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ORIGINAL ARTICLE

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Stereochemistry and biosynthesis of 8-*O*-4′ neolignans in *Eucommia ulmoides*: diastereoselective formation of guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether

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Abstract Stereochemistry and biosynthesis of guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether (GGSE), an 8-*O*-4′ neolignan, which consists of coniferyl and sinapyl alcohol moieties, in *Eucommia ulmoides* were investigated. Four 8-*O*-4′ neolignans, GGSE, syringylglycerol-8-*O*-4′-(coniferyl alcohol) ether (SGCE), guaiacylglycerol-8-*O*-4′-(coniferyl alcohol) ether (GGCE), and syringylglycerol-8-*O*-4′-(sinapyl alcohol) ether (SGSE), were synthesized. Their *erythro* and *threo* diastereomers were separated through acetonide derivatives, intermediates of the synthesis, and identified by means of nuclear magnetic resonance (NMR) spectroscopy. All of the *erythro*-acetonide derivatives have larger coupling constants (ca 9 Hz) for the C_7-H resonances than those of the *threo* ones (1.5–2 Hz). In the case of the four 8-*O*-4′ neolignans, the C_7-H coupling constants of the *threo*-isomers are not smaller than those of the *erythro* ones. GGSE isolated previously from this plant was identified as the *erythro* isomer by comparison of the ^{13}C-NMR data with synthetic *erythro*-GGSE and *threo*-GGSE and the other 8-*O*-4′ neolignans mentioned as above. Administration of a mixture of [8-^{14}C]coniferyl alcohol and [8-^{14}C]sinapyl alcohol to excised shoots of *E. ulmoides* was carried out and the incorporation of ^{14}C into *erythro*-[^{14}C]GGSE was found to be higher than that in *threo*-[^{14}C]GGSE. The occurrence of diastereoselective formation of *erythro*-GGSE by cross coupling of coniferyl and sinapyl alcohols is suggested.

Key words *Eucommia ulmoides* · Neolignan · Diastereomer · Cross coupling · Biosynthesis

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Introduction

Lignans and neolignans have been isolated and identified from many higher plants. However, many articles about 8-*O*-4′ neolignans have not described the identification of diastereomers.[1,2] There was confusion about *cis* and *trans* forms of the benzofuran ring in naturally occurring 8-5′ neolignans in some studies because the 8-5′ neolignans were reported as *cis* forms without strict evidence.[3] In addition, biosynthesis of neolignans, especially 8-*O*-4′ neolignans has hardly been studied. *Eucommia ulmoides* Oliver (Eucommiaceae), Tochu in Japanese (Du-Zhong in Chinese), is a medicinal woody plant whose bark is used as a crude drug in China and whose leaves are used as tea in Japan. Recently, it was reported that this plant has pharmacological effects on coronary blood flow, pain relief, diuresis, blood pressure, and lipid metabolism and other bioactivities.[4,5] Many bioactive compounds including lignans and neolignans are present in *E. ulmoides*.[1,6] Deyama and coworkers[1] isolated citrusin B, guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether (GGSE) 4-*O*-glucoside, from this plant, and was identified by its hydrolysis and subsequent spectrometric analysis of the resulting (+)-GGSE. However, its diastereomer, the *erythro* or *threo* isomer, has not yet been clarified. Citrusin B was first isolated from peels of *Citrus hassaku* Hort. and *Citrus sinensis* Osbeck. by Sawabe and others[2] with citrusin A [guaiacylglycerol-8-*O*-4′-(coniferyl alcohol) ether (GGCE) 4-*O*-glucoside]. However, the stereochemistries of citrusin A and B have not been identified. Recently, Katayama and Kado[7] discovered that incubation of cell-free extracts from *E. ulmoides* with coniferyl alcohol in the presence of hydrogen peroxide gave (+)-*erythro*- and (−)-*threo*-GGCE (diastereomeric ratio, 3:2). This is the first report on enzymatic formation of optically active 8-*O*-4′ neolignans from an achiral monolignol. However, few biosynthetic studies on syringyl 8-*O*-4′ neolignans or cross-coupling products of two heteromonolignols (coniferyl and sinapyl alcohols) have been reported.

In this article, to clarify the stereochemistry and biosynthetic pathway of GGSE, (an 8-*O*-4′ neolignan consisting of

coniferyl alcohol and sinapyl alcohol moieties), chemical synthesis of GGSE and its related neolignans, syringylglycerol-8-*O*-4′-(coniferyl alcohol) ether (SGCE), GGCE, and syringylglycerol-8-*O*-4′-(sinapyl alcohol) ether (SGSE), was done, and their *erythro* and *threo* isomers were obtained separately and identified by means of nuclear magnetic resonance (NMR) spectroscopy during the chemical synthesis. Labeled precursors were then administered to excised young shoots of *E. ulmoides* and diastereoselective formation of GGSE was observed.

Experimental

Instrumentation and chromatography materials

All reagents and solvents were reagent grade. Column chromatography was performed on the FMI high-performance low-to-medium pressure chromatograph equipped with a column of Merck silica gel 60 (230–400 ASTM mesh). Analytical and preparative thin-layer chromatographies (TLC) were done by using plates precoated with Merck silica gel 60 F254 (0.25-mm and 0.5-mm thickness, respectively). NMR spectra (400 MHz) were determined on a JNM Alpha 400 FT-NMR spectrometer. Mass spectra were taken on a JMS-SX102A mass spectrometer with electron impact ionization (EI-MS, 70 eV). Analytical HPLC was carried out on a Hitachi L-6200 equipped with a Hitachi L-4200 UV/Vis detector (280 nm) and a Shimadzu chromatopac C-R7A plus using a reversed phase column (Waters, Nova-Pak C_{18}, 150 × 3.9 mm, stainless steel, with guard pack insert Nova-Pak C_{18}). Compounds were separated at a flow rate of 1 ml/min using the following linear gradient solvent system: methanol (MeOH)/3% acetic acid (AcOH) in H_2O (v/v); at 0 min, 25 : 75; at 10 min, held at 25 : 75; at 15 min, 32 : 68 and at 40 min, held at 32 : 68. Radioactive samples were analyzed in liquid scintillation cocktail consisting of scintiblender-II/toluene/polyethylene glycol mono-*p*-isooctylphenyl ether (6/54/40; v/v/v) (Nacalai Tesque) and measured using a liquid scintillation counter (LSC-1000, Aloka).

