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Absolute Configuration of Syringylglycerol-8-*O*-4´-(Sinapyl Alcohol) Ethers, Neolignans as Well as Lignin Substructure Dimeric Compounds in Higher Plants

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ABSTRACT

Syringylglycerol-8-*O*-4'-(sinapyl alcohol) ethers (SGSEs) are lignin substructure dimeric compounds or 8-*O*-4' neolignans. To investigate biosynthesis of the 8-*O*-4' neolignans, incubation of sinapyl alcohol (SA) with enzyme preparations of *Eucommia ulmoides* was performed and formation of optically active SGSEs was found. To clarify the stereochemical mechanism of the SGSE formation, absolute configuration of four stereoisomers, (+)-*erythro*, (–)-*erythro*, (+)-*threo* and (–)-*threo* isomers, of SGSEs that contain a chiral secondary benzyl alcohol were determined as (7*R*, 8*S*), (7*S*, 8*R*), (7*S*, 8*S*) and (7*R*, 8*R*), respectively, by Mosher's method [^{1}H NMR analysis of tri-(*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetate (MTPA) of SGSEs] with our related empirical rules. Four stereoisomers of SGSEs were obtained by dehydrogenations of SA with $FeCl_3$ followed by reversed phase HPLC and chiral HPLC.

Key words: Stereochemistry, biosynthesis, *erythro*, *threo*, (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)

INTRODUCTION

Arylglycerol-**β**-aryl (or arylglycerol-8-*O*-4´-aryl) ether linkages are present in lignins and 8-*O*-4´ neolignans. The intermonomer linkages are the most abundant ones in natural products except for glycosidic linkages in carbohydrates. Mixtures of (±)-*erythro*- and (±)-*threo*- guaiacylglycerol-8(**β**)-*O*-4'-(coniferyl alcohol) ethers (GGCE) and syringylglycerol-8(**β**)-*O*-4'-(sinapyl alcohol) ethers (SGSE) are the dimeric intermediates of dehydrogenative polymerization of coniferyl alcohol (CA) and sinapyl alcohol (SA), respectively. This laboratory has investigated the biosynthesis of 8-*O*-4´ neolignans using *Eucommia ulmoides*. Katayama and Kado (1998) found enzymatic formation of (+)-*erythro*- and (–)-*threo*-guaiacylglycerol-8-*O*-4'-(coniferyl alcohol) ethers (GGCEs) by the incubation of CA with a cell-free extract (a soluble preparation) of *E. ulmoides* in the presence of hydrogen peroxide (H_2O_2). Katayama *et al*. (2005a) determined absolute configuration of the four stereoisomers, (+)-*erythro*-, (–)-*erythro*-, (+)-*threo*- and (–)-*threo*-GGCEs as (7*R*, 8*S*), (7*S*, 8*R*), (7*S*, 8*S*) and (7*R*, 8*R*), respectively, by Mosher's method (Dale *et al*., 1969; Dale and Mosher, 1973; Yamaguchi, 1985). They suggested that (+)-*erythro*-GGCE was formed by the selective water addition to the (8*R*)-enantiomer of the racemic quinonemethide (Katayama *et al*., 2005b).

