

Monohydroxy-Substituted Polyunsaturated Fatty Acids from *Swertia japonica*

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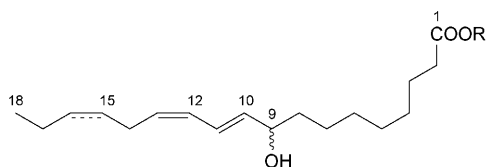
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Fourteen monohydroxy-substituted polyunsaturated fatty acids, including two new compounds, (9Z,12S,13E,15Z)-12-hydroxyoctadeca-9,13,15-trienoic acid (**10**) and (9Z,12Z,14E,16R)-16-hydroxyoctadeca-9,12,14-trienoic acid (**13**), and 12 known ones, *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.

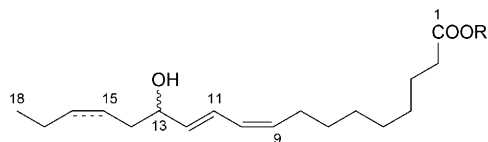
Introduction. – The whole plant of *Swertia japonica* MAKINO (Gentianaceae) is the crude drug *Swertia* herb, used as a stomachic or stimulant of appetite in Japan [1]. In previous articles, we reported the structure determination of fifteen new secoiridoid glycosides [2–4], a new unsaturated alcohol 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 H₂O and CHCl₃, and the CHCl₃-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; TMSCHN₂) 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**.

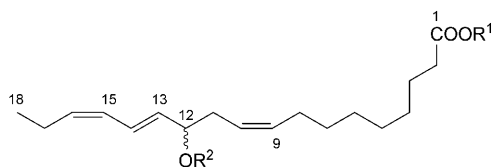
The known compounds were identified as (9R,10E,12Z)-9-hydroxyoctadeca-10,12-dienoic acid (**1**) [5], (9S,10E,12Z)-9-hydroxyoctadeca-10,12-dienoic acid (**2**) [6], (9R,10E,12Z, 15Z)-9-hydroxyoctadeca-10,12,15-trienoic acid (**3**) [5], (9S,10E,12Z, 15Z)-9-hydroxyoctadeca-10,12,15-trienoic acid (**4**)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



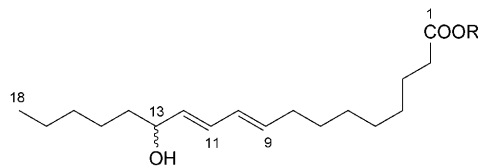
- 1** (9*R*) R = H
1a (9*R*) R = Me
2 (9*S*) R = H
2a (9*S*) R = Me
3 (9*R*) R = H, Δ^{15}
3a (9*R*) R = Me, Δ^{15}
4 (9*S*) R = H, Δ^{15}
4a (9*S*) R = Me, Δ^{15}



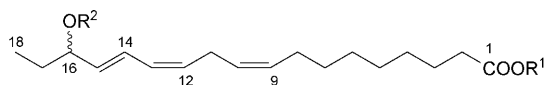
- 5** (13*R*) R = H
5a (13*R*) R = Me
6 (13*S*) R = H
6a (13*S*) R = Me
7 (13*R*) R = H, Δ^{15}
7a (13*R*) R = Me, Δ^{15}
8 (13*S*) R = H, Δ^{15}
8a (13*S*) R = Me, Δ^{15}



