Sesquiterpenoids from *Ligularia dentata*

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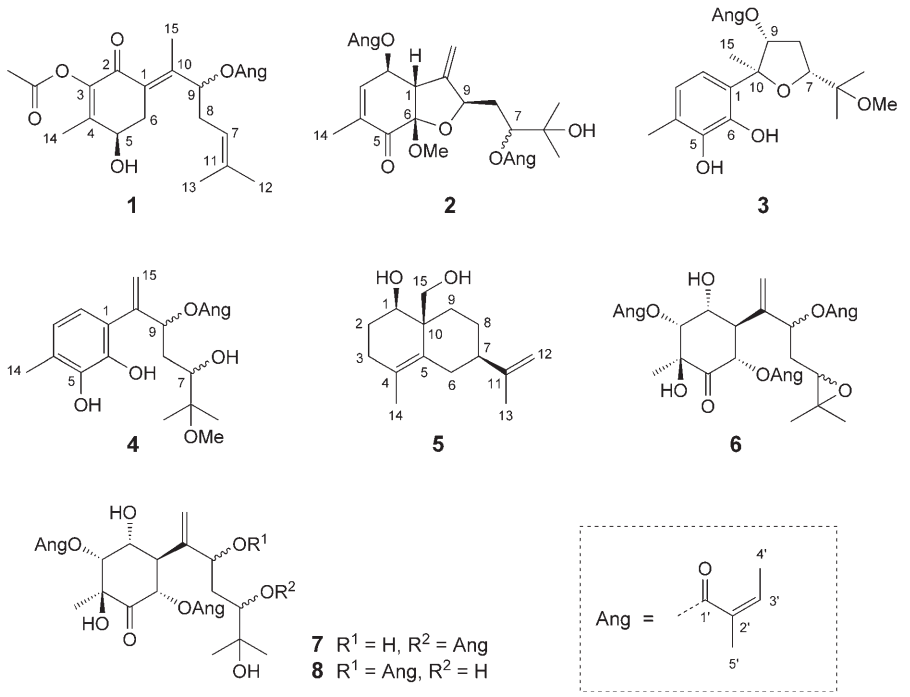
Five new compounds were isolated from the roots of *Ligularia dentata*, including four bisabolane-type sesquiterpenoids, **1**–**4**, as well a new eudesmane, **5**. The previously isolated 3α,6α,9-tris(angeloyloxy)-2α,4β-dihydroxy-7,11-epoxybisabol-10(15)-en-5-one (**6**), when left as an oil in a refrigerator over nine months, gave rise to a mixture of two positional isomers, **7** and **8**. Their formation is rationalized by means of epoxide ring opening and shift of an angeloyl (Ang) group. The structures of compounds **1**–**5**, **7**, and **8** were established by in-depth spectroscopic (UV, CD, IR, 1D- and 2D-NMR) as well as mass-spectrometric methods.

Introduction. – *Ligularia dentata* HARA (Compositae) has long been used as a medicinal herb in China to ease breathing, stimulate blood flow, reduce inflammation, alleviate pain, stop coughs, and to get rid of phlegm [1]. Recently, we reported the structure determination of five new bisabolane-type sesquiterpenoids and two new lactone derivatives from the roots of *L. dentata* [2]. In continuation of our phytochemical studies, we, herein, report the isolation and structure elucidation of five new constituents, **1**–**5**, from the roots of *L. dentata*. In addition, we report that the recently isolated compound **6** [2], when left in the refrigerator over longer periods of time, undergoes a chemical conversion.

Results and Discussion. – 1. *Structure Elucidation of Compounds* **1**–**5**. The Et_2O-soluble part of the MeOH extract of the roots of *L. dentata* yielded the new compounds **1**–**5** after repeated chromatographic purification.

Compound **1**, obtained as a colorless oil, had the molecular formula $C_{22}H_{30}O_6$, based on HR-EI-MS analysis (*m/z* 390.2062 (M^+; calc. 390.2043)). The IR spectrum showed the presence of an OH group (3479 cm^{-1}), a vinyl ester (1760, 1646 cm^{-1}), an α,β-unsaturated ester (1702, 1646 cm^{-1}), and a cross-conjugated C=O moiety (1673, 1602 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) exhibited signals due to four Me groups (δ(H) 1.44 (*s*), 1.54 (*s*), 1.81 (*s*), 2.11 (*d*, *J* = 1.8 Hz); δ(C) 13.8, 14.9, 17.8, 25.7), one AcO group (δ(H) 1.89 (*s*); δ(C) 19.9, 167.8), an angeloyl (Ang)[1]) group (δ(H) 1.71 (*dq*, *J* = 1.5, 1.5 Hz), 1.82 (*dq*, *J* = 7.0, 1.5 Hz), 5.63 (*qq*, *J* = 7.0, 1.5 Hz); δ(C) 15.9 (Me), 20.6 (Me), 127.6 (C_q), 139.9 (CH), 167.9 (C_q)) [3], two CH_2 moieties (δ(H) 2.12–2.14 (*m*), 2.34–2.39 (*m*), 2.52 (br. *dd*, *J* = 14.7, 3.7 Hz), 2.96 (*dd*, *J* = 14.7, 3.7); δ(C) 31.5, 35.6), two oxygenated CH groups (δ(H) 3.99 (*ddd*, *J* = 8.1, 3.7, 3.7 Hz), 5.54

[1]) Angelic acid (AngOH) = (*Z*)-2-methylbut-2-enoic acid.



(*t*, *J* = 7.3 Hz); δ(C) 68.7, 74.2), one C=CH moiety (δ(H) 4.96 (br. *t*, *J* = 6.3 Hz); δ(C) 118.7 (CH), 135.2 (C_q)), two fully substituted C=C groups (δ(C) 128.9, 143.8, 143.9, 144.2), and one OH group (δ(H) 3.88 (br. *d*, *J* = 8.1 Hz)). To accommodate eight degrees of unsaturation, compound **1** was proposed to have a monocyclic sesquiterpene skeleton, with an AngO and an AcO group, a cross-conjugated C=O moiety, and one C=CH moiety.