This laboratory has also studied the biosynthesis of syringyl lignans and syringyl neolignans. Lignans and neolignans (except for 9,9'-deoxy ones) are biosynthesized from coniferyl alcohol (which contains 4-hydroxy-3-methoxyphenyl group, so-called guaiacyl group) or sinapyl alcohol (which contains 4-hydroxy-3,5-dimethoxyphenyl group, so-called syringyl group). Biosynthesis of guaiacyl lignans/neolignans derived from coniferyl alcohol have been studied much more than that of syringyl lignans/neolignans from sinapyl alcohol. Especially there had been no study on biosynthesis of syringyl-8-*O*-4' neolignans. Therefore, Lourith *et al.* (2005) started the study and found the formation of optically active *erythro* and *threo*-SGSEs by feeding experiments using radiolabelled SA and the excised shoots of *E. ulmoides* as well as by the incubation of a mixture of SA and CA with an insoluble enzyme preparation from the plant. Recently, we have reported (Alam *et al.*, 2009) that incubation of SA with a cell-free extract (a soluble enzyme preparation) of *E. ulmoides* in the presence of hydrogen peroxide (H_2O_2) gave (–)-*erythro* and (–)-*threo*-SGSEs, whereas incubation of SA with a cell-wall residue (an insoluble enzyme preparation) in the absence of H_2O_2 afforded (+)-*erythro* and (–)-*threo*-SGSEs. To understand the stereochemical mechanism of the SGSE formation, absolute configurations of the four stereoisomers, (+)-*erythro*, (–)-*erythro*, (+)-*threo* and (–)-*threo* isomers, of SGSEs were required. However, the absolute configurations were unknown. In this study, we report determination of their absolute configurations by Mosher's method [^{1}H NMR spectroscopy of (*R*)-(+)-MTPA esters of SGSEs] and our empirical rules (Katayama *et al.*, 2005a).

MATERIALS AND METHODS

This research was conducted from April 2009 to March 2010.

Instrumentation and chromatography materials: All reagents and solvents were reagent grade. Analytical and preparative thin-layer chromatographies (TLC) were done by using plates precoated with Merck silica gel 60 F-254 (0.25 and 0.5 mm thickness, respectively). NMR spectra (600 MHz) were measured on a JEOL JNM-Delta-600 FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts and coupling constants (J) were expressed as δ (in ppm) and Hz, respectively. Analytical and preparative high performance liquid chromatographies (HPLC) were carried out on a Jasco PU-2089 equipped with a Jasco UV-2075 plus Intelligent UV/VIS detector and a Shimadzu Chromatopac C-R7A plus using a reversed phase column (TSK-GEL, ODS-80Ts, 250×4.6 mm), at a flow rate of 1.0 mL min^{-1} using the following linear gradient solvent system: methanol (MeOH) with 3% acetic acid (AcOH) in H_2O (v/v) starting with isocratic elution at 23:77 which was held for 10 min and then linearly increased to 28:72 within 5 min. This ratio was then held for the remainder of the analysis. Chiral (column) HPLC analysis was performed using the same HPLC system as above on a Daicel Chiralcel OD(H) column (250×46 mm) eluted with ethanol (EtOH)/*n*-hexane (23:77; v/v) at a flow 1.0 mL min^{-1} (for *erythro*-SGSE) rate and of 0.8 mL min^{-1} (for *threo*-SGSE). All detection was done at 280 nm.

Chemical synthesis: SGSE was prepared by the dehydrogenation of sinapyl alcohol with $FeCl_3$ (Lourith *et al.*, 2005; Tanahashi *et al.*, 1976) and the reaction mixture was purified by preparative TLC (7% MeOH in CH_2Cl_2) to give SGSE (24.7 mg, 27.6%) as a mixture of *erythro* and *threo* isomers. The diastereomeric ratio of this SGSE was quantified as (*erythro* : *threo* = 6:4) by reversed-phase HPLC and then diastereomeric separation was carefully carried out by preparative TLC [benzene/acetone 2:1 (x2)] to give *threo*-(R_f 0.38, 2.60 mg) and *erythro*-SGSE (R_f 0.35, 4.2 mg).

Preparative enantiomeric separation of (±)-*erythro*-SGSE and (±)-*threo*-SGSE was done by chiral column HPLC giving (+)-*erythro*- and (–)-*erythro*-SGSEs and (+)-*threo*- and (–)-*threo*-SGSEs, respectively.

Preparation of 4-*O*-methyl ether of (+)-*erythro*-syringylglycerol-8-*O*-4′-(sinapyl alcohol) ether (SGSE): To a stirred solution of (+)-*erythro*-SGSE (4.60 mg, 10.5 μmol) in MeOH (0.5 mL), an etherial yellow solution of diazomethane (CH_2N_2) (3 mL) (De Bore and Backer, 1963) was added at 0°C. This methyl etherification was observed by analytical TLC [benzene/acetone = 1:1 (×1)] at 30 min intervals. After 8 h the reaction mixture was evaporated to dryness in vacuo. The residue was purified by preparative TLC [benzene/acetone = 1:1 (×1)] to give 4-*O*-methyl ether of (+)-*erythro*-SGSE (3.5 mg, yield 97%). This structure was confirmed by ^{1}H NMR spectra (data not shown). The other stereoisomers [(–)-*erythro*, (+)-*threo* and (–)-*threo*-SGSEs] also converted to their 4-*O*-methyl SGSEs by the similar procedure as above.

