Monohydroxy-Substituted Polyunsaturated Fatty Acids from *Swertia japonica*

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Fourteen monohydroxy-substituted polyunsaturated fatty acids, including two new compounds, (9*Z*,12*S*,13*E*,15*Z*)-12-hydroxyoctadeca-9,13,15-trienoic acid (**10**) and (9*Z*,12*Z*,14*E*,16*R*)-16-hydroxyoctadeca-9,12,14-trienoic acid (**13**), and 12 known ones, *i.e.*, **1**–**9**, **11**, **12**, and **14**, were isolated from the whole plants of *Swertia japonica* MAKINO, and characterized as the corresponding methyl esters **1a**–**14a**. Their structures were elucidated by analysis of the corresponding spectroscopic data, and the absolute configurations of **10a** and **13a** were determined by the *Mosher*-ester method. The CD spectra (*Table*) of compounds **1a**–**14a** are briefly discussed. This is the first report on the isolation of monohydroxy-substituted polyunsaturated fatty acids from the *Swertia* genus in Gentianaceae.

Introduction. – The whole plant of *Swertia japonica* MAKINO (Gentianaceae) is the crude drug *Swertia* herb, used as a stomachic or stimulant of appetite in Japan [1]. In previous articles, we reported the structure determination of fifteen new secoiridoid glycosides [2–4], a new unsaturated alcohol glycoside [3], and a new lignan glycoside [3], all isolated from the whole plants of *S. japonica*. Here, we describe the isolation and structure elucidation of the monohydroxy-substituted polyunsaturated fatty acids contained in the whole plants of *S. japonica*.

Results and Discussion. – The dried whole plants of *S. japonica* were extracted with MeOH. The MeOH extract was partitioned between H_2O and $CHCl_3$, and the $CHCl_3$-soluble fraction was subjected to column chromatography (silica gel) to afford a fraction containing the free acids **1**–**14**. The latter was treated with (trimethylsilyl)-diazomethane (=(diazomethyl)trimethylsilane; $TMSCHN_2$) to prepare the corresponding methyl esters, which were purified by column chromatography (silica gel) followed by prep. HPLC (chiral column) to furnish the fourteen monohydroxy-substituted polyunsaturated fatty acid methyl esters **1a**–**14a**.

The known compounds were identified as (9*R*,10*E*,12*Z*)-9-hydroxyoctadeca-10,12-dienoic acid (**1**) [5], (9*S*,10*E*,12*Z*)-9-hydroxyoctadeca-10,12-dienoic acid (**2**) [6], (9*R*,10*E*,12*Z*, 15*Z*)-9-hydroxyoctadeca-10,12,15-trienoic acid (**3**) [5], (9*S*,10*E*,12*Z*, 15*Z*)-9-hydroxyoctadeca-10,12,15-trienoic acid (**4**) [7], (9*Z*,11*E*,13*R*)-13-hydroxyoctadeca-9,11-dienoic acid (**5**) [8], (9*Z*,11*E*,13*S*)-13-hydroxyoctadeca-9,11-dienoic acid (**6**) [6], (9*Z*,11*E*,13*R*,15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (**7**) [9], (9*Z*,11*E*,13*S*, 15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (**8**) [6], (9*Z*,12*R*,13*E*,15*Z*)-12-hydroxyoctadeca-9,13,15-trienoic acid (**9**) [7], (9*E*,11*E*,13*R*)-13-hydroxyoctadeca-9,11-dienoic

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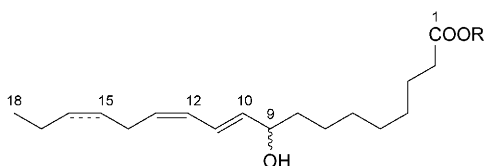
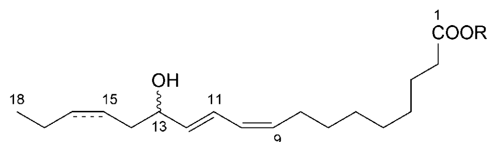
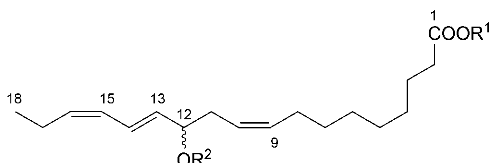