Chemical syntheses of guiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether

Sinapaldehyde was synthesized by the method of Tachibana et al.[8]

1-(4-Benzoyloxy-3-methoxyphenyl)-2-[4-(2-formylvinyl)-2, 6-dimethoxyphenoxy]ethanone (**1**, step a). To a stirred solution of sinapaldehyde (80.1 mg, 0.385 mmol) and 1-(4-benzoyloxy-3-methoxyphenyl)-2-bromoethanone (135.6 mg, 0.388 mmol) in 4 ml of *N*,*N*-dimethylformamide (DMF), powdered K_2CO_3 (53.8 mg, 0.389 mmol) and powdered KI (31.9 mg, 0.192 mmol) were added. The mixture was stirred at room temperature under nitrogen atmosphere for 5 h. The reaction mixture was filtered and the inorganic salts were washed with diethyl ether (Et_2O). The filtrate and washings were combined, and partitioned between Et_2O and H_2O. The organic layer was washed with H_2O and then saturated brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give a syrup containing compound **1** (180.1 mg, 98.3%) which was used for the next reaction without further purification. EI-MS *m/z* (%): 476 (49.9) $[M]^+$, 372 (8.2), 255 (14.2), 207 (40.7), 105 (100), 77 (33.7).

1-(4-Benzoyloxy-3-methoxyphenyl)-2-[4-(2-formylvinyl)-2,6-dimethoxyphenoxy]-3-hydroxy-1-propanone (**2**, step b). To a stirred solution of compound **1** (171.4 mg, 0.360 mmol) in 5 ml of dimethyl sulfoxide (DMSO), powdered (80%) paraformaldehyde (14.3 mg, 0.476 mmol) and powdered K_2CO_3 (16.4 mg, 0.119 mmol) were added. The reaction mixture was stirred for 7 h 30 min under nitrogen atmosphere at ambient temperature. The mixture was filtered and the precipitate was washed with 10 ml of ethyl acetate (EtOAc). The filtrate and washings were combined and then partitioned between EtOAc and H_2O (10 ml). The organic layer was washed with H_2O and then saturated brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC (EtOAc/*n*-hexane, 3 : 2) to give crude crystals of compound **2** (56.2 mg, 30.8%). ^{1}H-NMR δ ($CDCl_3$): 3.71 (1H, dd, J = 9.39, 4.27, 9-OH), 3.80 (6H, s, B-OCH_3), 3.89 (3H, s, A-OCH_3), 3.90 (1H, ddd, J = 12.44, 9.39, 3.17, H-9a), 4.05 (1H, ddd, J = 12.08, 7.69, 4.27, H-9b), 5.22 (1H, dd, J = 7.56, 3.17, H-8), 6.64 (1H, dd, J = 15.86, 7.81, H-8′), 6.80 (2H, s, H-2′, H-6′), 7.26 (1H, d, J = 8.29, H-5), 7.40 (1H, d, J = 15.86, H-7′), 7.53 (2H, br t, J = 7.69, BzH-3, BzH-5), 7.66 (1H, tt, J = 7.45, 1.50, BzH-4), 7.71 (1H, dd, J = 8.29, 1.95, H-6), 7.77 (1H, d, J = 1.95, H-2), 8.21 (2H, br dd, J = 8.42, 1.35, BzH-2, BzH-6), 9.69 (1H, d, J = 7.56, H-9′). EI-MS *m/z* (%): 506 (0.4) $[M]^+$, 476 (9.6), 105 (100), 77 (33.7).

1-(4-Benzoyloxy-3-methoxyphenyl)-2-[4-(3,3-dimethoxy-1-propenyl)-2,6-dimethoxyphenoxy]-3-hydroxy-1-propanone (**3**, step c). To a stirred solution of **2** in a mixed solution of tetrahydrofuran (THF) (1 ml) and MeOH (0.5 ml), methyl orthoformate (121 μl, 1.110 mmol, d_4^{25} = 0.97) and a solution of *p*-toluenesulfonic acid (316 μg, 1.664 μmol) in MeOH (0.1 ml) were added. The reaction solution was stirred under nitrogen atmosphere at ambient temperature for 5 min, and then neutralized by the addition of powdered $NaHCO_3$. The mixture was stirred for 3 min and filtered. The filtrate was partitioned between EtOAc and brine, the combined EtOAc solution was washed twice with saturated brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to afford a syrup containing **3** (56.2 mg, 91.7%) which was used for the next step without further purification.

1-(4-Benzoyloxy-3-methoxyphenyl)-2-[4-(3,3-dimethoxy-1-propenyl)-2,6-dimethoxyphenoxy]-1,3- propanediol (**4**, step d). Crude **3** was dissolved in a mixed solution of THF (0.5 ml) and MeOH (0.5 ml). The solution was cooled to 0°C. To the stirred cold solution, $NaBH_4$ (28.7 mg, 0.759 mmol) was added under nitrogen atmosphere and stirred for 10 min. The reaction mixture was extracted with EtOAc three times. The combined EtOAc solution was

washed twice with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo to yield a syrup containing **4** (50.4 mg, 89.4%). This material was used in the next step without further purification.