Preparation of 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*-syringylglycerol -8-*O*-4′-(sinapyl alcohol) ether (SGSE): To a stirred solution of 4-*O*-methyl ether of (+)-*erythro*-SGSE (3.5 mg, 7.7 μmol) in 2 mL of dry CH_2Cl_2 a solution of dicyclohexylcarbodiimide [(DCC), 19 mg, 92 μmol], (*R*)-(+)-MTPA esters (17 mg, 72 μmol) and dimethylaminopyridine [(DMAP), 8 mg, 65 μmol] in 3 mL of dry CH_2Cl_2 were added at room temperature and then reaction solution was warmed to 35°C. After stirring for 15 h under N_2 atmosphere, the reaction mixture was cooled at room temperature. The mixture was filtered and the residue was washed with CH_2Cl_2. The filtrate and the washings were combined and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (CH_2Cl_2) to afford 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*-SGSE (4.10 mg, 48.4%). The other stereoisomers also converted to their tri-(*R*)-MTPA esters of 4-*O*-methyl SGSEs by the similar procedure as above.

7,9,9'-Tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*-SGSE: ^{1}H NMR ($CDCl_3$): *δ* 3.678 (3H, s, 7-MTPA-OCH_3), 3.423 (3H, s, 9-MTPA-OCH_3), 3.583 (3H, d, J=0.84, 9′-MTPA-OCH_3), 3.616 [(exchangeable), 6H, s, 3 and 5-OCH_3], 3.642 [(exchangeable), 6H, s, 3′ and 5′-OCH_3], 3.795 (3H, s, 4-OCH_3), 4.863 (1H, dd, J=11.94, 6.72 , 9a-H), 4.411 (1H, dd, J=12.12, 3.30, 9b-H), 4.731 (1H, m, 8-H), 6.161 (1H, d, J=3.24, 7-H), 6.204 (1H, dt, J=15.66, 6.60, 8′-H), 6.593 (1H, d, J=15.66, 7′-H), 4.967 (2H, d, J=6.60, 9′-H_2), 6.283 [(exchangeable), 2H, s, 2 and 6-H], 6.531 [(exchangeable), 2H, s, 2′ and 6′-H], 7.30-7.56 (15H, m, MTPA-Ar-H).

7,9,9'-Tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*erythro*-SGSE: ^{1}H NMR ($CDCl_3$): δ 3.370 (3H, s, 7-MTPA-OCH_3), 3.549 (3H, s, 9-MTPA-OCH_3), 3.574 (3H, s, 9′-MTPA-OCH_3), 3.595 [(exchangeable), 6H, s, 3 and 5-OCH_3], 3.764 [(exchangeable), 6H, s, 3′ and 5′-OCH_3], 3.814 (3H, s, 4-OCH_3), 4.625 (1H, dd, J=11.94, 3.42, 9a-H), 4.391 (1H, dd, J=11.91, 4.53, 9b-H), 4.804 (1H, m, 8-H), 6.124 (1H, d, J=6.30, 7-H), 6.143 (1H, dt, J = 15.36, 6.60, 8′-H), 6.530 (1H, d, J=15.42, 7′-H), 4.940 (2H, d, J=6.54, 9′-H_2), 6.412 [(exchangeable), 2H, s, 2 and 6-H], 6.614 [(exchangeable), 2H, s, 2′ and 6′-H], 7.25-7.56 (15H, m, MTPA-Ar-H).

