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SESQUITERPENE DIMERS FROM *CHLORANTHUS JAPONICUS**

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Key Word Index—*Chloranthus japonicus*; Chloranthaceae; roots; sesquiterpene dimer; macrocyclic ester; lindenane skeleton; 2D NMR.

Abstract—Five novel dimeric sesquiterpenes, shizukaols E–I, consisting of two lindenane units were isolated from the roots of *Chloranthus japonicus*. Their structures were elucidated principally by 1D- and 2D-NMR methods. Three of them have a unique pendent macrocyclic ester ring found previously in shizukaol B.

INTRODUCTION

In the course of our continuing search for sesquiterpenes in plants of the Chloranthaceae, a series of unusual sesquiterpene lactones having a lindenane skeleton have been isolated from *Chloranthus japonicus* Sieb. [2–4] and *C. serratus* Roem. et Schult. [4, 5]. Lindenane sesquiterpenes are characteristic constituents of the chloranthaceous plants [6]. Recently, a series of dimeric lindenanes, shizukaols A (**1**), B (**2**), C (**3**) and D (**4**) [7, 8] and cycloshizukaol A (**5**) [1], were isolated from *Chloranthus* spp. Further investigation of the chemical constituents of *C. japonicus* yielded five novel lindenane dimers named shizukaols E (**6**), F (**7**), G (**8**), H (**9**) and I (**10**).

RESULTS AND DISCUSSION

Shizukaol E (**6**) showed a molecular ion at *m/z* 562 (FD-MS) and the molecular formula was determined to be $C_{33}H_{38}O_8$ with the aid of the ^1H and ^{13}C NMR data. The presence of a significant fragment peak at *m/z* 274 in the FD-mass spectrum indicated **6** to be a lindenane dimer as found in the previously known shizukaols [7, 8]. The ^1H NMR spectrum of **6** (Table 1) was quite similar to that of shizukaol D (**4**) [8] except that the 13′-hydroxymethyl signal disappeared and an allyl methyl (δ_H 1.80) appeared in place of it. The ^{13}C NMR (Table 2) showed a methyl signal in the high field region (δ8.6), characteristic of 13-methyl in the series of lindenane sesquiterpene lactones [2, 5–8]. The difference in molecular formula of the two compounds is one oxygen. Hence, **6** was concluded to be 13′-deoxyshizukaol D.

Shizukaol F (**7**) had a molecular formula of $C_{40}H_{44}O_{13}$ (FD-MS: $[M]^+$ at *m/z* 732) and should, therefore, be an isomer of shizukaol B (**2**) [8]. The ^1H NMR spectrum of **7** was similar to that of **2** except that the olefinic proton in the hydroxytiglate residue of **2** (δ6.62, *br dd*, J = 6.9 and 4.7 Hz) was shifted upfield and sharpened in **7** (δ5.98, *br s*). Furthermore, the non-equivalent methylene protons included in the hydroxytiglyl moiety in **2** coupled to the olefinic proton were replaced with an isolated methylene (δ4.38 and 5.06, each *br d*, J = 17.1 Hz) in **7**. These facts indicated that **7** had a γ-hydroxysenecioic acid moiety ($-OCH_2-C(Me)=CH-CO-$) in place of the γ-hydroxytiglic acid moiety ($-OCH_2-C(Me)=CH-CO-$) in **2**. The stereochemistry of the double bond was shown to be the *E*-configuration by the presence of an NOE between the oxymethylene (H-d) and olefinic (H-b) protons. The assignments of two pairs of methylene protons in the succinic ester residue were determined by the results of the heteronuclear NOE experiments [9]. The irradiation of the outer pair (δ2.62, *ddd*, J = 16.2, 6.4, and 4.5 Hz, and 2.94, *ddd*, J = 16.2, 9.7 and 4.5 Hz) caused the enhancement of a carbonyl carbon at δ171.7 (C-f) which was unambiguously assigned through an HMBC cross peak with the methylene protons (H-d) of the hydroxysenecioic residue. On the other hand, the irradiation of the inner pair (δ2.78, *ddd*, J = 17.4, 9.7 and 4.5 Hz, and 2.86, *ddd*, J = 17.4, 6.4 and 4.5 Hz) enhanced the intensity of the other carbonyl (C-i, δ172.0) which was also assigned by an HMBC correlation with H-13′. The details for the heteronuclear NOE experiments will be discussed elsewhere.