The ^{1}H,^{1}H-COSY spectrum of **1** (*Fig. 1,a*) implied connectivities of H–C(5)[2]) to both CH_2(6) and to an OH group, of H–C(9) to CH_2(8), and of CH_2(8) to H–C(7). The HMBC spectrum of **1** (*Fig. 1,a*) showed correlations between CH_2(6) and C(1), between H–C(9) and the Ang C=O group, between Me(12) and C(7), between Me(13) and C(7), between Me(15) and C(1), C(9), and C(10), and between Me(14) and C(3), C(4), and C(5), respectively. The presence of an AcO group at C(3) was indicated by a NOESY cross-peak between the Me group of the AcO moiety and Me(14) (*Fig. 1,a*). Therefore, the constitution of **1** was deduced, the parent framework being a bisabolatrienone.

The relative configuration of **1** was determined as follows. The coupling constants for H–C(5) (J(5,6α) = 3.7 Hz, J(5,6β) = 3.7 Hz) suggested that the OH group was in pseudo-axial β-position, which was supported by the NOESY cross-peaks between H–C(5) and both H_α–C(6) and H_β–C(6) (*Fig. 1,b*). The C=C bond between C(1) and C(10) was (*E*)-configured, based on a NOESY cross-peak between H_β–C(6) and

[2]) Arbitrary numbering.

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 600/150 MHz, resp.; δ in ppm, J in Hz. Asterisks (*) mark interchangeable signals.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1		128.9	3.26 (*ddd*, J = 6.6, 2.9, 1.5)	55.5
2		n.d.[c])	5.54 (*ddq*, J = 6.6, 3.3, 1.8)	68.8
3		143.8*	6.66 (*dq*, J = 3.3, 1.5)	142.8
4		143.9*		137.8
5	3.99 (*ddd*, J = 8.1, 3.7, 3.7)	68.7		192.5
6	2.52 (br. *dd*, J = 14.7, 3.7, H_α), 2.96 (*dd*, J = 14.7, 3.7, H_β)	35.6		101.5
7	4.96 (br. *t*, J = 6.3)	118.7	5.18–5.19 (*m*)	78.0
8	2.12–2.14 (*m*), 2.34–2.39 (*m*)	31.5	1.96–1.97 (*m*, H_a), 2.23 (*ddd*, J = 15.0, 4.0, 2.2, H_b)	37.1
9	5.54 (*t*, J = 7.3)	74.2	4.70 (*dddd*, J = 8.1, 4.0, 2.2, 1.8)	81.4
10		144.2		150.1
11		135.2		72.6
12	1.54 (*s*)	25.7	1.209*	24.8*
13	1.44 (*s*)	17.8	1.213*	28.2*
14	1.81 (*s*)	14.9	1.81 (*dd*, J = 1.5, 1.5)	15.5
15	2.11 (*d*, J = 1.8)	13.8	5.17 (*dd*, J = 1.8, 1.5, H_a), 5.20 (*dd*, J = 2.9, 2.2, H_b)	109.3
1′		167.9		168.2
1″				169.2
2′		127.6		128.8
2″				129.1
3′	5.63 (*qq*, J = 7.0, 1.5)	139.9	6.10 (*qq*, J = 7.3, 1.5)	138.8
3″			6.22 (*qq*, J = 7.3, 1.5)	140.7
4′	1.82 (*dq*, J = 7.0, 1.5)	15.9	1.94 (*dq*, J = 7.3, 1.5)	16.0
4″			2.00 (*dq*, J = 7.3, 1.5)	16.1
5′	1.71 (*dq*, J = 1.5, 1.5)	20.6	1.92 (*dq*, J = 1.5, 1.5)[d])	20.7
5″				20.8
5-OH	3.88 (br. *d*, J = 8.1)			
6-MeO			3.47 (*s*)	52.4
3-AcO	1.89 (*s*)	19.9		
		167.8		

[a]) In C_6D_6. [b]) In CD_3OD. [c]) Not detected. [d]) 5′- and 5″-Signals (2 Me).

H–C(9) (*Fig. 1, b*). The absolute configuration at C(5) was determined to be (*R*) from the circular-dichroism (CD) spectrum, in which a positive *Cotton* effect was observed at 244 nm ($\Delta\varepsilon = +2.56$) [4]. The absolute configuration at C(9) remains to be established. Accordingly, the structure of **1** was determined as (1*E*,5*R*)-3-acetoxy-9-(angeloyloxy)-5-hydroxybisabola-3,1(10),7(11)-trien-2-one[3]).

Compound **2**, obtained as a colorless oil, had the molecular formula $C_{26}H_{36}O_8$, based on HR-EI-MS analysis (*m*/*z* 476.2413 (M^+; calc. 476.2410)). The IR spectrum

[3]) For systematic names, see *Exper. Part*.

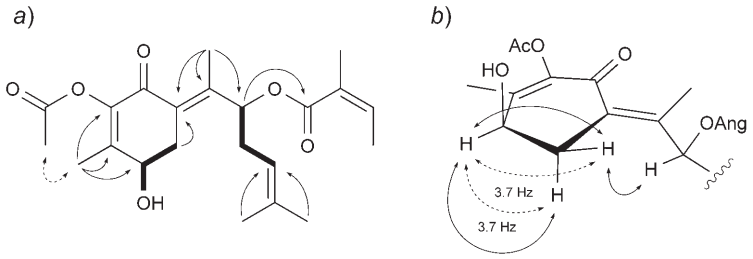


Fig. 1. a) *^{1}H,^{1}H-COSY* (▬), *HMBC* (→), *and NOESY* (⇢⇢⇢) *correlations for* **1**. *b*) *Selected coupling constants* (⇢⇢⇢) *and further NOEs* (↔) *for* **1**.