Guaiacylglycerol-8-*O*-4′-sinapaldehyde ether 4-benzoate (**5**, step e). Crude **4** was dissolved in 90% AcOH in H_2O (1 ml) at room temperature. The solution was stirred for 10 min, and then extracted three times with EtOAc. The combined EtOAc solution was washed twice with saturated brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by preparative TLC developed with 2% MeOH in CH_2Cl_2 (×3), giving a high R_f band (major) and a low R_f band (minor) which were identified as *threo*-**5** (17.7 mg, 38.3%) and *erythro*-**5** (3.8 mg, 8.2%) as syrups, respectively. *Threo*-**5**: ^{1}H-NMR δ ($CDCl_3$): 3.18 (1H, br, 9-OH), 3.45 (1H, br d, J = 13.66, H-9a), 3.63 (1H, dd, J = 12.93, 2.93, H-9b), 3.83 (3H, s, A-OCH_3), 3.95 (6H, s, B-OCH_3), 4.03 (1H, dt, J = 8.29, 2.44, H-8), 4.19 (1H, br, 7-OH), 5.18 (1H, d, J = 8.05, H-7), 6.66 (1H, dd, J = 15.83, 7.56, H-8′), 6.84 (2H, s, H-2′, H-6′), 7.10 (1H, dd, J = 8.06, 1.70, H-6), 7.14 (1H, d, J = 8.04, H-5), 7.16 (1H, d, J = 1.72, H-2), 7.41 (1H, d, J = 15.87, H-7′), 7.51 (2H, br t, J = 7.56, BzH-3, BzH-5), 7.63 (1H, tt, J = 7.44, 1.55, BzH-4), 8.21 (2H, br dd, J = 8.06, 1.46, BzH-2, BzH-6), 9.70 (1H, d, J = 7.56, H-9′). EI-MS *m/z* (%): 508 (3.9) $[M]^+$, 476 (0.5), 405 (6.6), 300 (0.2), 105 (100), 77 (26.2). *Erythro*-**5**: ^{1}H-NMR δ ($CDCl_3$): 3.08 (1H, br, 9-OH), 3.58 (1H, dd, J = 12.32, 2.08, H-9a), 3.83 (3H, s, A-OCH_3), 3.94 (6H, s, B-OCH_3), 3.96 (1H, dd, J = 13.18, 6.10, H-9b), 3.95–3.99 (1H, o, 7-OH), 4.28 (1H, m, H-8), 5.12 (1H, d, J = 3.91, H-7), 6.68 (1H, dd, J = 15.86, 7.57, H-8′), 6.86 (2H, s, H-2′, H-6′), 6.92 (1H, dd, J = 8.17, 1.83, H-6), 7.12 (1H, d, J = 8.05, H-5), 7.13 (1H, d, J = 2.20, H-2), 7.43 (1H, d, J = 15.86, H-7′), 7.51 (2H, t, J = 7.81, BzH-3, BzH-5), 7.63 (1H, tt, J = 7.32, 1.42, BzH-4), 8.20 (2H, dd, J = 8.17, 1.34, BzH-2, BzH-6), 9.71 (1H, d, J = 7.81, H-9′).

Guaiacylglycerol-8-*O*-4′-sinapaldehyde ether 4-benzoate 7,9-*O*-isopropylideneketal (acetonide derivative, A-GGSE, **6**) (step f). To a stirred solution of *threo*-**5** (17.7 mg, 0.035 mmol) in 1 ml of acetone, 2,2-dimethoxypropane (341 μl, 2.78 mmol) and a solution of camphorsulfonic acid (CSA) (0.485 mg, 0.002 mmol) in acetone were added. The reaction solution was stirred at ambient temperature for 4 h, and then neutralized by the addition of powdered $NaHCO_3$. The mixture was stirred for 3 min and filtered. The filtrate was partitioned between EtOAc and brine. The aqueous layer was extracted with EtOAc. The combined EtOAc solution was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC (EtOAc/*n*-hexane = 1:1, ×2) to afford *threo*-**6** (16.8 mg, 88%). Similarly, *erythro*-**5** was transformed into *erythro*-**6** (3 mg, 74%). *Threo*-**6**: Data of ^{1}H-NMR δ ($CDCl_3$) were shown in Table 1 and as follows (acetonide methyl and benzoyl groups): 1.59 (3H, s, C-CH_3), 1.65 (3H, s, C-CH_3), 7.50 (1H, t, J = 7.57, BzH-3, BzH-5), 7.62 (1H, tt, J = 7.44, 1.55, BzH-4), 8.20 (2H, dd, J = 8.42, 1.35, BzH-2, BzH-6). EI-MS *m/z* (%): 548 (4.9) $[M]^+$, 460 (0.5), 355 (0.5), 341 (2), 234 (82), 207 (13.7), 105 (100), 77 (22). *Erythro*-**6**: Data of ^{1}H-NMR δ ($CDCl_3$) were shown in Table 1 and as follows (acetonide methyl and benzoyl groups): 1.50 (3H, s, C-CH_3), 1.67 (3H, s, C-CH_3), 7.50 (2H, t, J = 7.93, BzH-3, BzH-5), 7.63 (1H, tt, J = 7.44, 1.42, BzH-4), 8.19 (2H, dd, J = 8.06, 0.98, BzH-2, BzH-6).

Guaiacylglycerol-8-*O*-4′-sinapaldehyde ether 4-benzoate (**5**, step g). *Threo*-**6** (10.8 mg) was dissolved in 90% AcOH in H_2O (1 ml), and the reaction solution was stirred for 4 h at room temperature and then extracted three times with EtOAc. The combined EtOAc solution was washed twice with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo to give a syrup of crude *threo*-**5** (8.4 mg, 83.9%). This material was used for the next step without further purification. *Erythro*-**5** (1.9 mg, 73%) was also obtained from *erythro*-**6** (2.8 mg) by a similar procedure.

Guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether 4-benzoate (**7**, step h). Crude *threo*-**5** (7 mg, 0.014 mmol) was dissolved in a mixed solvent of THF (0.5 ml) and MeOH (0.5 ml) and the resulting solution was cooled to 0°C. To the stirred cold solution, $NaBH_4$ (15.8 mg, 0.418 mmol) was added under nitrogen atmosphere, and the stirring was continued for 5 min. The reaction mixture was extracted three times with EtOAc, and the combined EtOAc solution was washed twice with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. The residue was purified by preparative TLC (5% MeOH in CH_2Cl_2) to give *threo*-**7** (6.7 mg, 95.4%). A similar reaction was also done with *erythro*-**5** to afford *erythro*-**7** (1.8 mg, 95%). *Threo*-**7**: ^{1}H-NMR δ ($CDCl_3$): 1.70 (1H, br s, 9′-OH), 3.35 (1H, br d, J = 10.49, 9-OH), 3.41 (1H, br dd, J = 11.46, 10.0, H-9a), 3.64 (1H, br d, J = 11.47, H-9b), 3.83 (3H, s, A-OCH_3), 3.88–3.95 (1H, m, H-8), 3.91 (6H, s, B-OCH_3), 4.32 (2H, br t, J = 5.37, H-9′), 4.37 (1H, d, J = 0.98, 7-OH), 5.15 (1H, d, J = 8.29, H-7), 6.31 (1H, td, J = 15.85, 5.52, H-8′), 6.55 (1H, d, J = 15.85, H-7′), 6.65 (2H, s, H-2′, H-6′), 7.12 (1H, br d, J = 8.29, H-6), 7.14 (1H, d, J = 8.05, H-5), 7.16 (1H, br s, H-2), 7.50 (2H, t, J = 7.69, BzH-3, BzH-5), 7.64 (1H, dt, J = 7.44, 1.22, BzH-4), 8.21 (2H, dd, J = 7.44, 0.85, BzH-2, BzH-6). *Erythro*-**7**: ^{1}H-NMR δ ($CDCl_3$): 1.48 (1H, br, 9′-OH), 3.15 (1H, dd, J = 7.93, 4.27, 9-OH), 3.54 (1H, ddd, J = 11.35, 8.36, 2.57, H-9a), 3.83 (3H, s, A-OCH_3), 3.90 (6H, s, B-OCH_3), 3.94 (1H, ddd, J = 11.71, 6.58, 4.14, H-9b), 4.10 (1H, br d, J = 3.17, 7-OH), 4.19 (1H, ddd, J = 6.34, 3.41, 2.93, H-8), 4.35 (2H, br t, J = 5.25, H-9′), 5.10 (1H, br t, J = 3.30, H-7), 6.33 (1H, td, J = 15.85, 5.61, H-8′), 6.58 (1H, d, J = 15.85, H-7′), 6.68 (2H, s, H-2′, H-6′), 6.91 (1H, dd, J = 8.17, 1.83, H-6), 7.110 (1H, d, J = 8.05, H-5), 7.111 (1H, d, J = 1.71, H-2), 7.50 (2H, t, J = 7.81, BzH-3, BzH-5), 7.63 (1H, t, J = 7.44, BzH-4), 8.21 (2H, d, J = 7.32, BzH-2, BzH-6).

Guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether (GGSE, **8**) (step i). To a stirred solution of *threo*-**7** (6.7 mg, 0.013 mmol) in benzene (0.3 ml, freshly distilled over anhydrous $CaCl_2$), *n*-butylamine (65 μl, 0.656 mmol) was added. The stirring was continued at room temperature under

nitrogen atmosphere for 78 h. The reaction solution was partitioned between EtOAc and 1*N* HCl. The organic layer was washed successively with 1*N* HCl (twice) and saturated brine (twice), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by preparative TLC (5% MeOH in CH_2Cl_2) to give *threo*-**8** (2.8 mg, 52.6%). Similarly, *erythro*-**7** was also deprotected to afford *erythro*-**8** (0.7 mg, 42%). ^{1}H-NMR and ^{13}C-NMR data of both diastereomers are shown in Tables 2 and 3, respectively. EI-MS *m/z* (%): *threo*: 406 (1.7) $[M]^+$, 358 (3.4), 298 (1.1), 210 (100), 107 (12.3), 93 (24.5), 77 (14); *erythro*: 406 (2.2) $[M]^+$, 358 (45), 210 (100), 107 (40.4), 93 (19.1), 77 (56.2).

Other syntheses. SGCE and SGSE were synthesized similarly to GGSE. Synthesis of GGCE was summarized in our previous paper.[9] [8-^{14}C]Coniferyl alcohol (5.51 MBq/mmol) and [8-^{14}C]sinapyl alcohol (5.76 MBq/mmol) were synthesized by the method of Katayama et al.[10] and Katayama and Ogaki[11], respectively.

Plant materials

Eucommia ulmoides plants obtained from Sanyo Nouen were maintained at the Faculty of Agriculture, Kagawa University.

Feeding experiment

Ten excised young shoots of *E. ulmoides* were each administered 140 μl of a mixed solution of 12.5 mM [8-^{14}C]coniferyl alcohol (5.51 MBq/mmol) and 12.5 mM sinapyl alcohol (5.76 MBq/mmol) in potassium phosphate (K-Pi) buffer (0.1 M, pH 7). The shoots were then allowed to metabolize for 3 h at 25°C in an environment-controlled room.

Leaves and stems were divided, immediately frozen in liquid nitrogen, individually freeze-dried, reduced into small size pieces (~2 mm) by means of scissors and extracted five times with MeOH at 65°C. The extractives were combined, concentrated to small volume (one tenth) and to this was added water. The whole was centrifuged (1500 rpm, 200 *g*, 20°C for 20 min) and the supernatant was partitioned between EtOAc (containing unlabelled *erythro*-GGSE and *threo*-GGSE as cold carriers) and water. The aqueous layers were extracted twice with EtOAc. The EtOAc solutions were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuo. This fraction was named as the "organic layer."

The aqueous layer was freeze-dried and the resulting powder was treated with a mixture of cellulase [(700 mg) (Wako; from *Trichoderma viride*, 1000 units/mg)] and β-glucosidase [(200 mg) (Oriental Yeast; from sweet almond, 34 units/mg)] in sodium acetate buffer (20 mM, pH 4.5) for 24 h at 50°C under nitrogen atmosphere.[12] The whole was extracted with EtOAc (containing unlabelled *erythro*-GGSE and *threo*-GGSE as cold carriers), and the aqueous layers extracted twice with EtOAc. The EtOAc solutions were combined, washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. This fraction was named as the "aqueous layer."

Both of the EtOAc extracts of the organic layer and aqueous layer were subjected to preparative TLC [benzene/acetone = 2:1 (×5)] to isolate [^{14}C]GGSE, which was then reconstituted in MeOH (400 μl) and filtered. An aliquot (10 μl) of the filtrate was subjected to reversed phase C_{18} HPLC. The eluate was collected every 30 s for liquid scintillation counting.

Results and discussion

Synthesis of 8-*O*-4′ neolignans, separation and identification of diastereomers

The synthetic route to GGSE is illustrated in Fig. 1. The route is a modification of the methods of Adler and Eriksoo,[13] Katayama et al.,[14] and Kawai et al.[15] Nucleophilic substitution of 1-(4-benzoyloxy-3-methoxyphenyl)-2-bromoethanone with sinapaldehyde in the presence of K_2CO_3 and KI in DMF gave compound **1**. Aldol-type condensation of **1** with paraformaldehyde and K_2CO_3 in DMSO afforded compound **2**. Then the terminal aldehyde of **2** was protected by dimethyl acetal formation with methyl orthoformate in the presence of *p*-toluenesulfonic acid as a catalyst to give compound **3**, whose ketone was reduced with $NaBH_4$ to afford diol **4** as a mixture of *erythro* and *threo* isomers. The terminal acetal of **4** was deprotected to yield compound **5**. These three steps from **2** to **5** were carried out successively without chromatographic purification of **3** and **4** due to the poor stability of the dimethyl acetal.