The FD-mass spectrum of shizukaol G (**8**) showed a molecular ion peak at *m/z* 748 giving a molecular formula of $C_{40}H_{44}O_{14}$. The ^1H NMR spectrum was quite similar to that of shizukaol B (**2**) except for the relatively less complex pattern of the side chain methylene region

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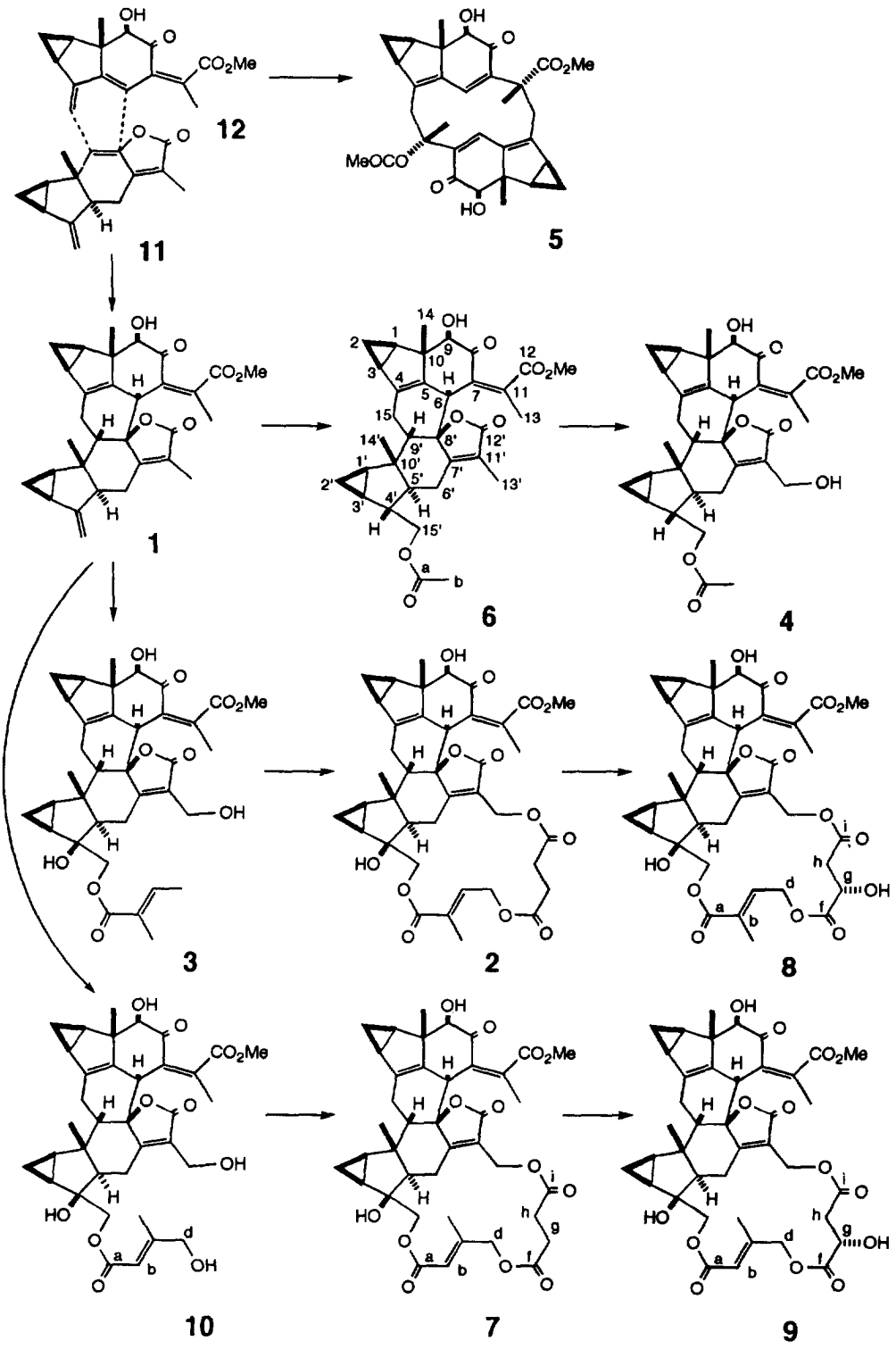