showed the presence of an OH group (3525 cm^{-1}), an α,β-unsaturated ester (1713, 1647 cm^{-1}), and an α,β-unsaturated C=O moiety (1692, 1563 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 1*) exhibited signals due to three Me groups (δ(H) 1.209 (*s*), 1.213 (*s*), 1.81 (*dd*, *J* = 1.5, 1.5 Hz); δ(C) 15.5, 24.8, 28.2), two AngO groups (δ(H) 1.92 (*dq*, *J* = 1.5, 1.5 Hz, 6 H), 1.94 (*dq*, *J* = 7.3, 1.5 Hz), 2.00 (*dq*, *J* = 7.3, 1.5 Hz), 6.10 (*qq*, *J* = 7.3, 1.5 Hz), 6.22 (*qq*, *J* = 7.3, 1.5 Hz); δ(C) 16.0 (Me), 16.1 (Me), 20.7 (Me), 20.8 (Me), 128.8 (C_q), 129.1 (C_q), 138.8 (CH), 140.7 (CH), 168.2 (C_q), 169.2 (C_q)), a CH_2= group (δ(H) 5.17 (*dd*, *J* = 1.8, 1.5 Hz), 5.20 (*dd*, *J* = 2.9, 2.2 Hz); δ(C) 109.3 (CH_2), 150.1 (C_q)), one MeO function (δ(H) 3.47 (*s*); δ(C) 52.4), three oxygenated CH groups (δ(H) 4.70 (*dddd*, *J* = 8.1, 4.0, 2.2, 1.8 Hz), 5.18–5.19 (*m*), 5.54 (*ddq*, *J* = 6.6, 3.3, 1.8 Hz); δ(C) 68.8, 78.0, 81.4), two oxygenated, quaternary, sp^3-type C-atoms (δ(C) 72.6, 101.5), one C=CH moiety (δ(H) 6.66 (*dq*, *J* = 3.3, 1.5 Hz); δ(C) 137.8 (C_q), 142.8 (CH)), and one C=O group (δ(C) 192.5).

The ^{1}H,^{1}H-COSY spectrum of **2** (*Fig. 2*,*a*) implied connectivities of H–C(2)^2) to both H–C(3) and H–C(1), of H–C(9) to CH_2(8), and of CH_2(8) to H–C(7). The HMBC spectrum showed correlations between Me(12) and both C(7) and C(11), between Me(13) and both C(7) and C(11), between CH_2(15) and both C(1) and C(9), between Me(14) and C(3), C(4), and C(5), and between the MeO group and C(6). By considering the chemical shifts of H–C(2) (δ(H) 5.54) and H–C(7) (δ(H) 5.18–5.19), the linking positions of the two AngO groups were determined to be C(2) and C(7).

According to the molecular formula of **2**, there were nine degrees of unsaturation. Two Ang groups, an α,β-unsaturated C=O moiety, and an exocyclic C=C bond

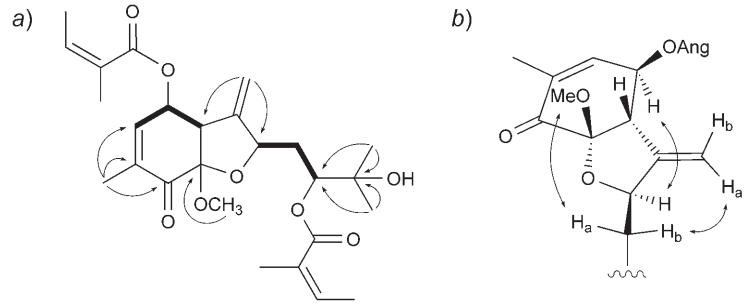


Fig. 2. a) *^{1}H,^{1}H-COSY* (▬) *and HMBC* (→) *correlations for* **2**. b) *NOESY* (↔) *Correlations for* **2**.

accounted for eight of those. The remaining degree of unsaturation was assumed to be due to a tetrahydrofuran ring formed between C(6) and C(9), as inferred from the ^{13}C-NMR data (δ(C) 81.4 (C(9)), 101.5 (C(6))) [5][6]. Therefore, the constitution of **2** was deduced.

The relative configuration of **2** was determined by NOESY experiments. The NOE cross-peaks observed between H–C(2) and H–C(9), between MeO and H_a–C(8), and between H_b–C(8) and H_a–C(15) implied that the 2-AngO group, the 6-MeO group, H–C(1), and the side chain at C(9) were on the β-face of the ring system (*Fig. 2*,*b*). The absolute configuration of **2** was determined as (1*S*,2*R*,6*S*,9*R*), based on the CD spectrum, in which a positive *Cotton* effect was observed at 241 nm ($\Delta\varepsilon = +10.58$) [4]. The absolute configuration at C(7) remains to be established. Accordingly, the structure of **2** was determined as (1*S*,2*R*,6*S*,9*R*)-2,7-bis(angeloyloxy)-6,9-epoxy-11-hydroxy-6-methoxybisabola-3,10(15)-dien-5-one[3]).

Compound **3**, obtained as a colorless oil, had the molecular formula $C_{21}H_{30}O_6$, based on HR-EI-MS analysis (*m*/*z* 378.2049 (M^+; calc. 378.2042)). The UV spectrum of **3** showed the typical absorption maxima of a benzene chromophore at 204 and 280 nm. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table 2*) exhibited signals due to a substituted i-Pr group (δ(H) 0.92 (*s*), 0.98 (*s*); δ(C) 20.2 (Me), 21.0 (Me), 74.9 (C_q)), one Me group (δ(H) 1.53 (*s*); δ(C) 24.2), an AngO group (δ(H) 1.83 (*dq*, $J = 1.5$, 1.5 Hz), 2.02 (*dq*, $J = 7.3$, 1.5 Hz), 5.75 (*qq*, $J = 7.3$, 1.5 Hz); δ(C) 16.0 (Me), 20.8 (Me), 127.5 (C_q), 139.9 (CH), 166.9 (C_q)), one Me group attached to a benzene ring (δ(H) 2.33 (*s*); δ(C) 15.6), one MeO group (δ(H) 2.98 (*s*); δ(C) 49.5), two oxygenated CH (δ(H) 3.67 (*dd*, $J = 8.1$, 8.1 Hz), 5.92 (*dd*, $J = 7.3$, 3.3 Hz); δ(C) 77.8, 82.6), an oxygenated, quaternary, sp^3-type C-atom (δ(C) 89.5), a benzene ring (δ(H) 6.63 (*d*, $J = 8.4$ Hz), 6.77 (*d*, $J = 8.4$ Hz); δ(C) 112.1 (CH), 116.4 (CH), 123.9 (C_q), 124.3 (C_q), 142.3 (C_q), 144.4 (C_q)), and two OH groups (δ(H) 5.94 (*s*), 9.60 (*s*)).