Fortunately, in the case of the small scale used, compound **5** was sufficiently separated into the *erythro* and *threo* isomers by means of preparative TLC (2% MeOH in CH_2Cl_2, ×3). One isomer (R_f 0.75) had a coupling constant of 8.05 Hz for the C_7-H doublet, while the other isomer (R_f 0.63) showed a coupling constant of 3.91 Hz for the equivalent resonance. The diastereomers were identified as the *threo* and *erythro* isomers, respectively, by independent transformation into acetonide derivatives (**6**) and ^{1}H-NMR analysis as described below. In the case of a normal preparative-scale reaction, preparative separation of *erythro*-**5** and *threo*-**5** is difficult. Preparation of acetonide derivatives is a useful method not only for separation but also for identification of the diastereomers.[14] The separation of *erythro*-**6** and *threo*-**6** [acetonide derivatives of GGSE (A-GGSE)] as well as the other acetonide derivatives of SGCE (A-SGCE), GGCE (A-GGCE), and SGSE (A-SGSE), was successively achieved by silica gel chromatography.

The acetonide derivatives of the four neolignans give characteristic coupling constants (*J* value) at the C_7-H resonance (doublet) in the ^{1}H-NMR spectra, from which deductions of dihedral angles between the H-C_7-C_8 and C_7-C_8-H planes can be made (the vicinal Karplus correlation),[16] and identification of the diastereomers becomes possible

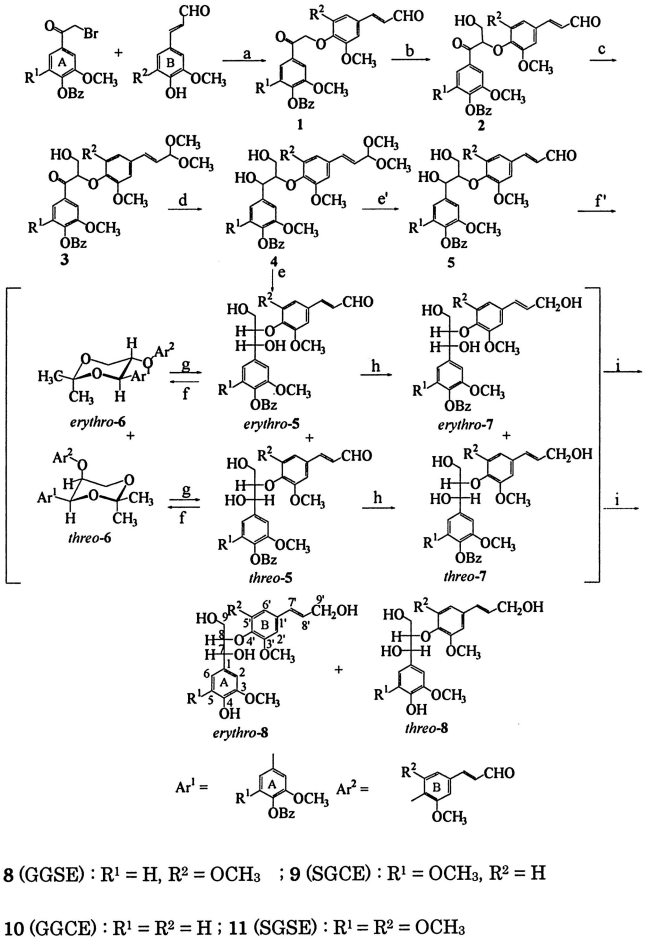

Fig. 1. Synthetic route for guaiacylglycerol-8-*O*-4′-(sinapyl alcohol) ether (GGSE, **8**) and the structures of other 8-*O*-4′ neolignans. Reaction conditions: (a) K_2CO_3/KI/DMF/rt, (b) -$(CH_2O)_n$-/K_2CO_3/DMSO/rt, (c) $CH(OCH_3)_3$/*p*-toluenesulfonic acid/THF/MeOH/rt, (d) $NaBH_4$/THF/MeOH/0°C, (e) 90% AcOH/rt; separation of *erythro* and *threo* isomers by preparative TLC (SiO_2), (f) $(CH_3)_2C(OCH_3)_2$/CSA/acetone/rt, (e′) 90% AcOH/rt, (f′) $(CH_3)_2C(OCH_3)_2$/CSA/acetone/rt; separation of *erythro* and *threo* isomers by column chromatography (SiO_2), (g) 90% AcOH/rt, (h) $NaBH_4$/THF/MeOH/0°C, (i) *n*-butylamine/benzene/rt. *Bz*, (C_6H_5CO-)

(Table 1). The C_8-H resonance is not useful due to its complex splitting pattern. One diastereomer (higher R_f) has a larger *J* value than the other (lower R_f) (A-GGSE: 9.27 Hz and 1.71 Hz, A-SGCE: 9.02 Hz and 1.46 Hz, A-GGCE: 8.8 Hz and 1.6 Hz, and A-SGSE: 9.02 Hz and 1.46 Hz, respectively), which are, due to the rigid orientation of the six-membered cyclic ketal, correlated to the larger dihedral angle (160°) and the smaller one (60°) of the H-C_7-C_8-H vicinal plane of the *erythro* isomer and that of the *threo* isomer, respectively. On the other hand, stable conformation of aryl (A) and aryloxy (B) groups of *erythro*-acetonide is diequatorial (Fig. 1) (dihedral angle of H-C_7-C_8-H is 180°) and those of the *threo* isomer are equatorial and axial (dihedral angle of H-C_7-C_8-H is 60°). Therefore, it was identified that the diastereomers with $J = 8.8$–9.3 Hz are *erythro* isomers and those with $J = 1.46$–1.71 Hz are *threo* isomers.

The acetonide derivatives were then deprotected with aqueous acetic acid to give diol **5**, whose terminal aldehyde was reduced with $NaBH_4$ to yield alcohol **7**. The benzoyl group of **7** was removed with *n*-butylamine to afford the final 8-*O*-4′ neolignan, GGSE (**8**). The ^{1}H and ^{13}C-NMR data of the neolignans are shown in Tables 2 and 3, respectively.

The three 8-*O*-4′ neolignans SGCE, GGCE, and SGSE were synthesized and their *J* values of the C_7-H resonance that could distinguish the *erythro* isomer from the *threo* isomer showed the same phenomenon as GGSE (Table 2). It is obvious that the *J* value of the C_7-H resonance of the *erythro*-acetonide is larger than that of the *erythro*-diol. In contrast, this value of the *threo*-acetonide is less than that of the *threo*-diol.