(δ2.5–3.0) compared to that of **2**. An oxymethine (H-g, δ4.63, *ddd*, J = 7.0, 6.8 and 2.6 Hz) and its coupled methylene (H-h, δ2.90, *dd*, J = 17.7 and 2.6 Hz and 2.98, *dd*, J = 17.7 and 7.0 Hz) indicated the presence of a malic ester moiety in place of the succinic ester in **2**. The orientation of the malic residue was again determined by a heteronuclear NOE (H-h → C-i) as well as by an HMBC cross-peak between OH-g (δ3.84, *d*, J = 6.8 Hz) and C-f (δ173.4). The presence of a malic ester residue and its absolute configuration were confirmed as follows. Alkaline hydrolysis of **8** with cesium carbonate followed by methylation and subsequent esterification with an

Table 1. ^{1}H NMR spectral data of compounds **6–10** (500 MHz, $CDCl_3$)

	6	**7**	**8**	**9**	**10**
1	2.04 *ddd* (7.4, 5.5, 3.9)	2.08 *ddd* (8.3, 5.7, 4.3)	2.06 *ddd* (8.5, 6.8, 3.1)	2.08 *ddd* (8.4, 5.7, 4.0)	2.08 *ddd* (8.2, 5.9, 4.2)
2α	0.98 *ddd* (7.4, 7.1, 3.2)	1.02 *ddd* (8.3, 8.0, 4.5)	1.02 *ddd* (8.6, 8.5, 4.7)	1.03 *ddd* (9.1, 8.4, 4.2)	1.01 *ddd* (8.2, 7.4, 4.1)
2β	0.27 *ddd* (3.9, 3.4, 3.2)	0.38 *ddd* (4.5, 4.3, 3.8)	0.32 *ddd* (5.0, 4.7, 3.1)	0.34 *ddd* (4.2, 4.2, 4.0)	0.33 *ddd* (4.2, 4.1, 3.2)
3	1.83 *ddd* (7.1, 5.5, 3.4)	1.86 *ddd* (8.0, 5.7, 3.8)	1.86 *ddd* (8.6, 6.8, 5.0)	1.86 *ddd* (9.1, 5.7, 4.2)	1.87 *ddd* (7.4, 5.9, 3.2)
6	3.87 *br d* (3.3)	3.95 *br d* (3.8)	3.95 *br d* (2.5)	3.93 *br d* (3.5)	3.93 *br d* (4.0)
9	4.05 *s*	3.95 *s*	3.89 *s*	3.97 *s*	4.00 *s*
13	1.80 *s*	1.93 *s*	1.87 *s*	1.85 *s*	1.92 *s*
14	1.01 *s*	1.01 *s*	1.03 *s*	1.01 *s*	1.00 *s*
15α	2.72 *dd* (16.5, 1.7)	2.82 *dd* (16.2, 1.6)	2.78 *dd* (16.3, 1.6)	2.77 *dd* (16.3, 1.5)	2.82 *dd* (16.3, 1.5)
15β	2.61 *ddd* (16.5, 5.7, 3.3)	2.57 *ddd* (16.2, 6.0, 3.8)	2.59 *ddd* (16.3, 5.9, 2.5)	2.59 *ddd* (16.3, 6.0, 3.5)	2.57 *ddd* (16.3, 6.2, 4.0)
1′	1.41 *ddd* (8.3, 8.1, 4.2)	1.63 *ddd* (8.7, 7.4, 4.0)	1.60 *ddd* (8.5, 7.6, 3.6)	1.59 *ddd* (8.8, 8.0, 4.4)	1.63 *ddd* (8.4, 7.8, 3.9)
