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ABSOLUTE CONFIGURATION OF 8-*O*-4′-NEOLIGNANS FROM *MYRISTICA FRAGRANS*

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Key Word Index—*Myristica fragrans*; Myristicaceae; seeds; (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan; (−)-*erythro*-(7*R*,8*S*)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan; (−)-(8*R*)-$\Delta^{8'}$-4,-hydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan; (+)-(8*S*)-$\Delta^{8'}$-4-hydroxy-3,3′,5′-trimethoxy)-8-*O*-4′-neolignan; absolute configuration; α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA); Mosher's (^{1}H) method.

Abstract—The absolute configuration of (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan and (−)-*erythro*-(7*R*,8*S*)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan was determined by the application of Mosher's (^{1}H) method. Furthermore, $LiAlH_4$ reduction of the MTPA ester of *erythro*-(7*S*,8*R*)-$\Delta^{8'}$-4-acetoxy-7-hydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan afforded (−)-(8*R*)-$\Delta^{8'}$-4-hydroxy-3,3′,5′-trimethoxy)-8-*O*-4′-neolignan, and that of the MTPA ester of *erythro*-(7*R*,8*S*)-$\Delta^{8'}$-4-acetoxy-7-hydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan afforded (+)-(8*S*)-$\Delta^{8'}$-4-hydroxy-3,3′,5′-trimethoxy)-8-*O*-4′-neolignan, respectively.

INTRODUCTION

In a previous report, we investigated the biotransformation of *erythro*-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan [= (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan] (**1**) in rats and by faecal intestinal bacteria, and revealed that **1** was specifically reduced to $\Delta^{8'}$-4-hydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan [= (4-hydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan] (**2**) [1].

Up to now, *erythro*-$\Delta^{8'}$-4, 7-dihydroxy-3,3′,5′- trimethoxy-8-*O*-4′-neolignan (**1**) has been isolated from seeds [2] and arils [3] of *Myristica fragrans*; **2** was obtained from arils [3]. Although a derivative of **1**, (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,5,3′,5′-pentamethoxy-8-*O*-4′-neolignan showed $[\alpha]_D$ + 12 ($CHCl_3$; *c* 1.0), **1** showed $[\alpha]_D^{20}$ + 1.02 ($CHCl_3$; *c* 2.0) and in the previous report [2] showed no optical activity. The specific optical rotation obviously indicated that **1** was obtained from a racemic mixture. However, there are no reports on the absolute configuration and specific optical rotations of **1** and **2**. The present paper deals with the absolute configuration assignment of **1**, **2** and 4-methylated **1** (**3**).

RESULTS AND DISCUSSION

erythro-$\Delta^{8'}$-4,7-Dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan (**1**) was isolated from seeds of *M. fragrans* by previously reported methods [4]. The NMR spectral data clearly corresponded with those of the *erythro*-form isolated from arils of this species [3, 5]. In order to determine the absolute configuration at the C-7 and C-8 positions, **1** was esterified with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) after protection of the phenolic hydroxyl group by methylation. Diastereomers were detected on TLC and readily separated by silica gel CC. The respective enantiomers were obtained by alkali hydrolysis. Each enantiomers was then esterified with (*S*)-(−)-MTPA using the same method. The absolute configurations at the C-7 and C-8 positions were then determined from (*S*)-(−)-MTPA and *R*)-(+)-MTPA ester of 4-methylated **1** (**3**) by Mosher's (^{1}H) method [6, 7].

The (+)-enantiomer of **3** (**3a**) showed $[\alpha]_D^{23}$ + 12.6 $CHCl_3$; *c* 1.0) and $\Delta\delta$ ($\delta_S - \delta_R$) values for the MTPA ester of **3a** as shown in Scheme 1. These $\Delta\delta$ values, except for that of the 8-methine proton, allowed us to assign **3a** as (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan. The $\Delta\delta$ values for the MTPA ester of **3b** were opposite; therefore **3b** was (−)-*erythro*-(7*R*, 8*S*)-$\Delta^{8'}$-7-hydroxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan, **3b** had $[\alpha]_D^{23}$ −12.68 ($CHCl_3$; *c* 2.0).