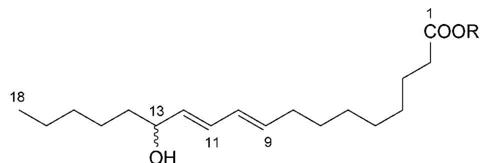
1	(9*R*)	R = H
1a	(9*R*)	R = Me
2	(9*S*)	R = H
2a	(9*S*)	R = Me
3	(9*R*)	R = H, Δ^{15}
3a	(9*R*)	R = Me, Δ^{15}
4	(9*S*)	R = H, Δ^{15}
4a	(9*S*)	R = Me, Δ^{15}



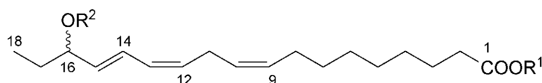
5	(13*R*)	R = H
5a	(13*R*)	R = Me
6	(13*S*)	R = H
6a	(13*S*)	R = Me
7	(13*R*)	R = H, Δ^{15}
7a	(13*R*)	R = Me, Δ^{15}
8	(13*S*)	R = H, Δ^{15}
8a	(13*S*)	R = Me, Δ^{15}



9	(12*R*)	R^1 = H, R^2 = H
9a	(12*R*)	R^1 = Me, R^2 = H
9b	(12*R*)	R^1 = Me, R^2 = (*R*)-MTPA
10	(12*S*)	R^1 = H, R^2 = H
10a	(12*S*)	R^1 = Me, R^2 = H
10b	(12*S*)	R^1 = Me, R^2 = (*R*)-MTPA



11	(13*R*)	R = H
11a	(13*R*)	R = Me
12	(13*S*)	R = H
12a	(13*S*)	R = Me



13	(16*R*)	R^1 = H, R^2 = H
13a	(16*R*)	R^1 = Me, R^2 = H
13b	(16*R*)	R^1 = Me, R^2 = (*R*)-MTPA
14	(16*S*)	R^1 = H, R^2 = H
14a	(16*S*)	R^1 = Me, R^2 = H
14b	(16*S*)	R^1 = Me, R^2 = (*R*)-MTPA

acid (**11**) [10], (9*E*,11*E*,13*S*)-13-hydroxyoctadeca-9,11-dienoic acid (**12**) [10], and (9*Z*,12*Z*,14*E*,16*S*)-16-hydroxyoctadeca-9,12,14-trienoic acid (**14**) [6] by comparison of their physical and spectroscopic data with the published data.

Compound **10a** had the molecular formula $C_{19}H_{32}O_3$, based on the HR-EI-MS ($M^{+\bullet}$ at *m*/*z* 308.2360). The IR spectrum showed the presence of an OH (3602 cm^{-1}) and an ester group (1731 cm^{-1}). In the UV spectrum (*Table*), the typical absorption maximum of a conjugated diene appeared at 232 nm [11]. Comparison of the ^{1}H- and ^{13}C-NMR

spectra ($CDCl_3$), EI-MS, and circular-dichroism (CD) data (*Table*) of **10a** with those of ester **9a** established their enantiomeric relationship.

Table. *UV and CD Data of Compounds* **1a** – **14a**

	Configuration of stereogenic center	UV λ_{max} (log ε)[a])	CD $\Delta\varepsilon$ (λ)[a])
1a	*R*	232 (4.2)	−1.6 (232)
2a	*S*	232 (4.2)	+1.8 (233)
3a	*R*	234 (4.3)	−1.9 (234)
4a	*S*	233 (4.0)	+1.8 (235)
5a	*R*	232 (4.4)	−2.6 (231)
6a	*S*	232 (4.4)	+2.7 (232)
7a	*R*	232 (4.4)	−0.4 (243)
8a	*S*	232 (4.4)	+0.5 (239)
9a	*R*	232 (4.2)	−0.2 (243)
10a	*S*	232 (4.3)	+0.3 (243)
11a	*R*	229 (4.0)	−0.8 (229)
12a	*S*	229 (4.0)	+0.7 (228)
13a	*R*	229 (4.0)	−0.8 (229)
14a	*S*	229 (4.0)	+0.7 (228)

[a]) Measurements were carried out in MeOH. λ in nm.