2′α	0.76 *ddd* (8.3, 8.2, 5.4)	0.73 *ddd* (8.9, 8.7, 5.8)	0.73 *ddd* (9.3, 8.5, 4.8)	0.73 *ddd* (8.8, 8.8, 5.6)	0.71 *ddd* (9.1, 8.4, 5.7)
2′β	0.83 *ddd* (5.4, 4.2, 4.2)	1.31 *ddd* (5.8, 4.0, 3.7)	1.33 *ddd* (4.8, 4.7, 3.6)	1.33 *ddd* (5.6, 4.4, 3.7)	1.28 *ddd* (5.7, 3.9, 3.6)
3′	1.09 *dddd* (8.2, 8.1, 4.2, 3.2)	1.40 *ddd* (8.9, 7.4, 3.7)	1.39 *ddd* (9.3, 7.6, 4.7)	1.38 *ddd* (8.8, 8.0, 3.7)	1.47 *ddd* (9.1, 7.8, 3.6)
4′	1.57 *dddd* (11.1, 8.0, 6.8, 3.2)	—	—	—	—
5′	1.75 *ddd* (13.4, 11.1, 6.1)	1.96 *dd* (13.5, 6.4)	1.86 *dd* (13.6, 6.1)	1.86 *m*	1.92 *dd* (13.6, 6.0)
6′α	2.40 *ddq* (18.0, 6.1, 2.0 (q))	2.67 *br dd* (19.2, 6.4)	2.60 *dd* (18.9, 6.1)	2.84 *m*	2.32 *br dd* (18.3, 6.0)
6′β	2.25 *dd* (18.0, 13.4)	2.78 *dd* (19.2, 13.5)	2.81 *dd* (18.9, 13.6)	2.82 *m*	2.72 *dd* (18.3, 13.6)
9′	1.82 *dd* (5.7, 1.7)	1.94 *dd* (6.0, 1.6)	1.81 *dd* (5.9, 1.6)	1.80 *dd* (6.0, 1.5)	1.92 *dd* (6.2, 1.5)
13′	1.80 *br s*	4.71 *d* (12.6)	4.43 *d* (11.9)	4.55 *d* (12.4)	4.33 *d* (13.1)
13′	—	4.91 *dd* (12.6, 1.0)	5.14 *d* (11.9)	5.02 *d* (12.4)	4.40 *d* (13.1)
14′	0.61 *s*	0.80 *s*	0.82 *s*	0.79 *s*	0.87 *s*
15′	3.77 *dd* (11.0, 8.0)	3.51 *d* (11.8)	3.62 *d* (11.9)	3.45 *d* (12.0)	3.81 *d* (11.8)
15′	3.95 *dd* (11.0, 6.8)	4.79 *d* (11.8)	4.63 *d* (11.9)	4.82 *d* (12.0)	4.26 *d* (11.8)
OMe	3.79 *s*	3.76 *s*	3.69 *s*	3.75 *s*	3.76 *s*
b	2.09 *s*	5.98 *br q* (1.4)	—	6.26 *q* (1.4)	6.01 *q* (1.0)
c	—	—	6.73 tq (5.8 (*t*), 1.0 (*q*))	—	—
d	—	4.38 *br d* (17.1)	4.91 *dd* (14.9, 5.8)	4.43 *br d* (17.2)	4.18 *s*
d	—	5.06 *br d* (17.1)	5.17 *dd* (14.9, 5.8)	5.26 *br d* (17.2)	—
e	—	2.14 *s*	1.93 *d* (1.0)	2.17 *s*	2.11 *d* (1.0)
g	—	2.62 *ddd* (16.2, 6.4, 4.5)	4.63 *ddd* (7.0, 6.8, 2.6)	4.54 *ddd* (9.4, 4.4, 4.0)	—
g	—	2.94 *ddd* (16.2, 9.7, 4.5)	—	—	—
h	—	2.78 *ddd* (17.4, 9.7, 4.5)	2.90 *dd* (17.7, 2.6)	3.05 *dd* (17.7, 4.4)	—
h	—	2.86 *ddd* (17.4, 6.4, 4.5)	2.98 *dd* (17.7, 7.0)	3.08 *dd* (17.7, 4.0)	—
g-OH	—	—	3.84 *d* (6.8)	3.97 *d* (9.4)	—

Table 2. ^{13}C NMR spectral data of compounds **6**–**10** (125 MHz, $CDCl_3$)