- 9** (12*R*) R¹ = H, R² = H
9a (12*R*) R¹ = Me, R² = H
9b (12*R*) R¹ = Me, R² = (*R*)-MTPA
10 (12*S*) R¹ = H, R² = H
10a (12*S*) R¹ = Me, R² = H
10b (12*S*) R¹ = Me, R² = (*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*) R¹ = H, R² = H
13a (16*R*) R¹ = Me, R² = H
13b (16*R*) R¹ = Me, R² = (*R*)-MTPA
14 (16*S*) R¹ = H, R² = H
14a (16*S*) R¹ = Me, R² = H
14b (16*S*) R¹ = Me, R² = (*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 C₁₉H₃₂O₃, based on the HR-EI-MS (*M*⁺ 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 ¹H- and ¹³C-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 $\Delta\epsilon$ (λ) ^{a)}
1a	<i>R</i>	232 (4.2)	– 1.6 (232)
2a	<i>S</i>	232 (4.2)	+ 1.8 (233)
3a	<i>R</i>	234 (4.3)	– 1.9 (234)
4a	<i>S</i>	233 (4.0)	+ 1.8 (235)
5a	<i>R</i>	232 (4.4)	– 2.6 (231)
6a	<i>S</i>	232 (4.4)	+ 2.7 (232)
7a	<i>R</i>	232 (4.4)	– 0.4 (243)
8a	<i>S</i>	232 (4.4)	+ 0.5 (239)
9a	<i>R</i>	232 (4.2)	– 0.2 (243)
10a	<i>S</i>	232 (4.3)	+ 0.3 (243)
11a	<i>R</i>	229 (4.0)	– 0.8 (229)
12a	<i>S</i>	229 (4.0)	+ 0.7 (228)
13a	<i>R</i>	229 (4.0)	– 0.8 (229)
14a	<i>S</i>	229 (4.0)	+ 0.7 (228)

^{a)} Measurements were carried out in MeOH. λ in nm.

The ^1H - and ^{13}C -NMR spectra of **10a** exhibited signals due to one Me group ($\delta(\text{H})$ 1.00 (*t*, $J = 7.6$ Hz); $\delta(\text{C})$ 14.2 (Me)), one MeO group ($\delta(\text{H})$ 3.67 (*s*); $\delta(\text{C})$ 51.5), one oxygenated CH ($\delta(\text{H})$ 4.19–4.23 (*m*); $\delta(\text{C})$ 72.2 (CH)), three CH=CH moieties ($\delta(\text{H})$ 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); $\delta(\text{C})$ 124.4 (CH), 125.8 (CH), 127.1 (CH), 133.6 (CH), 134.6 (CH), and 135.0 (CH)), and one C=O group ($\delta(\text{C})$ 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 ($\Delta\epsilon = -0.2$); **10a**: max. at 243 nm ($\Delta\epsilon = +0.3$)), 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 ($=(\alpha R)$ - 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 ^1H -NMR signals of **10b** from those of **9b** resulted in positive $\Delta\delta$ values for $\text{CH}_2(8)$ ($\Delta\delta = 0.08$), $\text{H}-\text{C}(10)$ ($\Delta\delta = 0.12$), 1 H of $\text{CH}_2(11)$ ($\Delta\delta = 0.05$), and 1 H of $\text{CH}_2(11)$ ($\Delta\delta = 0.06$), and in negative $\Delta\delta$ values for $\text{H}-\text{C}(14)$ ($\Delta\delta = -0.09$), $\text{H}-\text{C}(15)$ ($\Delta\delta = -0.05$), $\text{CH}_2(17)$ ($\Delta\delta = -0.03$), 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^{+} 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 ^1H - 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 ^1H - and ^{13}C -NMR spectra of **13a** exhibited signals due to one Me group ($\delta(\text{H})$ 0.88 (t , $J = 7.1\text{ Hz}$); $\delta(\text{C})$ 9.7 (Me)), one MeO group ($\delta(\text{H})$ 3.67 (s); $\delta(\text{C})$ 51.5), one oxygenated CH ($\delta(\text{H})$ 4.09–4.13 (m); $\delta(\text{C})$ 74.2 (CH)), three $\text{CH}=\text{CH}$ moieties ($\delta(\text{H})$ 5.34–5.38 (m), 5.39–5.43 (m , 2 H), 5.69 (dd , $J = 15.0, 7.0\text{ Hz}$), 6.00 (t , $J = 11.0\text{ Hz}$) and 6.52 (dd , $J = 15.0, 11.0\text{ Hz}$); $\delta(\text{C})$ 125.7 (CH), 127.2 (CH), 127.8 (CH), 130.7 (CH), 130.8 (CH), and 136.1 (CH)), and one $\text{C}=\text{O}$ group ($\delta(\text{C})$ 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 ($\Delta\epsilon = -0.8$); **14a**: max. at 228 nm ($\Delta\epsilon = +0.7$)), 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 ^1H -NMR signals of **14b** from those of **13b** resulted in positive $\Delta\delta$ values for $\text{CH}_2(11)$ ($\Delta\delta = 0.04$), $\text{H}-\text{C}(12)$ ($\Delta\delta = 0.04$), $\text{H}-\text{C}(14)$ ($\Delta\delta = 0.07$), and $\text{H}-\text{C}(15)$ ($\Delta\delta = 0.10$), 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 ($250 \times 4.6\text{ mm}$; 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, $\Delta\epsilon$ in $\text{l mol}^{-1}\text{ cm}^{-1}$. NMR Spectra: Jeol JNM-LA-600 (at 600 (^1H) and 150 MHz (^{13}C)) and JNM-LA-400 (at 400 (^1H) 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 H₂O and CHCl₃. The CHCl₃-soluble fraction was concentrated to afford a residue (308.0 g). A part of this residue (17.8 g) was subjected to CC (SiO₂, CHCl₃/MeOH 19:1 → 2:1): *Fractions 1–20* (by TLC). *Fr. 13* was methylated with TMSCHN₂ to give the corresponding methyl esters. The methyl ester mixture was subjected to CC (SiO₂, hexane/AcOEt 4:1, CHCl₃/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).