The ^{1}H,^{1}H-COSY spectrum of **3** (*Fig. 3*,*a*) implied connectivities of H–C(2) to H–C(3), of H–C(9) to CH_2(8), and of CH_2(8) to H–C(7). The HMBC spectrum (*Fig. 3*,*a*) showed correlations between H–C(2) and C(1), between Me(12) and both C(7) and C(11), between Me(13) and both C(7) and C(11), between Me(15) and C(1), C(10), and C(9), between Me(14) and C(3), C(4), and C(5), between MeO and C(11), and between the OH groups and C(5) and C(6), respectively.

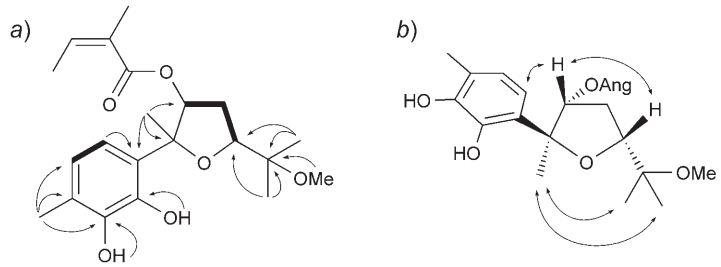


Fig. 3. a) *^{1}H,^{1}H-COSY* (—) *and HMBC* (→) *correlations for* **3**. b) *NOESY* (↔) *Correlations for* **3**.

By considering the chemical shift of H–C(9) (δ(H) 5.92), the linking position of the AngO group was determined to be at C(9). According to the molecular formula,

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3** *and* **4**. At 600/150 MHz, resp., in C_6D_6; δ in ppm, J in Hz. Asterisks (*) mark interchangeable signals.

Position	**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
1		123.9		123.3
2	6.77 (*d*, *J* = 8.4)	116.4	6.65 (*d*, *J* = 8.3)	120.4
3	6.63 (*d*, *J* = 8.4)	112.1	6.63 (*d*, *J* = 8.3)	122.2
4		124.3		124.7
5		144.4		144.2
6		142.3		141.4
7	3.67 (*dd*, *J* = 8.1, 8.1)	82.6	3.61 – 3.63 (*m*)	73.7
8	1.81 (*ddd*, *J* = 14.3, 8.1, 3.3), 2.05 (*ddd*, *J* = 14.3, 8.1, 7.3)	33.5	1.88 – 1.89 (*m*, 2 H)	37.1
9	5.92 (*dd*, *J* = 7.3, 3.3)	77.8	5.84 (*dd*, *J* = 7.0, 5.5)	74.6
10		89.5		149.2
11		74.9		76.6
12	0.92 (*s*)*	20.2*	0.80 (*s*)*	19.0*
13	0.98 (*s*)*	21.0*	0.84 (*s*)*	20.2*
14	2.33 (*s*)	15.6	2.32 (*s*)	15.7
15	1.53 (*s*)	24.2	5.14 (*d*, *J* = 1.1), 5.33 (*dd*, *J* = 1.1, 1.1)	114.6
1′		166.9		169.4
2′		127.5		127.6
3′	5.75 (*qq*, *J* = 7.3, 1.5)	139.9	5.71 (*qq*, *J* = 7.3, 1.5)	141.0
4′	2.02 (*dq*, *J* = 7.3, 1.5)	16.0	1.91 (*dq*, *J* = 7.3, 1.5)	16.1
5′	1.83 (*dq*, *J* = 1.5, 1.5)	20.8	1.80 (*dq*, *J* = 1.5, 1.5)	20.5
5-OH	5.94 (*s*)		5.98 (br. *s*)	
6-OH	9.60 (*s*)		9.39 (br. *s*)	
7-OH			1.95 (br. *s*)	
11-MeO	2.98 (*s*)	49.5	2.79 (*s*)	48.7

there were seven degrees of unsaturation in **3**. The Ang group and a benzene ring accounted for six of those. The remaining one was assumed to be due to a tetrahydrofuran ring formed between C(10) and C(7), on the basis of the ^{13}C-NMR data (δ(C) 82.6 (C(7)), 89.5 (C(10))) [5][6]. Therefore, the constitution of **3** was deduced.

The relative configuration of the substituents on the tetrahydrofuran ring were determined by NOESY experiments. The NOE cross-peaks observed between H–C(9) and both H–C(2) and H–C(7), and between Me(15) and both Me(12) and Me(13) implied that the Me group at C(10), the AngO group at C(9), and the MeO(Me_2)C substituent at C(7) occurred on the α-face of the ring system (*Fig. 3*,*b*). The absolute configuration of **3** could not be determined yet. From these data, the structure of **3** was elucidated as 9α-(angeloyloxy)-7β,10β-epoxy-11-methoxybisabola-1,3,5-triene-5,6-diol[3]).

Compound **4**, obtained as a colorless oil, had the molecular formula $C_{21}H_{30}O_6$, based on HR-EI-MS analysis (*m*/*z* 378.2047 (M^+; calc. 378.2042)). The UV spectrum showed the typical absorption maxima of a benzene chromophore at 203 and 279 nm.

The ^{1}H- and ^{13}C-NMR spectra of **4** (*Table 2*) exhibited signals due to a substituted i-Pr group (δ(H) 0.80 (*s*), 0.84 (*s*); δ(C) 19.0 (Me), 20.2 (Me), 76.6 (C_q)), an AngO group (δ(H) 1.80 (*dq*, J = 1.5, 1.5 Hz), 1.91 (*dq*, J = 7.3, 1.5 Hz), 5.71 (*qq*, J = 7.3, 1.5 Hz); δ(C) 16.1 (Me), 20.5 (Me), 127.6 (C_q), 141.0 (CH), 169.4 (C_q)), a Me group attached to a benzene ring (δ(H) 2.32 (*s*); δ(C) 15.7), a MeO group (δ(H) 2.79 (*s*); δ(C) 48.7), a CH_2= moiety (δ(H) 5.14 (*d*, J = 1.1 Hz), 5.33 (*dd*, J = 1.1, 1.1 Hz); δ(C) 114.6 (CH_2), 149.2 (C_q)), two oxygenated CH groups (δ(H) 3.61 – 3.63 (*m*), 5.84 (*dd*, J = 7.0, 5.5 Hz); δ(C) 73.7, 74.6), a benzene ring (δ(H) 6.63 (*d*, J = 8.3 Hz), 6.65 (*d*, J = 8.3 Hz); δ(C) 120.4 (CH), 122.2 (CH), 123.3 (C_q), 124.7 (C_q), 141.4 (C_q), 144.2 (C_q)), and three OH groups (δ(H) 1.95 (br. *s*), 5.98 (br. *s*), 9.39 (br. *s*)).