For the purpose of confirming the optical rotation of **1a** and **1b**, **1** was esterified with (*R*)-(+)-MTPA after acetylation for protection of the phenolic hydroxyl group; the diastereomers were readily separated. Each diastereomer was hydrolysed in the same way, but when refluxed in 5% NaOH in aqueous MeOH, the 7-

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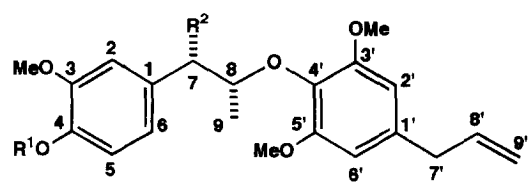
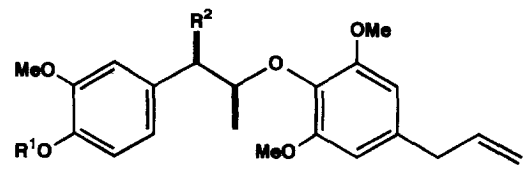
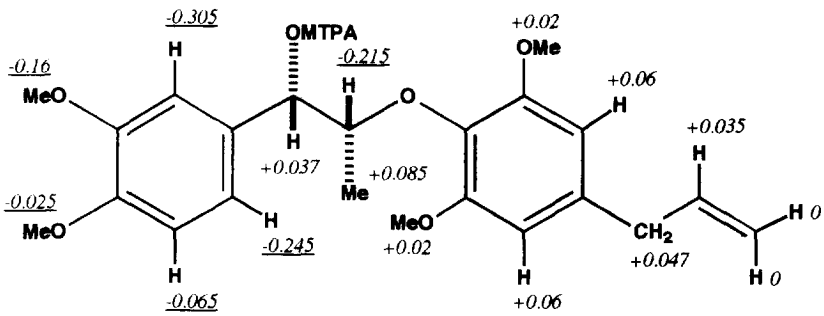


	R^1	R^2
1a	H	OH
2a	H	H
3a	Me	OH
4a	Ac	OH



	R^1	R^2
1b	H	OH
2b	H	H
3b	Me	OH
4b	Ac	OH



Scheme 1. $\Delta\delta$ values for MTPA ester of (+)-erythro-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,3',5'-tetramethoxy-8-*O*-4'-neolignan (**3a**). $\Delta\delta$ values given in PPM.

methoxy derivatives were obtained, instead of **1a** and **1b**. In addition, when each diastereomer was added in 5% NaOH in aqueous EtOH, 7-ethoxy derivatives were obtained immediately at room temperature. So, in order to obtain **1a** and **1b**, each diastereomer was cleaved with $LiAlH_4$. Reaction of the diastereomers with $LiAlH_4$ in diethyl ether afforded **1a** from **2a**, and **1b** from **2b**, respectively. **1a** and **1b** were then esterified with (*S*)-(−)-MTPA after acetylation for the protection of phenolic hydroxyl group and the $\Delta\delta$ values obtained reconfirmed that the absolute configuration of the (+)-enantiomer of **1** was *erythro*-(7*S*,8*R*)-$\Delta^{8'}$-4,7-dihydroxy-3,3',5'-trimethoxy-8-*O*-4'-neolignan (**1a**) and that the (−)-enantiomer of **1** was *erythro*-(7*R*,8*S*)-$\Delta^{8'}$-4,7-dihydroxy-3,3',5'-trimethoxy-8-*O*-4'-neolignan (**1b**). (+)-Enantiomer **1a** had $[\alpha]_D^{20}$ + 25.28 ($CHCl_3$; *c* 0.65,) and (−)-enantiomer **1b** had $[\alpha]_D^{20}$ − 25.53 ($CHCl_3$; *c* 0.75,), respectively.

Compound **2a** obtained from 4-acetylated **1a** was determined to be (−)-$\Delta^{8'}$-4-hydroxy-3,3',5'- trimethoxy)-8-*O*-4'-neolignan and **2b** from 4-acetylated **1b**, (+)-$\Delta^{8'}$-4-hydroxy-3,3',5'-trimethoxy)-8-*O*-4'-neolignan. Thus, **2a** and **2b** were the respective enantiomer and optically active compounds. These facts suggested that dehydroxylation at the 7-position proceeded and that the absolute configuration at the 8-position was unchanged. Therefore, **2a** was determined to be (−)-(8*R*)-$\Delta^{8'}$-4-hydroxy-3,3',5'-trimethoxy)-8-*O*-4'-neolignan and **2b**, (+)-(8S)-$\Delta^{8'}$-4-hydroxy-3,3',5'-trimethoxy)-8-*O*-4'-neolignan.

