 mg; **10**, 4.4 mg; M-HP-fr: **8**, 3.0 mg; **9**, 5.4 mg; **10**, not detected; C-Bu-HP(50)-fr: **8**, 45.1 mg; **9**, 21.1 mg; **10**, 673.3 mg.

Eugenyl β-glucoside (**8**). Amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 276.2 (3.38); ^{1}H NMR (pyridine-d_5): δ3.32 (2H, *d*, *J* = 6.5 Hz, H-7), 3.73 (3H, *s*, OMe), 4.10 (1H, *ddd*, *J* = 7.5, 5.0, 2.0 Hz, H-5′), 4.39 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-6′a), 4.54 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-6′b), 5.07 (1H, *ddt*, *J* = 10.0, 2.0, 1.0 Hz, H-9a), 5.10 (1H, *ddt*, *J* = 17.0, 2.0, 1.5 Hz, H-9b), 5.76 (1H, *d*, *J* = 6.0 Hz, H-1′), 6.00 (1H, *ddt*, *J* = 17.0, 10.0, 6.5 Hz, H-8), 6.77 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-5), 6.90 (1H, *d*, *J* = 2.0 Hz, H-3), 7.53 (1H, *d*, *J* = 8.0 Hz, H-6); FAB-MS *m/z* 349 $[M+Na]^+$.

Eugenyl β-rutinoside (**9**). Amorphous solid, UV λ_{max}^{MeOH} nm (log ε): 277.1 (3.40); ^{1}H NMR (pyridine-d_5): δ1.60 (3H, *d*, *J* = 6.0 Hz, H-6″), 3.27 (2H, *d*, *J* = 6.5 Hz, H-7), 3.70 (3H, *s*, OMe), 4.95 (1H, *s*, H-1″), 5.04 (1H, *ddt*, *J* = 10.0, 2.0, 1.0 Hz, H-9a), 5.07, (1H, *ddt*, *J* = 17.0, 2.0, 1.5 Hz, H-9b), 5.51 (1H, *d*, *J* = 7.5 Hz, H-1′), 5.90 (1H, *ddt*, *J* = 17.0, 10.0, 6.5 Hz, H-8), 6.84 (1H, *d*, *J* = 2.0 Hz, H-3), 6.91 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-5), 7.62 (1H, *d*, *J* = 8.0 Hz, H-6); FAB-MS *m/z* 495 $[M+Na]^+$.

Eugenyl β-gentiobioside (**10**). Amorphous solid, $[\alpha]_D^{28}$ −44° (pyridine; *c* 1.11); UV λ_{max}^{MeOH} nm (log ε): 276.0 (3.40); ^{1}H NMR (pyridine-d_5): δ3.30 (2H, *d*, *J* = 6.5 Hz, H-7), 3.73 (3H, *s*, OMe), 3.85 (1H, *ddd*, *J* = 9.0, 5.0, 2.5 Hz, H-5″), 4.06 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-2″), 4.35 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-6″b), 4.37 (1H, *dd*, *J* = 11.5, 5.0 Hz, H-6′b), 4.49 (1H, *dd*, *J* = 12.0, 2.5 Hz, H-6″a), 4.78 (1H, *dd*, *J* = 11.5, 1.5 Hz, H-6′a), 5.04 (1H, *ddt*, *J* = 10.0, 2.0, 1.0 Hz, H-9a), 5.07 (1H, *ddt*, *J* = 17.0, 2.0, 1.5 Hz, H-9b), 5.09 (1H, *d*, *J* = 8.0 Hz, H-1″), 5.58 (1H, *d*, *J* = 6.5 Hz, H-1′), 6.00 (1H, *ddt*, *J* = 17.0, 10.0, 6.5 Hz, H-8), 6.88 (1H, *d*, *J* = 2.0 Hz, H-3), 6.92 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-5), 7.67 (1H, *d*, *J* = 8.0 Hz, H-6); FAB-MS *m/z* 511 $[M+Na]^+$.

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