The ^{1}H,^{1}H-COSY spectrum of **4** (*Fig. 4*) implied connectivities of H–C(9) to CH_2(8), of CH_2(8) to H–C(7), and of H–C(7) to the 7-OH group. The HMBC spectrum (*Fig. 4*) showed correlations between Me(12) and both C(7) and C(11), between Me(13) and both C(7) and C(11), between CH_2(15) and both C(1) and C(9), between Me(14) and C(3), C(4), and C(5), between MeO and C(11), between the 5-OH group and C(4), and between the 6-OH group and C(5). By considering the chemical shift of H–C(9) (δ(H) 5.84), the linking position of the AngO group was determined to be at C(9). The absolute configurations at C(9) and C(7) remain to be established. Therefore, the structure of **4** was determined as 9-angeloyloxy-11-methoxybisabola-1,3,5,10(15)-tetraene-5,6,7-triol[2].

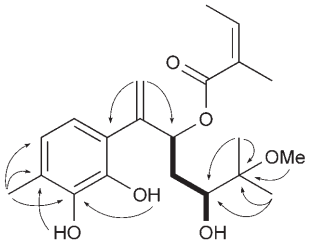


Fig. 4. *^{1}H,^{1}H-COSY* (—) *and HMBC* (→) *correlations for* **4**

Compound **5**, obtained as a colorless oil, had the molecular formula $C_{15}H_{24}O_2$, based on HR-EI-MS analysis (*m*/*z* 236.1789 (M^+; calc. 236.1776)). The IR spectrum showed the presence of OH groups (3503 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **5** (*Table 3*) exhibited signals due to two Me groups (δ(H) 1.63 (*s*), 1.75 (*s*); δ(C) 19.3, 20.7), an oxygenated CH group (δ(H) 3.67 (*dd*, J = 11.5, 3.9 Hz); δ(C) 79.8), an oxygenated CH_2 moiety (δ(H) 3.78 (*d*, J = 11.7 Hz), 3.87 (*d*, J = 11.7 Hz); δ(C) 63.0), an exocyclic CH= unit (δ(H) 4.72 (br. *s*, 2 H); δ(C) 108.6 (CH_2), 150.2 (C_q)), and a fully substituted C=C bond (δ(C) 127.8, 129.4).

The ^{1}H,^{1}H-COSY spectrum of **5** (*Fig. 5*,*a*) implied connectivities of H–C(1) to CH_2(2), of CH_2(2) to CH_2(3), of CH_2(6) to H–C(7), of H–C(7) to CH_2(8), and of CH_2(8) to CH_2(9). The HMBC spectrum (*Fig. 5*,*a*) showed correlations between CH_2(12) and C(7), between Me(13) and C(7), between CH_2(15) and C(1), and between Me(14) and C(3), C(4) and C(5), respectively. Therefore, the constitution of **5** was deduced.

The relative configuration of **5** was determined as follows. The coupling constants for H–C(1) (J(1,2β) = 11.5 Hz, J(1,2α) = 3.9 Hz) suggested that the OH group at C(1)

Table 3. *^{1}H- and ^{13}C-NMR Data of* **5**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

Position	δ(H)	δ(C)
1	3.67 (*dd*, $J = 11.5, 3.9$)	79.8
2	1.78–1.80 (*m*), 1.91–1.97 (*m*)	27.7
3	1.88 (*ddd*, $J = 11.5, 3.9, 3.7$, H_β), 2.06–2.10 (*m*, H_α)	31.4
4		129.4
5		127.8
6	1.76–1.77 (*m*, H_β), 2.55 (*ddd*, $J = 13.9, 3.4, 2.2$, H_α)	31.2
7	1.72–1.73 (*m*)	45.8
8	1.56–1.61 (*m*, H_β), 1.68–1.71 (*m*, H_α)	26.9
9	1.12 (*ddd*, $J = 13.2, 13.2, 3.9$, H_α), 2.60 (*ddd*, $J = 13.2, 3.4, 3.4$, H_β)	32.8
10		43.2
11		150.2
12	4.72 (br. *s*, 2 H)	108.6
13	1.75 (*s*)	20.7
14	1.63 (*s*)	19.3
15	3.78 (*d*, $J = 11.7$, H_a), 3.87 (*d*, $J = 11.7$, H_b)	63.0

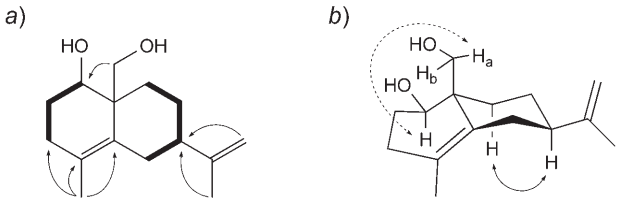


Fig. 5. a) *^{1}H,^{1}H-COSY* (▬) *and HMBC* (→) *correlations for* **5**. b) *NOEs* (↔) *and* W*-type couplings* (⇢⇢) *for* **5**.

was in pseudo-equatorial β-position, which was supported by a *W*-type coupling observed between H–C(1) and H_a–C(15) in the long-range ^{1}H,^{1}H-COSY spectrum (*Fig. 5*,*b*). The NOESY cross-peak observed between H–C(7) and H_α–C(9) implied that the isopropenyl group at C(7) was β-configured. Thus, the structure of **5** was determined as eudesma-4,11-diene-1β,15-diol, the absolute configuration of which remains to be established.

2. *Spontaneous Conversion of a Previously Isolated Bisabolane Derivative.* Recently, we reported the isolation of the bisabolane derivative **6** (= 3α,6α,9-tris(angeloyloxy)-2α,4β-dihydroxy-7,11-epoxybisabol-10(15)-en-5-one) from the roots of *L. dentata* [2]. Interestingly, when left as an oil in the refrigerator for a long time (nine months), we observed that **6** was partly converted into two new compounds identified as **7** and **8**, in yields of 13 and 25%, respectively.