Considering that *erythro*-$\Delta^{8'}$-4,7-dihydroxy-3,3',5'-trimethoxy-8-*O*-4'-neolignan (**1**) and $\Delta^{8'}$-4-hydroxy-3,3',5'-trimethoxy-8-*O*-4'-neolignan (**2**) possess antibacterial activity [8], it is of importance to extend the study of these neolignans.

EXPERIMENTAL

Preparation of neolignan. erythro-$\Delta^{8'}$-4,7-Dihydroxy-3,3',5'-trimethoxy-8-*O*-4'-neolignan (**1**) was isolated from the seed of *M. fragrans* as reported in ref. [4].

Methylation of **1**. Neolignan **1** (500 mg) was reacted with CH_2N_2 for 3 days in Et_2O at room temp. The product was then purified by silica gel CC and identified as *erythro*-$\Delta^{8'}$-hydroxy-3,4,3',5'-tetramethoxy-8-*O*-4'-neolignan (**3**).

MTPA ester of **3**. Monomethyl ester **3** (500 mg, 1.3 $\times 10^{-3}$ mol), (dimethylamino)pyridine (630 mg, 5.2×10^{-3} mol), and Et_3N (130 mg, 1.3×10^{-3} mol) in 3 mol of $CHCl_3$ was treated with (+)-MTPA chloride (650 mg, 2.6×10^{-3} mol), and stirred at room temp. for 10 hr. After addition of 3-[(dimethylamino)propyl] amine (130 mg, 1.3×10^{-3} mol) to the mixt., solvent was evapd under red. pres. and the residue subjected to silica gel CC (hexane–$CHCl_3$; 10–40%) to give the (+)-MTPA ester of **3a** and **3b**.

Cleavage of (+)-*MTPA ester of* **3a** *and* **3b**. (+)-MTPA ester of **3a** (50 mg) was refluxed in 5% NaOH in aq. MeOH for 3 hr. The soln was then dild with H_2O,

extracted ×3 with Et_2O and the combined Et_2O extracts then evapd under red. pres. The residue was subjected to silica gel CC (hexane–$CHCl_3$; 10–40%) to give **3a** (15 mg). (+)-MTPA ester of **3b** (50 mg) was also treated in the same way and converted to **3b** (16 mg).

Acetylation of **1**. Neolignan **1** (500 mg) was reacted with Ac_2O–pyridine at room temp. for 3 hr. The product was purified by silica gel CC and identified as *erythro*-$\Delta^{8'}$-4-acetoxy-7-hydroxy-3,3′,5′-trimethoxy)-8-*O*-4′-neolignan (**4**).

MTPA ester of **4**. Monoacetate **4** (500 mg, 1.2×10^{-3} mol), (dimethylamino)pyridine (585 mg, 4.8×10^{-3} mol) and Et_3N (121 mg, 1.2×10^{-3} mol) in 3 ml of $CHCl_3$ was treated with (+)-MTPA chloride (600 mg, 2.4×10^{-3} mol), and stirred at room temp. for 10 hr. After addition of 3-[(dimethylamino)propyl]amine (123 mg, 1.2×10^{-3} mol) to the mixt., solvent was evapd under red. pres. and the residue was subjected to silica gel CC (hexane–$CHCl_3$; 10–40%) to give (+)-MTPA ester of **4a** and **4b**.

Cleavage of (+)-MTPA ester of **4a** *and* **4b**. (+)-MTPA ester of **4a** (90 mg) was dissolved, in Et_2O and treated with $LiAlH_4$ (120 mg, 3.16×10^{-3} mol) at room temp. for 3 hr. Then EtOAc was carefully added, washed with 1M HCl, and the solvent evapd under red. pres. The residue was subjected to silica gel CC (hexane–$CHCl_3$; 10–40%) to give **1a** (13 mg) and **2a** (26 mg). (+)-MTPA ester of **4b** (90 mg) was treated in the same way to give **1b** (15 mg) and **2b** (20 mg).