7,9,9'-Tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*threo*-SGSE: ^{1}H NMR ($CDCl_3$): δ 3.458 (3H, s, 7-MTPA-OCH_3), 3.492 (3H, s, 9-MTPA-OCH_3), 3.5772 (3H, d, J=1.08, 9′-MTPA-OCH_3),

3.601 [(exchangeable), 6H, s, 3 and 5-OCH_3], 3.767 [(exchangeable), 6H, s, 3′ and 5′-OCH_3], 3.855 (3H, s, 4-OCH_3), 3.921 (1H, dd, J=12.24, 3.96, 9a-H), 4.517 (1H, dd, J=12.09, 3.57, 9b-H), 4.785 (1H, dt, J=6.30, 3.84, 8-H), 6.297 (1H, dt, J=6.30, 7-H), 6.172 (1H, dt, J=15.96, 6.60, 8′-H), 6.563 (1H, d, J = 15.66, 7′-H), 4.952 (2H, dd, J=6.60, 1.08, 9′-H_2), 6.474 [(exchangeable), 2H, s, 2 and 6-H], 6.643 [(exchangeable), 2H, s, 2′ and 6′-H], 7.30-7.56 (15H, m, MTPA-Ar-H).

7,9,9'-Tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*-SGSE: ^{1}H NMR ($CDCl_3$): δ 3.718 (3H, s, 7-MTPA-OCH_3), 3.641 (3H, s, 9-MTPA-OCH_3), 3.585 (3H, s, 9′-MTPA-OCH_3), 3.507 [(exchangeable), 6H, s, 3 and 5-OCH_3], 3.664 [(exchangeable), 6H, s, 3′ and 5′-OCH_3], 3.806 (3H, s, 4-OCH_3), 4.70-4.78 (3H, m, 8-H, 9a-H and 9b-H), 6.318 (1H, d, J=8.76, 7-H), 6.207 (1H, dt, J=15.72, 6.51, 8′-H), 6.598 (1H, d, J=15.66, 7′-H), 4.968 (2H, d, J=6.6, 9′-H_2), 6.371 [(exchangeable), 2H, s, 2 and 6-H], 6.539 [(exchangeable), 2H, s, 2′ and 6′-H], 7.18-7.61 (15H, m, MTPA-Ar-H).

RESULTS AND DISCUSSION

A mixture of (±)-*erythro*- and (±)-*threo*-SGSEs was prepared by dehydrogenative dimerization of SA with $FeCl_3$ in dioxane-H_2O (10:1) and purified by preparative TLC. (±)-*Erythro*-SGSE and (±)-*threo*-SGSE were separated carefully by preparative TLC and identified by ^{1}H NMR in acetone-d_6 (data not shown) and HPLC using authentic samples (Lourith et al., 2005). The diastereomeric ratio (*erythro*:*threo* = 6:4) was determined by reversed phase HPLC. Their enantiomers were separated by chiral column HPLC to afford (+)- and (–)-*erythro*-SGSEs and (+)- and (–)-*threo*-SGSEs. Each phenolic hydroxyl group of the four stereoisomers was methylated with diazomethane (^{1}H NMR data not shown) separately. The resulting four stereoisomers were converted to 7, 9, 9'-tri-(*R*)-MTPA esters of 4-*O*-methyl SGSEs and their structures were confirmed by ^{1}H NMR spectra in $CDCl_3$. According to the definition of *erythro* and *threo* diastereomers, absolute configuration of *erythro*-SGSE and its 4-*O*-methyl ether is (7*R*, 8*S*) or (7*S*, 8*R*) and that of *threo*-SGSE and its 4-*O*-methyl ether is (7*R*, 8*R*) or (7*S*, 8*S*).