Compound **7**, obtained as a colorless oil, had the molecular formula $C_{30}H_{44}O_{11}$, based on HR-EI-MS analysis (*m*/*z* 580.2877 (M^+; calc. 580.2883)). The ^{1}H- and

^{13}C-NMR spectra of **7** (see *Exper. Part*) were very similar to those of **6**, but lacked the signals due to the epoxide ring of **6**; instead, the signals due to an oxygenated CH (δ(H) 4.22 (*dd*, J = 8.3, 4.6 Hz); δ(C) 73.4) and an oxygenated, quaternary, sp^3-type C-atom (δ(C) 78.7 (C)) were found. The ^{1}H,^{1}H-COSY spectrum of **7** (*Fig. 6*) implied connectivities of H–C(9) to CH_2(8), and of CH_2(8) to H–C(7). The HMBC spectrum (*Fig. 6*) showed correlations between Me(12) and both C(7) and C(11), between Me(13) and both C(7) and C(11), and between CH_2(15) and C(9). By considering the chemical shift of H–C(7) (δ(H) 5.01), the linking position of the AngO group was determined to be C(7). The absolute configurations at C(9) and C(7) remain to be established. Accordingly, the structure of **7** was determined as 3α,6α,7-tris(angeloyloxy)-2α,4β,9,11-tetrahydroxybisabol-10(15)-en-5-one[3]), which is a new compound.

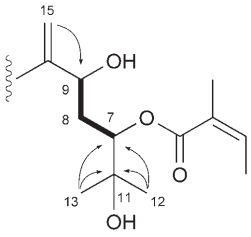
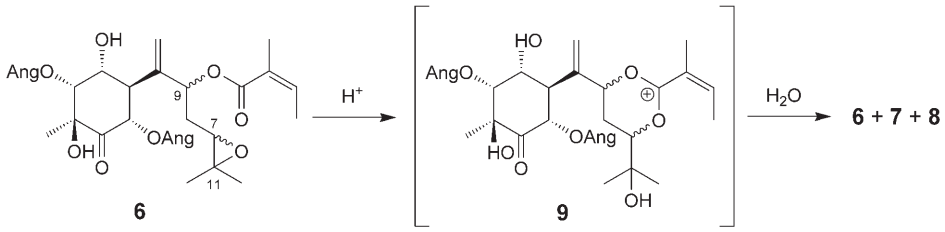


Fig. 6. *^{1}H,^{1}H-COSY* (—) *and HMBC* (→) *correlations for* **7**

The molecular formula of **8** was determined as $C_{30}H_{44}O_{11}$, based on HR-FAB-MS analysis (*m*/*z* 579.2785 ([*M* – H]$^-$; calc. 579.2805)). The spectroscopic data of **8** were very similar to those of **7** (see *Exper. Part*). The ^{1}H-NMR spectrum of **8** showed a downfield-shifted signal for H–C(9), and an upfield-shifted one for H–C(7), relative to those of **7**. The ^{13}C-NMR spectrum of **8** also showed a downfield-shifted signal for C(9) and an upfield-shifted one for C(7). The chemical shifts of all the other H- and C-atoms of **8** were basically identical to those of **7**. This clearly indicated that these two compounds were positional isomers. Thus, the structure of **8** was determined as 3α,6α,9-tris(angeloyloxy)-2α,4β,7,11-tetrahydroxybisabol-10(15)-en-5-one. Notably, this same compound has been isolated before from the roots of *L. dentata* [7]. The absolute configurations at C(9) and C(7) also remain to be established.

A proposed pathway for the formation of **7** and **8** from **6** is shown in the *Scheme*. Under hydrolytic conditions owing to atmospheric moisture, the reaction is expected to proceed *via* the dioxonium ion **9**, which is generated *via* a 6-*exo* ring closure by means of rearrangement of the epoxide ring and the AngO group at C(9) of **6** [8]. Subsequent

Scheme



direct hydrolysis of the intermediate dioxonium ion **9** then leads to the observed hydrolysis products **7** and **8** [8].

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

Experimental Part

General. Column chromatography (CC): silica gel (230–400 mesh; *Merck*). Prep. HPLC: *CCPD* pump (*Tosoh*), *TSKgel ODS-120T* column (300 × 7.8 mm; *Tosoh*), *RI-8010* detector (*Tosoh*). Optical rotations: *Jasco DIP-360* digital polarimeter. UV Spectra: *Beckman DU-64* spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *Jasco J-720* spectropolarimeter; $\Delta\varepsilon$ in l mol^{-1} cm^{-1} (λ in nm). IR Spectra: *Perkin-Elmer Spectrum-One* FT-IR spectrophotometer; in cm^{-1}. NMR Spectra: *Jeol JNM-LA 600* (600/150 MHz, resp.) and *JNM-LA 400* (400/100 MHz, resp.) spectrometers; *J* in Hz, δ in ppm rel. to residual solvent signals: C_6D_6: δ(H) 7.16, δ(C) 128.0; $CDCl_3$: δ(H) 7.27, δ(C) 77.0; CD_3OD: δ(H) 3.31, δ(C) 49.0. EI- and FAB-MS: *Jeol JMS-DX-303* and *JMS-700* mass spectrometers; in *m*/*z* (rel. %).

Plant Material. The roots of *Ligularia dentata* were collected in Sendai City, Miyagi Prefecture, Japan, in May 2004. A voucher specimen (LDB-2004-01) was deposited at the Laboratory of Molecular Structural Analysis, Tohoku Pharmaceutical University, Japan.