(+)-*Erythro*-(7S,8R)-$\Delta^{8'}$-4, 7-*dihydroxy*-3,3′,5′-*trimethoxy*-8-O-4′-*neolignan* (**1a**). $[\alpha]_D^{20}$ + 25.28 ($CHCl_3$; *c* 0.65,). EIMS (GC, 70 eV) *m/z* (rel. int.): 374 $[M]^+$ (2), 356 (2), 221 (10), 194 (100), 179 (4), 164 (6), 151 (4), 131 (3), 91 (4), 83 (18). IR ν_{max}^{KBr} cm^{-1}: 3497 (OH), 3004, 2980, 2939, 2841, 1639, 1591, 1517, 1504, 1464, 1426, 1272, 1241, 1229, 1126, 1036.

(+)-*Erythro*-(7R,8S)-$\Delta^{8'}$-4, 7-*dihydroxy*-3,3′,5′-*trimethoxy*-8-O-4′-*neolignan* (**1b**). $[\alpha]_D^{20}$ − 25.53 $CHCl_3$; *c* 0.75).

(−)-(8R)-$\Delta^{8'}$-4-*Hydroxy*-3,3′,5′-*trimethoxy*)-8-O-4′-*neolignan* (**2a**). $[\alpha]_D^{23}$ − 7.0 ($CHCl_3$; *c* 0.2,). EIMS (GC, 70 eV) *m/z* (rel. int.): 358$[M]^+$ (2), 256 (18), 194 (58), 165 (100), 137 (11), 103 (10). IR ν_{max}^{KBr} cm^{-1}: 3491 (OH), 2935, 2839, 1589, 1516, 1504, 1463, 1423, 1270, 1240, 1037.

(+)-(8S)-$\Delta^{8'}$-4-*Hydroxy*-3,3′,5′-*trimethoxy*)-8-O-4′-*neolignan* (**2b**). $[\alpha]_D^{23}$ + 10.0 ($CHCl_3$; *c* 0.45).

(+)-*Erythro*-(7S,8R)-$\Delta^{8'}$-7-*hydroxy*-3,4,3′,5′-*tetramethoxy*-8-O-4′-*neolignan* (**3a**). $[\alpha]_D^{23}$ + 12.6 ($CHCl_3$; *c* 1.0). ^1H NMR δ($CDCl_3$, 270.05 MHz): 6.96 *d* (1H, *J* = 1.5 Hz, H-2), 6.80 *d* (1H, *J* = 8 Hz, H-5), 6.77 *dd* (1H, *J* = 1.5, 8 Hz, H-6), 4.81 *d* (1H, *J* = 2, H-7), 4.36 *ddd* (1H, *J* = 3, 6.5, 13 Hz, H-8), 1.13 *d* (3H, *J* = 6.5, H-9), 6.46 *s* (2H, H-2′, 6′), 3.36 *d* (2H, *J* = 6.5, H-7′), 5.98 *m* (1H, H-8′), 5.11 *ddd* (1H, *J* = 1.5, 3, 10, H-9′a), 5.13 *ddd* (1H, *J* = 2, 3.5, 10, H-9′b), 4.12 *d* (1H, *J* = 2, OH), 3.88 *s* (3H, 3-OMe), 3.84 *s* (3H, 4-OMe), 3.87 *s* (6H, 3′, 5′-OMe). HRMS *m/z*: 388.1915 ($[M]^+$, calcd for $C_{22}H_{28}O_6$: 388.1886). EIMS (GC, 70 eV) *m/z* (rel. int.): 388 $[M]^+$ (4), 370 (32), 327 (10), 296 (20), 286 (8), 194 (100), 178 (15), 164 (6), 151 (33), 131 (7). IR ν_{max}^{KBr} cm^{-1}: 3484 (OH), 2939, 1590, 1516, 1463, 1421, 1265, 1228, 1029.

(−)-*Erythro*-(7S, 8R)-$\Delta^{8'}$-7-*hydroxy*-3,4,3′,5′-*tetramethoxy*-8-O-4′-*neolignan* (**3b**). $[\alpha]_D^{23}$ − 12.68 ($CHCl_3$; *c* 2.0).

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