According to the Mosher's method (Dale *et al*., 1969; Dale and Mosher, 1973; Yamaguchi, 1985), because a preferred conformation of the MTPA esters has α-CH_3, the >C=O of the MTPA ester and the benzyl C-H in an eclipsed arrangement, the (*R*)-MTPA-OCH_3 of (*R*)-MTPA ester of an (7*S*)-secondary benzyl alcohol [(*R*, 7*S*)-MTPA ester] is located on the aromatic (3,4,5-trimethoxyphenyl) ring and the C8-H of the X group is on the benzene ring of the MTPA moiety (Fig. 1), that is, the (*R*)-MTPA-OCH_3 and the C8-H receive shielding effects. In contrast, the (*R*)-MTPA-OCH_3 of (*R*)-MTPA ester of an (7*R*)-secondary benzyl alcohol [(*R*, 7*R*)-MTPA ester] is located not on the aromatic ring but on the C8-H and the benzene ring is on the aromatic ring (Fig. 1), that is the (*R*)-MTPA-OCH_3 and the C8-H have no shielding effect. Therefore, the ^{1}H NMR chemical shift (δs) of the (*R*)-MTPA-OCH_3 of the (*R*, 7*S*)-MTPA ester is upfield relative to that (δ_R) of the (*R*)-MTPA-OCH_3 of the (*R*, 7*R*)-MTPA ester. The Δδ value in the Mosher's method was defined as the absolute value of the difference in the ^{1}H chemical shifts of the peak between the diastereomers, $|\delta_S - \delta_R|$. [Note: the relations between (*R*)- and (*S*)-isomers and between (+)- and (–)-isomers are enantiomers, whereas the relations between (*R*)-MTPA ester of (*R*)-isomer and that of (*S*)-isomer and between (*R*)-MTPA ester of (+)-isomer and that of (–)-isomer are diastereomers.]

Furthermore, our empirical rules (Katayama *et al*., 2000, 2005a) on the ^{1}H NMR chemical shifts of (*R*)-MTPA-OCH_3 of arylglycerol-β(8-*O*-4')-aryl ethers are as below. Rule-1, the Δδ values of 7-MTPA-OCH_3s were larger than those of 9-MTPA-OCH_3s, because one diastereomer's