(*9Z,12S,13E,15Z*)-12-Hydroxyoctadeca-9,13,15-trienoic Acid Methyl Ester (**10a**): Colorless oil. UV (MeOH): *Table*. CD (MeOH): *Table*. IR (CHCl₃): 3602, 1731. ¹H-NMR (600 MHz, CDCl₃): 1.00 (*t*, *J* = 7.6, Me(18)); 2.05 (br. *q*, *J* = 7.1, CH₂(8)); 2.18–2.24 (*m*, CH₂(17)); 2.30 (*t*, *J* = 7.6, CH₂(2)); 2.28–2.32 (*m*, CH₂(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)). ¹³C-NMR (150 MHz, CDCl₃): 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*⁺, C₁₉H₃₂O₃⁺; calc. 308.2351).

(*9Z,12Z,14E,16R*)-16-Hydroxyoctadeca-9,12,14-trienoic Acid Methyl Ester (**13a**): Colorless oil. UV (MeOH): *Table*. CD (MeOH): *Table*. IR (CHCl₃): 3599, 1731. ¹H-NMR (600 MHz, CDCl₃): 0.88 (*t*, *J* = 7.1, Me(18)); 2.06 (br. *q*, *J* = 7.0, CH₂(8)); 2.31 (*t*, *J* = 7.7, CH₂(2)); 2.93 (br. *t*, *J* = 7.7, CH₂(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)). ¹³C-NMR (150 MHz, CDCl₃): 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*⁺, C₁₉H₃₂O₃⁺; 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: ¹H-NMR (400 MHz, CDCl₃): 0.98 (*t*, *J* = 7.6, Me(18)); 2.01 (br. *q*, *J* = 7.1, CH₂(8)); 2.11–2.15 (*m*, CH₂(17)); 2.43–2.47 (*m*, 1 H of CH₂(11)); 2.49–2.53 (*m*, 1 H of CH₂(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: ¹H-NMR (400 MHz, CDCl₃): 1.00 (*t*, *J* = 7.6, Me(18)); 1.93 (br. *q*, *J* = 7.1, CH₂(8)); 2.16–2.20 (*m*, CH₂(17)); 2.38–2.42 (*m*, 1 H of CH₂(11)); 2.43–2.47 (*m*, 1 H of CH₂(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: ¹H-NMR (400 MHz, CDCl₃): 0.85 (*t*, *J* = 7.1, Me(18)); 2.91 (br. *t*, *J* = 7.7, CH₂(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: ¹H-NMR (400 MHz, CDCl₃): 0.94 (*t*, *J* = 7.1, Me(18)); 2.87 (br. *t*, *J* = 7.7, CH₂(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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