The ^{1}H- and ^{13}C-NMR spectra of **10a** exhibited signals due to one Me group (δ(H) 1.00 (*t*, $J = 7.6$ Hz); δ(C) 14.2 (Me)), one MeO group (δ(H) 3.67 (*s*); δ(C) 51.5), one oxygenated CH (δ(H) 4.19–4.23 (*m*); δ(C) 72.2 (CH)), three CH=CH moieties (δ(H) 5.39 (*dt*, $J = 10.7, 7.6$ Hz), 5.45 (*dt*, $J = 11.0, 7.6$ Hz), 5.56 (*dt*, $J = 10.7, 7.3$ Hz), 5.69 (*dd*, $J = 15.1, 6.6$ Hz), 5.94 (*t*, $J = 11.0$ Hz), and 6.53 (*dd*, $J = 15.1, 11.0$ Hz); δ(C) 124.4 (CH), 125.8 (CH), 127.1 (CH), 133.6 (CH), 134.6 (CH), and 135.0 (CH)), and one C=O group (δ(C) 174.3). The EI-MS of **10a** with the fragment-ion peak at *m/z* 81 (cleavage of the C-chain on either side of the OH group) indicated the position of the OH group at C(12) [12]. These spectral data were identical to those of **9a**, except for the CD data. The CD spectra of **9a** and **10a** exhibited mirror images (**9a**: max. at 243 nm ($\Delta\varepsilon = -0.2$); **10a**: max. at 243 nm ($\Delta\varepsilon = +0.3$)), confirming their enantiomeric relationship.

The absolute configuration at C(12) of **10a** was determined by the *Mosher*-ester method. While the *Mosher*-ester method is usually applied in a manner that the (*R*)- and (*S*)-MTPA esters (=(αR)- and (αS)-α-methoxy-α-(trifluoromethyl)benzeneacetates) of a given secondary alcohol are prepared for the determination of its absolute configuration [13], in this case, the (*R*)-MTPA esters **9b** and **10b** of the two enantiomeric compounds **9a** and **10a** were prepared to obtain the same information [14]. Thus, the absolute configuration at C(12) of **10a** should be (*S*). From these data, the structure of **10a** was determined to be (9*Z*,12*S*,13*E*,15*Z*)-12-hydroxyoctadeca-9,13,15-trienoic acid methyl ester. Consequently, the structure of **10** is (9*Z*,12*S*,13*E*,15*Z*)-12-hydroxyoctadeca-9,13,15-trienoic acid.

The subtraction of corresponding ^{1}H-NMR signals of **10b** from those of **9b** resulted in positive $\Delta\delta$ values for $CH_2(8)$ ($\Delta\delta = 0.08$), H−C(10) ($\Delta\delta = 0.12$), 1 H of $CH_2(11)$ ($\Delta\delta = 0.05$), and 1 H of $CH_2(11)$ ($\Delta\delta = 0.06$), and in negative $\Delta\delta$ values for H−C(14) ($\Delta\delta = -0.09$), H−C(15) ($\Delta\delta = -0.05$), $CH_2(17)$ ($\Delta\delta = -0.03$), and Me(18) ($\Delta\delta = -0.02$).

Compound **13a** had the molecular formula $C_{19}H_{32}O_3$, based on the HR-EI-MS ($M^{+\cdot}$ at *m/z* 308.2363). The IR spectrum showed the presence of an OH (3599 cm^{-1}) and an ester group (1731 cm^{-1}). In the UV spectrum (*Table*), the typical absorption maximum of a conjugated diene appeared at 229 nm [11]. Comparison of the ^{1}H- and ^{13}C-NMR spectra ($CDCl_3$), EI-MS, and CD data (*Table*) of **13a** with those of ester **14a** established their enantiomeric relationship.