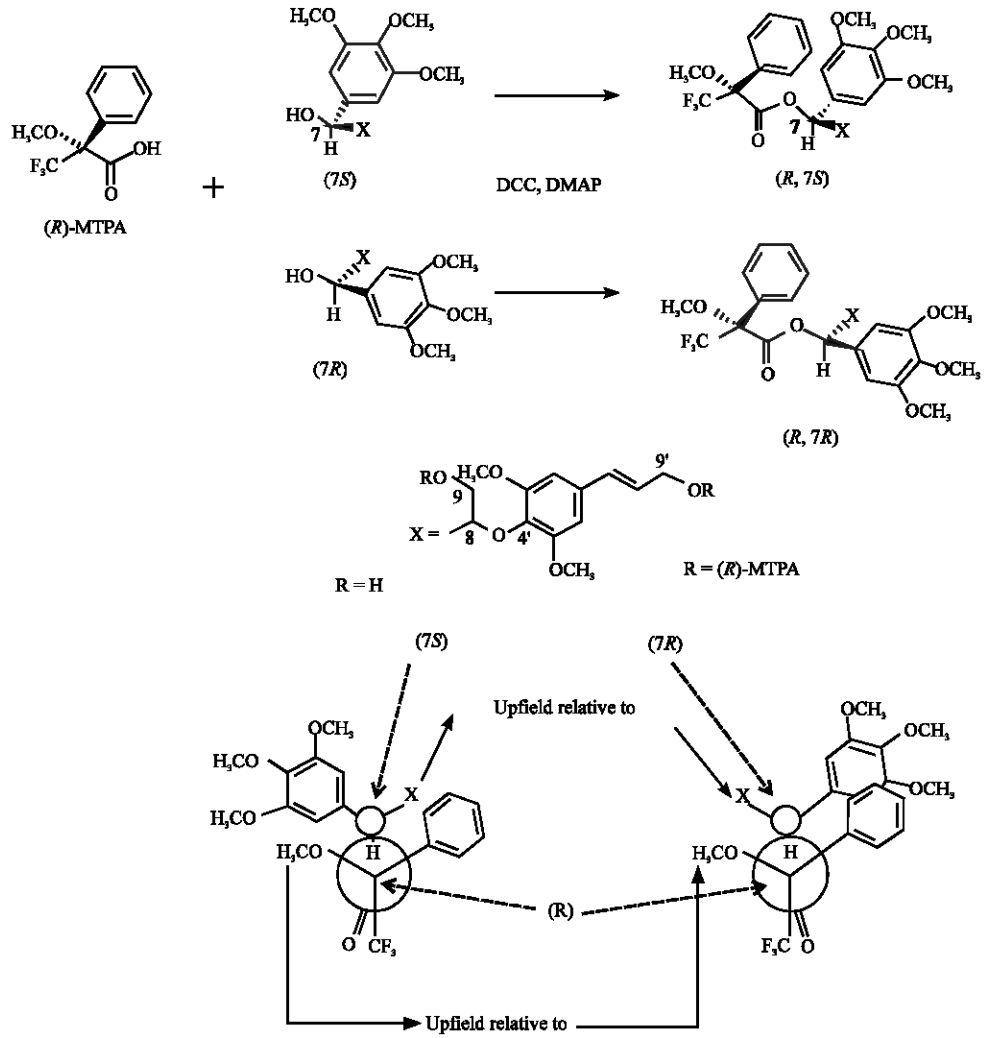

Fig. 1: Reaction of (7*R*)- and (7*S*)-secondary benzyl alcohols in 4-*O*-methylsyringylglycerol-8-*O*-4′-(sinapyl alcohol) ether (SGSE) with (*R*)-(+)-MTPA and preferred conformation of the resulting (*R*, 7*R*) and (*R*, 7*S*)-MTPA esters. The left-hand Newman projection formula (*R*, 7*S*) shows shielding effects of the 3,4,5-trimethoxyphenyl ring on the MTPA-OCH_3 and of the benzene ring on the X moiety. Ether oxygen atoms in the MTPA esters are omitted

7-MTPA-OCH_3s are on the aromatic rings and receive the shielding effect and the others are not on the rings nor have the shielding effect. Such shielding effect was not expected for 9-MTPA-OCH_3s.

Rule-3, 7*S*-Hs of the (*R*)-MTPA esters of the *erythro*-arylglycerol-β(8-*O*-4')-aryl ethers [veratrylglycerol-β-(methyl vanillate) ether, 3,4,5-trimethoxyphenylglycerol-β-(methyl vanillate) ether and 4-*O*-methyl GGCE] that have the shielding effect gave J = 6 Hz, whereas 7*R*-Hs of those of the *erythro*-ones that have not the effect gave J = 4 Hz. And 7*S*-Hs of those of the *threo*-ones that have the shielding effect gave J = 7 Hz, whereas 7*R*-Hs of those of the *threo*-ones that have not the effect gave J = 8.0-8.6 Hz.

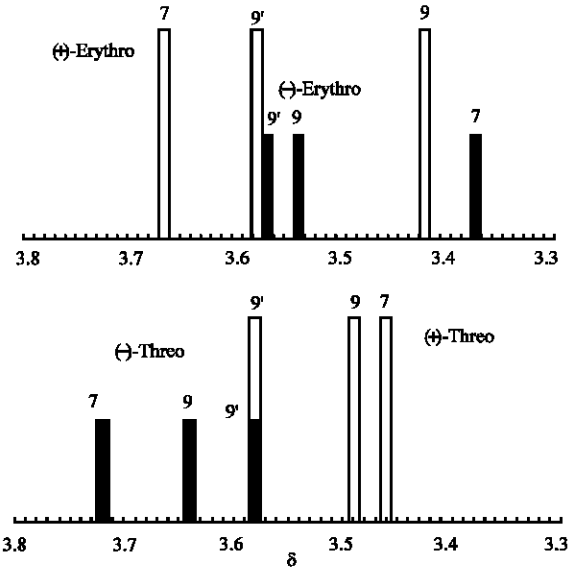

Fig. 2: ^{1}H NMR chemical shifts of 7, 9, 9'-tri-(*R*)-MTPA-OCH$_3$ peaks of 4-*O*-methyl and 7, 9,9'-tri-(*R*)-MTPA ester derivatives of four stereoisomers of syringylglycerol-8-*O*-4´-(sinapyl alcohol) ethers (SGSEs). The long white columns correspond to (+)-*erythro* and (+)-*threo* isomers and short black columns correspond to (–)-*erythro* and (–)-*threo* isomers