	6	7	8	9	10
1	25.4	26.4	25.9	25.9	26.0
2	15.8	16.1	16.0	16.0	16.0
3	24.7	24.9	24.8	24.8	24.8
4	142.3	142.7	142.4	142.7	142.5
5	131.5	132.9	132.0	132.0	132.1
6	40.5	41.7	40.9	41.1	41.0
7	131.8	130.5	131.6	130.9	131.2
8	200.6	200.2	200.9	200.5	200.0
9	80.1	79.7	80.0	80.0	79.7
10	51.2	51.0	50.9	51.1	51.0
11	147.1	147.5	147.4	147.6	147.2
12	170.8	170.4	170.5	171.0	171.6
13	20.3	19.9	19.8	19.9	20.4
14	15.1	15.6	15.2	15.3	15.5
15	25.6	25.4	25.4	25.4	25.2
1′	24.3	26.2	25.6	26.0	25.6
2′	16.6	11.7	11.7	11.9	11.7
3′	21.8	27.5	27.8	27.4	28.3
4′	43.0	77.8	77.2	78.0	77.4
5′	59.3	61.0	61.4	61.6	60.1
6′	25.0	24.7	24.0	25.1	22.6
7′	165.5	174.2	175.3	175.5	168.6
8′	92.5	93.5	93.4	93.4	93.4
9′	54.7	54.8	55.9	55.6	55.0
10′	44.0	44.8	45.0	44.6	44.8
11′	124.2	123.5	122.9	122.6	127.2
12′	173.4	171.5	171.8	171.7	172.4
13′	8.6	55.2	54.8	55.7	54.8
14′	23.9	25.8	26.1	26.1	26.3
15′	66.2	71.1	72.2	71.2	71.4
OMe	52.6	52.5	52.8	53.0	52.9
a	171.0	166.1	167.3	166.4	166.8
b	20.8	112.8	129.0	113.7	112.4
c	—	154.0	135.7	152.9	159.9
d	—	65.6	62.3	66.1	67.1
e	—	15.6	13.1	15.7	15.7
f	—	171.7	173.4	172.3	—
g	—	29.1	67.1	67.8	—
h	—	29.0	38.8	39.1	—
i	—	172.0	170.6	170.9	—

optically active (−)-MTPA chloride [10] of the resultant acidic products gave a mixture of the MTPA esters of dimethyl malate and methyl γ-hydroxytiglate which were easily separated by preparative TLC. The ^1H NMR spectrum of the resulting (*S*)-MTPA-dimethyl malate was superimposable on that of the authentic (*S*)-MTPA ester of dimethyl L-malate. Hence, the stereochemistry of the malic ester residue of **8** was elucidated to be the L-form.

Shizukaol H (**9**) was an isomer of **8**. Analysis of the NMR spectra revealed the difference in structure of **9** and **8** corresponded to that of shizukaols F (**7**) and B (**2**). Thus, shizukaol H contained γ-hydroxysenecioic and malic acids in the macrocyclic ester chain. The selective INEPT experiments [11] supported the direction of the malic residue by the fact that the C-f peak (δ172.3) appeared when a hydroxyl proton (δ3.97, *d*, *J* = 9.4 Hz, OH-g) was selectively excited.

Shizukaol I (**10**) showed a molecular ion at *m/z* 650 by FD-mass spectrometry. The molecular formula was concluded to be $C_{36}H_{42}O_{11}$ with the help of the NMR data. The ^1H NMR spectrum showed the lack of C_4-acid unit found in shizukaols B and F–H. The presence of a γ-hydroxysenecioic residue was supported by the similarity in the NMR pattern compared to that of shizukaol F (**7**). Therefore, it was concluded that **10** was a des-succinyl derivative of **7**.