Identification of diastereomer of natural GGSE by ^{13}C-NMR

Table 3 shows that the characteristic ^{13}C-NMR peaks that distinguish between the *erythro* and *threo* isomers are those of C_7 and C_8, which are chiral centers, as well as that of C_9 for the four neolignans. It also shows that the differences between *erythro*-GGSE and *threo*-GGSE and between *erythro*-SGCE and *threo*-SGCE are clear, as well as that the differences between GGSE and SGCE are apparent.

Because Deyama et al.[1] reported the ^{13}C-NMR data of natural GGSE in DMSO-d_6, the ^{13}C-NMR spectra of synthetic *erythro*-GGSE and *threo*-GGSE were determined in DMSO-d_6 and compared with those of natural GGSE as shown in Table 4. The diastereomer of natural GGSE was identified as the *erythro* isomer. It is clear that natural GGSE is different from both *erythro*-SGCE and *threo*-SGCE (Table 5).

Feeding experiment

The feeding experiment using labeled precursors was performed and the results are shown in Table 6. Lignans and neolignans would be extracted in the organic layer and their glycosides in the aqueous layer. When the mixture of [8-^{14}C]coniferyl alcohol and [8-^{14}C]sinapyl alcohol was administered into excised young shoots of *Eucommia ulmoides*, the radioactivity was incorporated into *erythro*-GGSE to a degree that was three times higher than that in *threo*-GGSE from the organic layer of stems, but such incorporation could not be detected in leaves. However, the radioactivity of their glucosides was detected in both stems and leaves even in small amounts. Overall, formation of the *erythro* isomer was 2.4 times higher than that of the *threo* isomer.

This result is consistent with the identification of natural GGSE as the *erythro* isomer, as described above. Thus, it was suggested that *erythro*-GGSE is diastereoselectively produced by cross coupling of coniferyl and sinapyl alcohols in *E. ulmoides*.

Future work will require chiral analysis of each diastereomer to clarify enantioselective formation of 8-*O*-4′

Table 1. Partial ^{1}H nuclear magnetic resonance (NMR) spectra of four acetonide derivatives: comparison between *erythro* and *threo* isomers

Proton no.	A-GGSE		A-SGCE		A-GGCE[a]		A-SGSE	
	Erythro	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*
2	7.10, o	7.28, d, J = 1.46	6.82, s	6.80, s	6.98, d, J = 1.6	7.19, d, J = 2.0	6.75, s	6.82, s
5	7.05, d, J = 7.81	7.06, d, J = 7.81	–	–	6.84, d, J = 8.0	6.83, d, J = 7.7	–	–
6	7.09, dd, J = 7.81, 1.71	7.04, dd, J = 8.30, 1.47	6.82, s	6.80, s	7.00, dd, J = 8.0, 2.0	6.88, dd, J = 8.4, 2.0	6.75, s	6.82, s
7	4.96, d, J = 9.27	5.14, d, J = 1.71	5.00, d, J = 9.02	5.14, d, J = 1.46	4.91, d, J = 8.8	5.09, d, J = 1.6	4.93, d, J = 9.02	5.11, d, J = 1.46
8	4.32, td, J = 8.79, 5.37	4.39, dd, J = 4.15, 1.95	4.27, td, J = 8.12, 5.37	4.24–4.28, o	4.26, m	4.22, m	4.33, ddd, J = 11.71, 8.42, 5.61	4.40, d (with shoulder), J = 1.95
9a	4.05, dd, J = 11.71, 5.61	4.08, dd, J = 12.93, 1.95	4.03, dd, J = 11.71, 8.54	4.24–4.28, o	4.01, dd, J = 12.0, 8.0	4.17, dd, J = 13.0, 2.6	4.06, dd, J = 11.71, 5.61	4.09, dd, J = 12.93, 2.20
9b	4.11, dd, J = 11.95, 8.66	4.14, dd, J = 12.93, 1.95	4.19, dd, J = 11.83, 5.25	4.24–4.28, o	4.16, dd, J = 12.0, 4.0	4.22, dd, J = 13.0, 2.0	4.11, dd, J = 11.71, 8.29	4.15, dd, J = 12.93, 1.96
2′	6.65, s,	6.69, s	7.01, o	7.01, o	6.98, d, J = 1.6	7.00, d, J = 2.0	6.67, s	6.70, s
5′	–	–	6.57, d, J = 8.30	6.48, d, J = 8.05	6.55, d, J = 8.4	6.48, d, J = 8.4	–	–
6′	6.65, s	6.69, s	7.00–7.04, o, $J_{2'6'}$ = 1.95	7.01–7.04, o, $J_{2'6'}$ = 1.95	6.95, dd, J = 8.0, 1.6	6.95, dd, J = 8.0, 2.0	6.67, s	6.70, s
7′	7.33, d, J = 15.85	7.34, d, J = 15.86	7.36, d, J = 15.86	7.36, d, J = 15.85	7.33, d, J = 15.9	7.33, d, J = 15.6	7.34, d, J = 15.86	7.35, d, J = 15.85
8′	6.60, dd, J = 15.86, 7.81	6.59, dd, J = 15.74, 7.69	6.58, dd, J = 15.74, 7.69	6.58, dd, J = 15.86, 8.05	6.56, dd, J = 15.8, 8.0	6.55, dd, J = 15.6, 7.6	6.60, dd, J = 15.85, 7.56	6.60, dd, J = 15.85, 7.80
9′	9.65, d, J = 7.56	9.65, d, J = 7.81	9.65, d, J = 7.80	9.64, d, J = 7.81	9.63, d, J = 8.0	9.63, d, J = 8.0	9.66, d, J = 7.81	9.65, d, J = 7.60
OCH_3(A)	3.76, s	3.79, s	3.75, s	3.77, s	3.84, s	3.89, s	3.75, s	3.77, s
OCH_3(B)	3.71, s	3.74, s	3.84, s,	3.81, s	3.85, s	3.82, s	3.73, s	3.74, s

Spectra recorded in $CDCl_3$. ^{1}H NMR peaks of acetonide moieties, benzoyl groups (A-GGSE, A-SGCE and A-SGSE) and phenolic hydroxyl groups (A-GGCE) are omitted
s, Singlet; d, doublet; t, triplet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, doublet of triplet; td, triplet of doublet; tt, triplet of triplet; o, overlapping; br, broad; m, multiplet
[a] Acetonide derivatives of GGCE (A-GGCE) had been prepared as guaiacylglycerol-8-*O*-4′-(coniferaldehyde) ether 7,9-*O*-isopropylideneketals that have a free 4-phenolic hydroxyl group

Table 2. ^{1}H NMR spectra of four 8-*O*-4′ neolignans: comparison between *erythro* and *threo* isomers