Table 1: ^{1}H NMR Chemical shifts (δ) of MTPA-OCH$_3$ peaks of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of synthetic (+)- and (–)-*erythro*- and (+)- and (–)-*threo*-syringylglycerol-8-*O*-4'-(sinapyl alcohol) ethers (SGSE) and coupling constants of their 7-H peaks

	MTPA-OCH$_3$ (δ)			
Stereoisomers	7(α)	9(γ)	9'(γ')	7-H $J_{7,8}$ (Hz)
(+)-*Erythro*	3.6779	3.4229	3.5834	3.24
(–)-*Erythro*	3.3699	3.5489	3.5741	6.30
|Δδ|	0.308	0.126	0.009	
(+)-*Threo*	3.4582	3.4916	3.5772	6.30
(–)-*Threo*	3.7178	3.6409	3.5851	8.76
|Δδ|	0.2596	0.1493	0.0079	

Rule-4, the 9'-MTPA-OCH$_3$s have the smallest Δδ, almost 0, among the three (7, 9 and 9') MTPA-OCH$_3$s, because the 9'-MTPA-OCH$_3$ groups are located farthest from the two chiral centers, 7-C and 8-C, among the three MTPA-OCH$_3$s. Rules 1 and 4 indicate the equation: Δδ (7)>Δδ (9)> Δδ (9') ~ 0.

***Erythro* isomer:** Because Fig. 2 showed that, 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*erythro*-SGSE had a ^{1}Ĥ NMR peak of MTPA-OCH$_3$ markedly upfield, it was suggested that the peak was due to α-MTPA-OCH$_3$ with (7*S*)-configuration and thus 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*-SGSE have (7*R*)-configuration. The assignments in Table 1 were consistent with rules 1, 3 and 4 as follows. The Δδ value for 7-MTPA-OCH$_3$ in the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro* and (–)-*erythro*-SGSEs ($|\delta_R - \delta_S|$) was 0.308 ppm which was apparently larger than that of 9-MTPA-OCH$_3$: 0.126 ppm. The Δδ value for 9'-MTPA-OCH$_3$ in the tri-(*R*)-MTPA esters was 0.009 ppm, that meant almost 0.

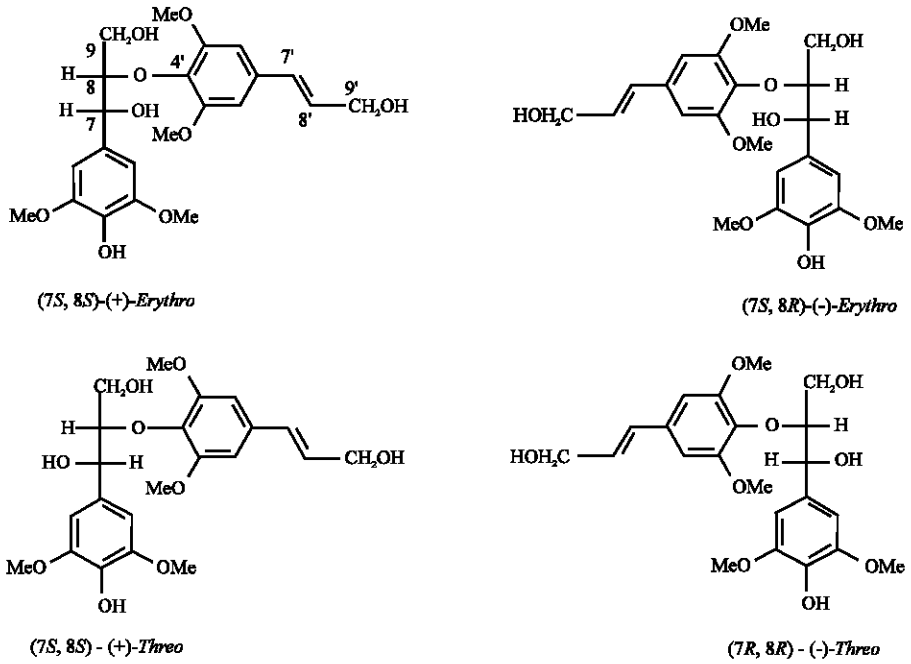

Fig. 3: Absolute configuration of four stereoisomers of syringylglycerol-8-*O*-4′-(sinapyl alcohol) ethers (SGSEs)