Extraction and Isolation. The roots of *L. dentata* (2.3 kg) were extracted three times (14 d each) with MeOH at r.t. The MeOH extract was filtered, concentrated under reduced pressure, and the residue (138 g) was suspended in a small excess of H_2O. The aq. suspension was extracted with Et_2O, and the Et_2O-soluble fraction was concentrated under atmospheric pressure to afford a residue (16.6 g), which was subjected to CC (SiO_2; 1. hexane/AcOEt 4:1 → 1:4, 2. AcOEt, 3. $CHCl_3$/MeOH 4:1 → 1:1, 4. MeOH): 44 fractions (*Fr. 1–44*) according to TLC. *Fr. 16* was purified by prep. HPLC (MeOH/H_2O 2:1, 1.5 ml/min) to afford 0.9 mg of **2** (t_R 72.7 min). *Fr. 18*, after purification by prep. HPLC (MeOH/H_2O 2:1, 1.0 ml/min), gave 0.6 mg of **5** (t_R 55.8 min) and 0.6 mg of **1** (t_R 65.7 min). *Fr. 20*, after purification by prep. HPLC (MeOH/H_2O 2:1, 1.0 ml/min), gave 0.4 mg of **4** (t_R 63.0 min) and 0.2 mg of **3** (t_R 90.3 min).

*(1*E*,5*R*)-3-Acetoxy-9-(angeloyloxy)-5-hydroxybisabola-3,1(10),7(11)-trien-2-one* (=*1-{(1*E*)-1-[(5*R*)-3-Acetoxy-5-hydroxy-4-methyl-2-oxocyclohex-3-en-1-ylidene]ethyl}-4-methylpent-3-en-1-yl (2*Z*)-2-Methylbut-2-enoate*; **1**). Colorless oil. $[\alpha]_D^{21} = -48.4$ ($c = 0.06$, MeOH). UV (MeOH): 203 (4.2), 218 sh (4.1), 279 (3.8). CD (MeOH): −2.65 (286), +2.56 (244), +9.87 (204). IR ($CHCl_3$): 3479, 1760, 1702, 1673, 1646, 1602. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 390 (1, M^+), 330 (20), 230 (50), 215 (51), 161 (100), 149 (30), 83 (41). HR-EI-MS: 390.2062 (M^+, $C_{22}H_{30}O_6^+$; calc. 390.2043).

*(1*S*,2*R*,6*S*,9*R*)-2,7-Bis(angeloyloxy)-6,9-epoxy-11-hydroxy-6-methoxybisabola-3,10(15)-dien-5-one* (=*2-Hydroxy-1-{[(2*R*,3a*S*,4*R*,7a*S*)-2,3,3a,4,7,7a-hexahydro-7a-methoxy-6-methyl-4-{[(2*Z*)-2-methylbut-2-enoyl]oxy}-3-methylidene-7-oxo-1-benzofuran-2-yl]methyl}-2-methylpropyl (2*Z*)-2-Methylbut-2-enoate*; **2**). Colorless oil. $[\alpha]_D^{21} = +11.5$ ($c = 0.09$, MeOH). UV (MeOH): 202 (4.1), 219 (4.1). CD (MeOH): −1.05 (356), +10.58 (241), −6.12 (216). IR ($CHCl_3$): 3525, 1713, 1692, 1647, 1563. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 476 (1, M^+), 276 (2), 244 (3), 83 (100). HR-EI-MS: 476.2413 (M^+, $C_{26}H_{36}O_8^+$; calc. 476.2410).

9α-(Angeloyloxy)-7β,10β-epoxy-11-methoxybisabola-1,3,5-triene-5,6-diol (=*(2*S**,3*R**,5*R**)-2-(2,3-Dihydroxy-4-methylphenyl)-2,3,4,5-tetrahydro-5-(1-methoxy-1-methylethyl)-2-methylfuran-3-yl (2*Z*)-2-Methylbut-2-enoate*; **3**). Colorless oil. $[\alpha]_D^{25} = +133.3$ ($c = 0.02$, MeOH). UV (MeOH): 204 (4.3), 220 sh (4.1), 280 (3.4). ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 378 (19, M^+), 346 (2), 305 (2), 278 (18), 263 (3), 246 (100), 231 (29), 203 (15), 175 (71), 83 (88). HR-EI-MS: 378.2049 (M^+, $C_{21}H_{30}O_6^+$; calc. 378.2042).

9-(Angeloyloxy)-11-methoxybisabola-1,3,5,10(15)-tetraene-5,6,7-triol (=*1-[1-(2,3-Dihydroxy-4-methylphenyl)ethenyl]-3-hydroxy-4-methoxy-4-methylpentyl (2*Z*)-2-Methylbut-2-enoate*; **4**). Colorless oil. $[\alpha]_D^{25} = +108.1$ ($c = 0.04$, MeOH). UV (MeOH): 203 (4.3), 211 sh (4.3), 279 (3.5). ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 378 (29, M^+), 346 (1), 305 (4), 278 (13), 260 (21), 246 (35), 228 (16), 175 (100), 162 (34), 83 (69). HR-EI-MS: 378.2047 (M^+, $C_{21}H_{30}O_6^+$; calc. 378.2042).

Eudesma-4,11-diene-1β,15-diol (=*(1*R**,6*R**,8a*R**)-1,2,3,5,6,7,8,8a-Octahydro-8a-(hydroxymethyl)-4-methyl-6-(1-methylethenyl)naphthalen-1-ol*; **5**). Colorless oil. $[\alpha]_D^{24} = +31.7$ ($c = 0.06$, MeOH). IR

($CHCl_3$): 3503. ^{1}H- and ^{13}C-NMR: see *Table 3*. EI-MS: 236 (5, M^+), 218 (34), 205 (25), 188 (100), 177 (13), 159 (17), 145 (89), 131 (84), 119 (23), 105 (57), 91 (51), 79 (35), 67 (25), 55 (39), 41 (65). HR-EI-MS: 236.1789 (M^+, $C_{15}H_{24}O_2^+$; calc. 236.1776).

3α,6α,7-Tris(angeloyloxy)-2α,4β,9,11-tetrahydroxybisabol-10(15)-en-5-one (*=(1*R*,2*S*,4*S*,5*R*,6*R*)-5-(2,5-Dihydroxy-5-methyl-4-{[(2Z)-2-methylbut-2-enoyl]oxy}-1-methylidenehexyl)-2,6-dihydroxy-2-methyl-3-oxocyclohexane-1,4-diyl (2Z,2′Z)Bis(2-methylbut-2-enoate)*; **7**) *and 3α,6α,9-Tris(angeloyloxy)-2α,4β,7,11-tetrahydroxybisabol-10(15)-en-5-one* (*=(1*R*,2*S*,4*S*,5*R*,6*R*)-5-(4,5-Dihydroxy-5-methyl-2-{[(2Z)-2-methylbut-2-enoyl]oxy}-1-methylidenehexyl)-2,6-dihydroxy-2-methyl-3-oxocyclohexane-1,4-diyl (2Z,2′Z)bis(2-methylbut-2-enoate)*; **8**). The oily compound **6** (4.0 mg) [2] was left for nine months in a refrigerator at 4°. The resulting product mixture was purified by prep. HPLC (MeOH/H_2O 2:1, 1.0 ml/min) to afford 0.5 mg (13%) of **7** (t_R 54.6 min), 1.0 mg of **8** (25%; t_R 56.7 min), and 2.3 mg of unchanged **6** (t_R 96.6 min).