Proton no.	GGSE		SGCE		GGCE		SGSE	
	Erythro	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*
2	7.05, d, J = 1.71	7.07, d, J = 1.95	6.78, s	6.79, s	7.11, d, J = 1.95	7.10, d, J = 1.95	6.73, s	6.78, s
5	6.78, d, J = 8.05	6.78, d, J = 8.30	–	–	6.77, d, J = 8.05	6.77, d, J = 8.29	–	–
6	6.84, ddd, J = 8.18, 1.79, 0.61	6.91, dd, J = 8.05, 1.95	6.78, s	6.79, s	6.89, ddd, J = 8.29, 1.83, 0.49	6.90, ddd, J = 7.93, 1.95, 0.49	6.73, s	6.78, s
7	4.98, d, J = 4.39	5.00, d, J = 7.32	4.89, d, J = 5.12	4.90, d, J = 5.61	4.90, d, J = 5.37	4.90, d, J = 5.85	4.99, d, J = 4.63	4.99, d, J = 7.07
8	4.18, ddd, J = 5.73, 4.52, 3.54	3.93–3.97, o	4.35, dt, J = 5.43, 4.03	4.32, dt, J = 5.49, 4.15	4.33, dt, J = 5.24, 4.03	4.27, dt, J = 5.69, 3.98	4.20–4.24, o	4.00, td, J = 7.07, 3.54
9a	3.45, dd, J = 11.95, 3.42	3.30, dd, J = 12.20, 3.42	3.73, dd, J = 11.83, 4.03	3.52, dd, J = 11.83, 5.73	3.71, dd, J = 11.71, 3.90	3.50, dd, J = 11.95, 5.61	3.49, dd, J = 11.96, 3.42	3.35, dd, J = 12.20, 3.41
9b	3.85, dd, J = 11.95, 5.85	3.67, dd, J = 12.20, 3.66	3.83, dd, J = 11.71, 5.37	3.74, dd, J = 11.71, 4.15	3.82, dd (o), J = 11.23, 6.01	3.71, dd, J = 11.71, 3.90	3.89, dd, J = 11.95, 5.61	3.70, dd, J = 12.20, 3.66
2′	6.81, s	6.82, s	7.07, d, J = 1.95	7.10, d, J = 2.20	7.07, d, J = 1.95	7.11, d, J = 2.20	6.82, s	6.82, s
5′	–	–	6.93, d, J = 8.05	7.09, d, J = 9.03	6.93, d, J = 8.29	7.10, d, J = 8.29	–	–
6′	6.81, s	6.82, s	6.88, dd, J = 8.54, 1.95	6.91, dd, J = 8.30, 1.95	6.88, dd, J = 8.29, 1.95	6.91, dd, J = 8.29, 2.07	6.82, s	6.82, s
7′	6.56, td, J = 15.86, 1.46	6.56, td, J = 15.86, 1.46	6.51, td, J = 16.10, 1.71	6.53, td, J = 16.10, 1.46	6.52, td, J = 15.86, 1.71	6.53, td, J = 16.10, 1.46	6.57, td, J = 15.86, 1.46	6.56, td, J = 16.00, 1.47
8′	6.39, td, J = 15.85, 5.13	6.39, td, J = 15.85, 5.37	6.28, td, J = 15.86, 5.37	6.30, td, J = 15.86, 5.37	6.28, td, J = 15.86, 5.37	6.30, td, J = 15.86, 5.37	6.39, td, J = 15.86, 5.25	6.39, td, J = 15.86, 5.25
9′	4.22, dd, J = 5.12, 1.47	4.22, dd, J = 5.12, 1.46	4.19, dd, J = 5.00, 1.10	4.20, dd, J = 5.00, 1.10	4.19, dd, J = 5.37, 1.22	4.20, dd, J = 5.49, 1.58	4.23, dd, J = 5.12, 1.46	4.22, dd, J = 5.36, 1.34
OCH_3(A)	3.84, s	3.82, s	3.80, s	3.80, s	3.82, s	3.82, s	3.82, s	3.81, s
OCH_3(B)	3.89, s	3.92, s	3.86, s	3.91, s	3.86, s	3.91, s	3.89, s	3.92, s

Spectra recorded in acetone-d_6 + D_2O

Table 3. ^{13}C NMR of four 8-*O*-4′ neolignans: comparison between *erythro* and *threo* isomers

Carbon no.	GGSE[a]		SGCE[b]		GGCE[c]		SGSE[b]	
	Erythro	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*	*Erythro*	*Threo*
1	133.773	133.691	[d]133.156	[e]132.802	[f]134.111	[g]133.798	[h]134.341	[i]134.472
2	110.885	111.478	105.373	105.299	111.864	111.749	104.888	105.348
3	148.006	147.965	148.319	148.368	148.738	148.870	148.376	148.360
4	146.484	146.821	135.986	135.970	147.052	147.216	135.600	135.937
5	115.246	115.237	148.319	148.368	115.674	115.871	148.376	148.360
6	120.009	120.766	105.373	105.299	121.062	120.848	104.888	105.348
7	73.328	74.052	73.806	73.575	74.159	74.061	73.452	73.937
8	88.039	89.972	85.941	87.257	86.220	87.150	87.619	89.388
9	60.914	61.342	61.671	61.646	62.255	61.934	60.848	61.309
1′	134.407	134.522	[d′]132.531	[e′]132.597	[f′]133.066	[g′]133.181	[h′]132.605	[i′]132.473
2′	104.616	104.534	110.885	110.754	111.395	111.288	104.616	104.542
3′	154.325	154.012	151.527	151.330	151.939	151.782	154.119	153.864
4′	136.019	136.472	148.475	148.985	148.985	149.290	135.904	136.439
5′	154.325	154.012	118.397	118.495	118.890	118.849	154.119	153.864
6′	104.616	104.534	120.281	120.363	120.692	120.774	104.616	104.542
7′	129.865	129.791	129.380	129.454	128.508	128.639	130.013	129.898
8′	130.902	130.992	129.931	129.906	131.503	131.453	130.597	130.778
9′	63.143	63.119	63.102	63.102	63.793	63.785	62.863	62.880
OCH_3(A)	56.241	56.208	56.594	56.578	56.356	56.364	56.611	56.603
OCH_3(B)	56.570	56.594	56.307	56.307	56.529	56.570	56.627	56.636