Coupling constant (J = 6.30 Hz) of the 7-H in the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*erythro*–SGSE that have the shielding effect was around 6 Hz, which was larger than that (J = 3.24), almost 4 Hz, of the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*–SGSE.

Thus, it was established that the 7-MTPA-OCH_3 of the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*erythro*–SGSE was affected by the shielding effect of the 3,4,5-trimethoxyphenyl ring, whereas that of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*–SGSE was not affected. Consequently, the C7 of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*erythro*–SGSE has an (*S*)-configuration, whereas the C7 of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*erythro*–SGSE has an (*R*)-configuration. So, the absolute configuration of (+)-*erythro*– and (–)-*erythro*–SGSEs are determined to be (7*R*, 8*S*) and (7*S*, 8*R*), respectively (Fig. 3).

***Threo* isomer:** Because Fig. 2 also showed that, 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*threo*-SGSE had a $^1\hat{H}$ NMR peak of MTPA-OCH_3 markedly upfield, it was suggested that the peak was due to α-MTPA-OCH_3 with (7*S*)-configuration and thus 7,9,9'-tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*-SGSE have (7*R*)-configuration. The assignments in Table 1 were consistent with rules 1, 3 and 4 as follows. The Δδ value for 7-MTPA-OCH_3 in the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*- and (+)-*threo*-SGSEs ($|\delta_R - \delta_S|$) was 0.260 ppm which was apparently larger than that of 9-MTPA-OCH_3: 0.149 ppm. The Δδ value for 9'-MTPA-OCH_3 in the tri-(*R*)-MTPA esters was 0.008 ppm, that also meant almost 0.

Coupling constant (J = 6.30 Hz) of the 7-H in the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*threo*-SGSE that have the shielding effect is around 7 Hz, which was larger than that (J = 8.76), almost 8.0-8.6 Hz, of the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*-SGSE.

Thus it was established that the 7-MTPA-OCH_3 of the tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*threo*-SGSE was affected by the shielding effect of the 3,4,5-trimethoxyphenyl ring, whereas

that of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*-SGSE was not affected. Consequently, the C7 of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (+)-*threo*-SGSE has an (*S*)-configuration, whereas the C7 of tri-(*R*)-MTPA esters of 4-*O*-methyl ether of (–)-*threo*-SGSE has an (*R*)-configuration. So, the absolute configuration of (+)-*threo*- and (–)-*threo*-SGSEs are determined to be (7*S*, 8*S*) and (7*R*, 8*R*), respectively (Fig. 3).

Rahman *et al.* (2007) determined absolute configurations of syringyl lignans, (+)- and (–)-5,5'-dimethoxylariciresinols as (8*R*, 8'*R*) and (8*S*, 8'*S*) with (+)- and (–)-5,5'-dimethoxysecoisolariciresinols as (8*S*, 8'*S*) and (8*R*, 8'*R*), respectively, which are intermediates of biosynthesis of lyoniresinol, a syringyl lignan. Katayama *et al.* (2005b) studied absolute configurations of four stereoisomers, (+)-*erythro*-, (–)-*erythro*-, (+)-*threo*- and (–)-*threo*-GGCEs, guaiacyl 8-*O*-4' neolignans and determined them as (7*R*, 8*S*), (7*S*, 8*R*), (7*S*, 8*S*) and (7*R*, 8*R*), respectively. These results were accidentally identical with those of SGSEs in this study. Kasahara *et al.* (1995) also determined absolute configuration of 9,9'-deoxy-8-*O*-4' neolignans. It could be assumed that our present report will play an important role to clarify the formation mechanism of syringyl 8-*O*-4' neolignans.

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