The ^{1}H- and ^{13}C-NMR spectra of **13a** exhibited signals due to one Me group (δ(H) 0.88 (*t*, J = 7.1 Hz); δ(C) 9.7 (Me)), one MeO group (δ(H) 3.67 (*s*); δ(C) 51.5), one oxygenated CH (δ(H) 4.09–4.13 (*m*); δ(C) 74.2 (CH)), three CH=CH moieties (δ(H) 5.34–5.38 (*m*), 5.39–5.43 (*m*, 2 H), 5.69 (*dd*, J = 15.0, 7.0 Hz), 6.00 (*t*, J = 11.0 Hz) and 6.52 (*dd*, J = 15.0, 11.0 Hz); δ(C) 125.7 (CH), 127.2 (CH), 127.8 (CH), 130.7 (CH), 130.8 (CH), and 136.1 (CH)), and one C=O group (δ(C) 174.4). The EI-MS of **13a** with the fragment ion peak at *m/z* 57 indicated the position of the OH group at C(16) [12]. These spectral data were identical to those of **14a** except for the CD data. The CD spectra of **13a** and **14a** exhibited mirror images (**13a**: max. at 229 nm ($\Delta\varepsilon$ = −0.8); **14a**: max. at 228 nm ($\Delta\varepsilon$ = +0.7)), confirming their enantiomeric relationship.

The absolute configuration at C(16) of **13a** was determined by the *Mosher*-ester method in the above-described manner. Thus, the absolute configuration at C(16) of **13a** should be (*R*). From these data, the structure of **13a** was determined to be (9*Z*,12*Z*,14*E*,16*R*)-16-hydroxyoctadeca-9,12,14-trienoic acid methyl ester. Therefore, the structure of **13** is (9*Z*,12*Z*,14*E*,16*R*)-16-hydroxyoctadeca-9,12,14-trienoic acid.

The subtraction of corresponding ^{1}H-NMR signals of **14b** from those of **13b** resulted in positive $\Delta\delta$ values for CH_2(11) ($\Delta\delta$ = 0.04), H−C(12) ($\Delta\delta$ = 0.04), H−C(14) ($\Delta\delta$ = 0.07), and H−C(15) ($\Delta\delta$ = 0.10), and in a negative $\Delta\delta$ value for Me(18) ($\Delta\delta$ = −0.09).

The CD data of esters **1a**–**14a** obtained in the present work are summarized in the *Table*. In the (*R*) series, the OH group has a negative *Cotton* effect at *ca.* 230 nm, whereas in the (*S*) series, it has a positive *Cotton* effect in this region. Thus, the absolute configuration of acyclic hydroxylated diene moieties of the monohydroxy-substituted polyunsaturated fatty acids can be deduced from a CD measurement.

This is the first report on the isolation of monohydroxy-substituted polyunsaturated fatty acids from the *Swertia* genus in Gentianaceae.

We are grateful to Mr. *S. Satoh* and Mr. *T. Matsuki* for NMR and MS measurements.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 230–400 mesh; *Merck*). Prep. HPLC: *CCPM* pump (*Tosoh*); *UV-8020* UV/VIS detector (*Tosoh*); if not stated otherwise, *Chiralcel-OD* column (250 × 4.6 mm; *Daicel Chemical Industries, Ltd.*), hexane/i-PrOH eluents, and detection at λ 235 nm; flow 1.0 ml/min; t_R in min. UV Spectra: *Beckman DU-64* spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *Jasco J-720* spectropolarimeter; λ in nm, $\Delta\varepsilon$ in l mol^{-1} cm^{-1}. NMR Spectra: *Jeol JNM-LA-600* (at 600 (^{1}H) and 150 MHz (^{13}C)) and *JNM-LA-400* (at 400 (^{1}H) and 100 MHz (^{13}C)) spectrometers; δ in ppm rel. to Me_4Si, J in Hz. EI- and HR-EI-MS: *Jeol JMS-DX-303* mass spectrometer; in *m/z*.

Plant Material. The dried whole plants of *Swertia japonica* were purchased from *Uchida Wakanyaku Co., Ltd.*, Japan, in 2002. A voucher specimen (SJ-2002-01) was deposited with the Laboratory of Molecular Structural Analysis, Tohoku Pharmaceutical University.