Data of **7**. Colorless oil. $[\alpha]_D^{20} = +19.6$ ($c = 0.05$, MeOH). ^{1}H-NMR (400 MHz, CD_3OD): 1.17 (*s*, Me(12)); 1.19 (*s*, Me(13)); 1.21 (*s*, Me(14)); 1.62 (*ddd*, *J* = 15.1, 8.3, 7.6, H–C(8)); 1.84 (*dq*, *J* = 1.5, 1.5, Me(5′)); 1.89 (*dq*, *J* = 1.5, 1.5, Me(5″)); 1.91 (*dq*, *J* = 1.5, 1.5, Me(5‴)); 1.94 (*dq*, *J* = 7.1, 1.5, Me(4′)); 1.96 (*dq*, *J* = 7.1, 1.5, Me(4″)); 1.98 (*dq*, *J* = 7.1, 1.5, Me(4‴)); 2.29 (*ddd*, *J* = 15.1, 4.6, 3.9, H–C(8)); 2.85 (*dd*, *J* = 12.2, 10.7, H–C(1)); 4.22 (*dd*, *J* = 8.3, 4.6, H–C(9)); 4.62 (*dd*, *J* = 10.7, 2.9, H–C(2)); 5.01 (*dd*, *J* = 7.6, 3.9, H–C(7)); 5.25 (*s*, 1 H of CH_2(15)); 5.39 (*d*, *J* = 2.9, H–C(3)); 5.43 (*s*, 1 H of CH_2(15)); 6.10 (*qq*, *J* = 7.1, 1.5 Hz, H–C(3′), H–C(3″)); 6.12 (*d*, *J* = 12.2, H–C(6)); 6.17 (*qq*, *J* = 7.1, 1.5, H–C(3‴)). ^{13}C-NMR (100 MHz, CD_3OD): 16.0 (C(4′), C(4″)); 16.2 (C(4‴)); 19.8 (C(14)); 20.8 (C(5′), C(5″)); 20.9 (C(5‴)); 25.5 (C(12)); 26.8 (C(13)); 36.9 (C(8); 48.5 (C(1)); 71.6 (C(2)); 72.8 (C(11)); 73.4 (C(9)); 75.8 (C(6)); 76.7 (C(4)); 78.7 (C(7)); 80.2 (C(3)); 112.2 (C(15)); 128.7 (C(2′)); 128.8 (C(2″)); 129.5 (C(2‴)); 138.8 (C(3′)), 139.2 (C(3″)); 140.3 (C(3‴)); 152.0 (C(10)); 167.9 (C(1′)), 168.4 (C(1″)); 169.2 (C(1‴)); 204.5 (C(5)). EI-MS: 580 (1, M^+), 562 (2), 544 (17), 480 (5), 462 (4), 444 (3), 380 (9), 362 (14), 344 (14), 280 (6), 262 (57), 244 (18), 83 (100). HR-EI-MS: 580.2877 (M^+, $C_{30}H_{44}O_{11}^+$; calc. 580.2883).

Data of **8**. Colorless oil. $[\alpha]_D^{21} = +9.6$ ($c = 0.1$, MeOH). ^{1}H-NMR (600 MHz, CD_3OD): 1.13 (*s*, Me(12)); 1.15 (*s*, Me(13)); 1.21 (*s*, Me(14)); 1.68–1.70 (*m*, H–C(8)); 1.85 (9 H, *dq*, *J* = 1.5, 1.5, Me(5′), Me(5″), Me(5‴)); 1.91 (*dq*, *J* = 7.3, 1.5, Me(4′)); 1.95 (*dq*, *J* = 7.3, 1.5, Me(4″)); 1.96 (*dq*, *J* = 7.3, 1.5, Me(4‴)); 2.22–2.26 (*m*, H–C(8)); 3.01 (*dd*, *J* = 12.5, 11.0, H–C(1)); 3.48 (*dd*, *J* = 9.5, 2.6, H–C(7)); 4.74 (*dd*, *J* = 11.0, 2.9, H–C(2)); 5.33 (*s*, H–C(15)); 5.44 (*d*, *J* = 2.9, H–C(3)); 5.47 (*s*, H–C(15)); 5.72 (*dd*, *J* = 7.0, 7.0, H–C(9)); 6.04 (*d*, *J* = 12.5, H–C(6)); 6.05 (*qq*, *J* = 7.3, 1.5, H–C(3′)); 6.11 (*qq*, *J* = 7.3, 1.5 Hz, H–C(3″)); 6.18 (*qq*, *J* = 7.3, 1.5, H–C(3‴)). ^{13}C-NMR (150 MHz, CD_3OD): 16.0 (C(4′), C(4″)); 16.2 (C(4‴)); 19.8 (C(14)); 20.8 (C(5′), C(5″)); 20.9 (C(5‴)); 25.1 (C(12)); 25.7 (C(13)); 36.8 (C(8); 50.8 (C(1)); 70.0 (C(2)); 72.8 (C(11)); 73.7 (C(9)); 74.8 (C(6)); 76.5 (C(4)); 76.6 (C(7)); 80.1 (C(3)); 117.6 (C(15)); 128.7 (C(2′)); 129.0 (C(2″)); 129.2 (C(2‴)); 138.5 (C(3′)); 139.9 (C(3″)); 140.4 (C(3‴)); 148.6 (C(10)); 167.8 (C(1′)); 168.3 (C(1″)); 168.5 (C(1‴)); 204.3 (C(5)). HR-FAB-MS: 579.2785 ($[M-H]^-$, $C_{30}H_{43}O_{11}^-$; calc. 579.2805).

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