So far, 10 lindenane dimers have been isolated from *Chloranthus* spp. Nine of them seem to be from the same biogenetic route starting from a Diels–Alder-type cycloaddition of **11** and **12**. An increasing number of naturally occurring Diels–Alder reaction products have been identified and the possibility for the enzyme-catalysed biosynthesis of such a molecule has recently been reported [12]. This biogenetic route for the series of lindenane dimers also strongly suggests the presence of the biological Diels–Alder reaction since consecutive hydroxylation and acylation occurred after the cyclo-addition. In addition, four dimers, shizukaols B (**2**), F (**7**), G (**8**) and H (**9**), carried an unusual macrocyclic ester ring as a pendent side chain composed of both C_5-hydroxy acid, γ-hydroxytiglic (**2**, **8**) or γ-hydroxysenecioic (**7**, **9**) acid, and C_4-dicarboxylic acid, succinic (**2**, **7**) or L-malic (**8**, **9**) acid.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured in $CDCl_3$ using TMS as int. standard. The air-dried roots (400 g) of *C. japonicus* were extracted with Et_2O at room temp. The extracts were washed with 5% $NaHCO_3$ and chromatographed over silica gel using an Et_2O–Me_2CO gradient. The first half of the Et_2O eluates were subjected to silica gel prep. TLC developed with hexane–EtOAc (2:1) to give **6** (18 mg), whereas prep. TLC ($CHCl_3$–MeOH, 50:1) of the latter half gave **7** (72 mg). The Et_2O–Me_2CO (9:1) and (7:3) eluates were separated by prep. TLC (Et_2O–MeOH, 25:1) to give **8** (25 mg), **9** (2 mg) and **10** (18 mg), respectively.

Shizukaol E (**6**). Oil, $[\alpha]_D^{23}$ −184° ($CHCl_3$; *c* 1.09); FD-MS *m/z* (rel. int.): 562 $[M]^+$ (100), 274 (14).

Shizukaol F (**7**). Oil, $[\alpha]_D^{23}$ −80° ($CHCl_3$; *c* 2.50); FD-MS *m/z* (rel. int.): 732 $[M]^+$ (41), 274 (100).

Shizukaol G (**8**). Oil, $[\alpha]_D^{23}$ −100° ($CHCl_3$; *c* 3.36); FD-MS *m/z* (rel. int.): 748 $[M]^+$ (100), 274 (79).

Shizukaol H (**9**). Oil, $[\alpha]_D^{23}$ −121° ($CHCl_3$; *c* 0.29); FD-MS *m/z* (rel. int.): 748 $[M]^+$ (100), 274 (77).

Shizukaol I (**10**). Oil, $[\alpha]_D^{23}$ −122° ($CHCl_3$; *c* 3.40); FD-MS *m/z* (rel. int.): 650 $[M]^+$ (100), 274 (94).

Determination of the absolute configuration of the malic residue in compound **8**. To a soln of **8** (2.8 mg) in 3 drops of MeOH was added 3 drops of 5% Cs_2CO_3 in MeOH–H_2O (3:1). After 2 hr, the reaction mixture was acidified (1 M HCl) and extracted with EtOAc. The extract was treated with ethereal CH_2N_2. After evapn, the residue was dissolved in dry pyridine (0.2 ml) and (−)-

MTPA chloride (30 μl) was added to it. The reaction mixture was left overnight and the usual work-up yielded the mixture of (*S*)-MTPA esters which were easily sepd by prep. TLC ($CHCl_3$) to give (*S*)-MTPA ester of dimethyl malate (0.3 mg) as an oil. EI-MS *m/z* (rel. int.): 348 $[M]^+$ (0.2), 309 (2), 216 (4), 189 (100). ^1H NMR (500 MHz, $CDCl_3$): δ2.88 (1H, *dd*, J = 16.7 and 8.8 Hz), 2.95 (1H, *dd*, J = 16.7 and 4.0 Hz), 3.60 (3H, *s*), 3.64 (3H, *s*), 3.82 (3H, *s*), 5.72 (1H, *dd*, J = 8.8 and 4.0 Hz), 7.41 (3H, *m*), 7.62 (2H, *m*). The physicochemical data agreed well with those of the authentic (*S*)-MTPA ester of dimethyl L-malate prepared from L-malic acid, but did not agree with those of the corresponding D-malate derivative. EI-MS *m/z* (rel. int.): 348 $[M]^+$ (0.3), 309 (2), 216 (4), 189 (100). ^1H NMR (500 MHz, $CDCl_3$): δ2.93 (1H, *dd*, J = 16.9 and 9,2 Hz), 3.02 (1H, *dd*, J = 16.9 and 3.7 Hz), 3.56 (3H, *s*), 3.70 (3H, *s*), 3.77 (3H, *s*), 5.71 (1H, *dd*, J = 9.2 and 3.7 Hz), 7.42 (3H, *m*), 7.58 (2H, *m*).

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