Extraction and Isolation. The dried whole plants of *Swertia japonica* (2.0 kg) were extracted three times (14 d each time) with MeOH at r.t., and the extracts were filtered. The MeOH extracts were then concentrated to give a residue (474.0 g), which was partitioned between H_2O and $CHCl_3$. The $CHCl_3$-soluble fraction was concentrated to afford a residue (308.0 g). A part of this residue (17.8 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 19:1 → 2:1): *Fractions 1–20* (by TLC). *Fr. 13* was methylated with $TMSCHN_2$ to give the corresponding methyl esters. The methyl ester mixture was subjected to CC (SiO_2, hexane/AcOEt 4:1, $CHCl_3$/MeOH 19:1 → 4:1): *Fr. 1′–20′* (by TLC). *Fr. 5′*, on prep. HPLC (hexane/i-PrOH 300:1) gave 0.4 mg of **11a** (t_R 105.0) and 0.4 mg of **12a** (t_R 126.3). *Fr. 6′*, on prep. HPLC (hexane/i-PrOH 60:1) gave 1.2 mg of **6a** (t_R 17.0), 0.2 mg of **1a** (t_R 19.3), 1.3 mg of **5a** (t_R 20.8), and 0.5 mg of **2a** (t_R 25.0). *Fr. 7′*, on prep. HPLC (hexane/i-PrOH 60:1) gave 0.6 mg of **13a** (t_R 16.5), 1.0 mg of **7a** (t_R 18.1), a mixture **9a/10a/14a** (t_R 21.0), 0.3 mg of **4a** (t_R 21.6), 1.1 mg of **8a** (t_R 24.4), and 1.0 mg of **3a** (t_R 34.4). The mixture **9a/10a/14a**, on prep. HPLC (hexane/ i-PrOH 300:1) gave 0.4 mg of **9a** (t_R 197.0), 0.4 mg of **10a** (t_R 266.0), and 0.3 mg of **14a** (t_R 338.0).

*(9*Z*,12*S*,13*E*,15*Z*)-12-Hydroxyoctadeca-9,13,15-trienoic Acid Methyl Ester* (**10a**): Colorless oil. UV (MeOH): *Table.* CD (MeOH): *Table.* IR ($CHCl_3$): 3602, 1731. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.00 (*t*, *J* = 7.6, Me(18)); 2.05 (br. *q*, *J* = 7.1, CH_2(8)); 2.18–2.24 (*m*, CH_2(17)); 2.30 (*t*, *J* = 7.6, CH_2(2)); 2.28–2.32 (*m*, CH_2(11)); 3.67 (*s*, MeO); 4.19–4.23 (*m*, H–C(12)); 5.39 (*dt*, *J* = 10.7, 7.6, H–C(10)); 5.45 (*dt*, *J* = 11.0, 7.6, H–C(16)); 5.56 (*dt*, *J* = 10.7, 7.3, H–C(9)); 5.69 (*dd*, *J* = 15.1, 6.6, H–C(13)); 5.94 (*t*, *J* = 11.0, H–C(15)); 6.53 (*dd*, *J* = 15.1, 11.0, H–C(14)). ^{13}C-NMR (150 MHz, $CDCl_3$): 14.2 (C(18)); 21.1 (C(17)); 24.9 (C(3)); 27.4 (C(8)); 29.1 (C(5), C(6), C(7)); 29.5 (C(4)); 34.1 (C(2)); 35.4 (C(11)); 51.5 (MeO); 72.2 (C(12)); 124.4 (C(10)); 125.8 (C(16)); 127.1 (C(15)); 133.6 (C(13)); 134.6 (C(14)); 135.0 (C(9)); 174.3 (C(1)). HR-EI-MS: 308.2360 ($M^{+\bullet}$, $C_{19}H_{32}O_3^{+\bullet}$; calc. 308.2351).