[a] Acetone-d_6
[b] Acetone-d_6 + D_2O
[c] CD_3OD
The assignments of d-d′, e-e′, f-f′, g-g′, h-h′, and i-i′ might be interchangeable

Table 4. Comparison of ^{13}C NMR (DMSO-d_6) between synthesized *erythro*- and *threo*-GGSE and that isolated from *Eucommia ulmoides* by Deyama et al.[1]

Carbon no.	*Erythro*-GGSE	Δ	$\lvert x - \Delta \rvert$	$\lvert x_{7,8,9} - \Delta \rvert$	Deyama's GGSE	*Threo*-GGSE	Δ	$\lvert x - \Delta \rvert$	$\lvert x_{7,8,9} - \Delta \rvert$
1	133.197	0.097	0.017		133.1	132.860	0.240	0.006	
2	110.819	0.381	0.267		111.2	110.885	0.315	0.069	
3	146.879	0.079	0.035		146.8	146.764	0.036	0.210	
4	145.192	0.008	0.106		145.2	145.209	0.009	0.237	
5	114.579	0.079	0.035		114.5	114.546	0.046	0.200	
6	119.227	0.027	0.087		119.2	119.104	0.096	0.150	
7	71.905	0.095	0.019	0.018	72.0	71.387	0.613	0.367	0.148
8	86.113	0.213	0.099	0.100	85.9	87.084	1.184	0.938	0.424
9	59.630	0.030	0.084	0.083	59.6	60.083	0.483	0.237	0.277
1′	132.226	0.026	0.088		132.2	132.276	0.076	0.170	
2′	103.538	0.262	0.148		103.8	103.522	0.278	0.032	
3′	152.654	0.054	0.060		152.6	152.531	0.069	0.177	
4′	134.810	0.090	0.024		134.9	135.468	0.568	0.322	
5′	152.654	0.054	0.060		152.6	152.531	0.069	0.177	
6′	103.538	0.262	0.148		103.8	103.522	0.278	0.032	
7′	128.409	0.009	0.105		[a]128.4	128.376	0.024	0.222	
8′	130.021	0.121	0.007		[a]129.9	130.071	0.171	0.075	
9′	61.350	0.250	0.136		61.1	61.350	0.250	0.004	
OCH_3(A)	55.401	0.099	0.015		55.5	55.418	0.082	0.164	
OCH_3(B)	55.837	0.037	0.077		55.8	55.837	0.037	0.209	
Totals			1.617	0.201				3.998	0.849

For total carbons (*erythro*) $\Sigma = 2.273$, $n = 20$, $x = 0.114$. SD = 0.081. For carbon no. 7, 8, 9 $\Sigma = 0.338$, $n = 3$, $x = 0.113$, SD = 0.067. For total carbons (*threo*) $\Sigma = 4.924$, $n = 20$, $x = 0.246$, SD = 0.200. For carbon no. 7, 8, 9 $\Sigma = 2.279$, $n = 3$, $x = 0.760$, SD = 0.283
[a] According to the original report by Deyama et al.[1], C-7′ and C-8′ were assigned as 129.9 and 128.4, respectively. However, the authors corrected their assignment as above

neolignans containing syringyl moieties or enantioselective cross coupling of coniferyl and sinapyl alcohols.

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Table 5. Comparison of ^{13}C NMR (DMSO-d_6) between synthesized *erythro*- and *threo*-SGCE and GGSE isolated from *Eucommia ulmoides* by Deyama et al.[1]

Carbon no.	*Erythro*-SGCE	Δ	$\lvert x - \Delta \rvert$	$\lvert x_{7,8,9} - \Delta \rvert$	Deyama's GGSE	*Threo*-SGCE	Δ	$\lvert x - \Delta \rvert$	$\lvert x_{7,8,9} - \Delta \rvert$
1	132.193	0.907	6.520		133.1	131.840	1.260	6.264	
2	104.583	6.617	0.810		111.2	103.999	7.201	0.323	
3	147.233	0.433	6.994		146.8	147.298	0.498	7.026	
4	134.300	10.900	3.473		145.2	134.225	10.975	3.451	
5	147.233	32.733	25.306		114.5	147.298	32.798	25.274	
6	104.583	14.617	7.190		119.2	103.999	15.201	7.677	
7	71.716	0.284	7.143	0.830	72.0	70.803	1.197	6.327	0.002
8	83.300	2.600	4.827	1.486	85.9	83.876	2.024	5.500	0.825
9	60.058	0.458	6.969	0.656	59.6	59.976	0.376	7.148	0.823
1′	129.791	2.409	5.018		132.2	129.939	2.261	5.263	
2′	109.626	5.826	1.601		103.8	109.569	5.769	1.755	
3′	149.487	3.113	4.314		152.6	149.487	3.113	4.411	
4′	147.438	12.538	5.111		134.9	147.611	12.711	5.187	
5′	115.130	37.470	30.043		152.6	115.073	37.527	30.003	
6′	118.898	15.098	7.671		103.8	118.948	15.148	7.624	
7′	128.327	0.073	7.354		[a]128.4	128.417	0.017	7.507	
8′	128.450	1.450	5.977		[a]129.9	128.450	1.450	6.074	
9′	61.506	0.406	7.021		61.1	61.506	0.406	7.118	
OCH_3(A)	55.731	0.231	7.196		55.5	55.689	0.189	7.335	
OCH_3(B)	55.426	0.374	7.053		55.8	55.443	0.357	7.167	
Totals			157.591	2.972				158.434	1.650

For total carbons (*erythro*) $\Sigma = 148.537$, $n = 20$, $x = 7.427$, SD = 7.880. For carbon no. 7, 8, 9 $\Sigma = 3.342$, $n = 3$, $x = 1.114$, SD = 0.990. For total carbons (*threo*) $\Sigma = 150.478$, $n = 20$, $x = 7.524$, SD = 7.922. For carbon no. 7, 8, 9 $\Sigma = 3.597$, $n = 3$, $x = 1.199$, SD = 0.550

[a] See Table 4

Table 6. Incorporation of radioactivity into [^{14}C]GGSE following administration of a mixture of [8-^{14}C]coniferyl alcohol and [8-^{14}C]sinapyl alcohol to excised shoots of *Eucommia ulmoides*

[^{14}C]GGSE	Stems		Leaves		Total
	Organic	Aqueous	Organic	Aqueous	
Erythro	0.303	0.002	nd	0.025	0.330
Threo	0.109	0.006	nd	0.022	0.137
Total	0.412	0.008	–	0.047	0.467

nd, not detected

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