*(9*Z*,12*Z*,14*E*,16*R*)-16-Hydroxyoctadeca-9,12,14-trienoic Acid Methyl Ester* (**13a**): Colorless oil. UV (MeOH): *Table.* CD (MeOH): *Table.* IR ($CHCl_3$): 3599, 1731. ^{1}H-NMR (600 MHz, $CDCl_3$): 0.88 (*t*, *J* = 7.1, Me(18)); 2.06 (br. *q*, *J* = 7.0, CH_2(8)); 2.31 (*t*, *J* = 7.7, CH_2(2)); 2.93 (br. *t*, *J* = 7.7, CH_2(11)); 3.67 (*s*, MeO); 4.09–4.13 (*m*, H–C(16)); 5.34–5.38 (*m*, H–C(10)); 5.39–5.43 (*m*, H–C(9), H–C(12)); 5.69 (*dd*, *J* = 15.0, 7.0, H–C(15)); 6.00 (*t*, *J* = 11.0, H–C(13)); 6.52 (*dd*, *J* = 15.0, 11.0, H–C(14)). ^{13}C-NMR (150 MHz, $CDCl_3$): 9.7 (C(18)); 25.0 (C(3)); 26.1 (C(11)); 27.2 (C(8)); 29.1 (C(5), C(6), C(7)); 29.5 (C(4)); 30.2 (C(17)); 34.1 (C(2)); 51.5 (MeO); 74.2 (C(16)); 125.7 (C(14)); 127.2 (C(10)); 127.8 (C(13)); 130.7 (C(12)); 130.8 (C(9)); 136.1 (C(15)); 174.4 (C(1)). HR-EI-MS: 308.2363 ($M^{+\bullet}$, $C_{19}H_{32}O_3^{+\bullet}$; calc. 308.2351).

MTPA Esterification of **9a**, **10a**, **13a**, *and* **14a**. To a soln. of each methyl ester **9a** (0.4 mg), **10a** (0.4 mg), **13a** (0.3 mg), and **14a** (0.3 mg) in pyridine (20 µl), (*S*)-MTPA-Cl (1.7 µl) was added. Each mixture was allowed to stand at r.t. for 24 h. Next, [(3-dimethylamino)propyl]amine (1.0 µl) was added, and after 30 min, the mixture was concentrated and the residue purified by prep. HPLC (*TSKgel-Silica-60* column (300 × 7.8 mm; *Tosoh*), hexane/acetone 9:1, λ 230 nm) to give the (*R*)-MTPA esters **9b** (0.3 mg), **10b** (0.3 mg), **13b** (0.2 mg), and **14b** (0.2 mg) from **9a**, **10a**, **13a**, and **14a**, resp.

Data of **9b**: ^{1}H-NMR (400 MHz, $CDCl_3$): 0.98 (*t*, *J* = 7.6, Me(18)); 2.01 (br. *q*, *J* = 7.1, CH_2(8)); 2.11–2.15 (*m*, CH_2(17)); 2.43–2.47 (*m*, 1 H of CH_2(11)); 2.49–2.53 (*m*, 1 H of CH_2(11)); 5.35 (*dt*, *J* = 10.7, 7.6, H–C(10)); 5.87 (*t*, *J* = 11.0, H–C(15)); 6.52 (*dd*, *J* = 15.1, 11.0, H–C(14)).

Data of **10b**: ^{1}H-NMR (400 MHz, $CDCl_3$): 1.00 (*t*, *J* = 7.6, Me(18)); 1.93 (br. *q*, *J* = 7.1, CH_2(8)); 2.16–2.20 (*m*, CH_2(17)); 2.38–2.42 (*m*, 1 H of CH_2(11)); 2.43–2.47 (*m*, 1 H of CH_2(11)); 5.23 (*dt*, *J* = 10.7, 7.6, H–C(10)); 5.92 (*t*, *J* = 11.0, H–C(15)); 6.61 (*dd*, *J* = 15.1, 11.0, H–C(14)).

Data of **13b**: ^{1}H-NMR (400 MHz, $CDCl_3$): 0.85 (*t*, *J* = 7.1, Me(18)); 2.91 (br. *t*, *J* = 7.7, CH_2(11)); 5.63 (*dd*, *J* = 15.0, 7.0, H–C(15)); 5.97 (*t*, *J* = 11.0, H–C(13)); 6.64 (*dd*, *J* = 15.0, 11.0, H–C(14)).

Data of **14b**: ^{1}H-NMR (400 MHz, $CDCl_3$): 0.94 (*t*, *J* = 7.1, Me(18)); 2.87 (br. *t*, *J* = 7.7, CH_2(11)); 5.53 (*dd*, *J* = 15.0, 7.0, H–C(15)); 5.93 (*t*, *J* = 11.0, H–C(13)); 6.57 (*dd*, *J* = 15.0, 11.0, H–C(14)).

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Received March 25, 2008