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Lipase inhibitory and LDL anti-oxidative triterpenes from *Abies sibirica*

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ABSTRACT

A methanol extract of *Abies sibirica* Ladeb, a Mongolian medicinal plant, had an inhibitory effect on both lipase activity in mouse plasma and LDL anti-oxidative activity, which are preventative factors for arteriosclerosis. The extract was fractionated by silica gel column chromatography and its active constituents were sought. From lipid soluble fractions, 20 terpenoids including seven hitherto unknown triterpenes were isolated. The latter triterpenes had either a γ-lactone ring with a lactol or a derivative thereof. Their chemical structures were determined by spectroscopic methods. The lipase inhibitory activity and LDL anti-oxidative activity of these compounds were evaluated. Some constituents (either lipase inhibitory or LDL anti-oxidative activities) had moderate inhibitory activities.

1. Introduction

Risk factors for cardiovascular disease have important meaning for the prevention and treatment of life style-related diseases, and our focus is on lipids and lipoproteins in bloods. Ingested triacylglycerols (TGs) and TGs in the bloodstream are hydrolyzed and resynthesized by lipases. For example, gastric and pancreatic lipases are enzymes which digest TGs to free fatty acids. After intestinal absorption, they are resynthesized into TGs by lipases in serums. Inhibitors and activators of these enzymes are expected to be key factors regulating lipid metabolism. Lipase inhibitors are considered to prevent digestion and intestinal absorption of fatty acids from dietary fats. Actually, lipase inhibitors such as orlistat have been used to treat of obesity, and extracts of various plants and their constituents were reported as anti-obesity agents based on their lipase inhibitory activity (Tucci et al., 2010; Yamada et al., 2010). Low-density lipoprotein (LDL) has important roles in the maintenance of the body. However, oxidized LDL is a factor for arteriosclerosis. So, LDL anti-oxidative activity is a target in the treatment of cardiovascular diseases. *Abies sibirica* Ladeb is a medicinal plant in Mongolia, used to treat tumors and diarrhea (Boldsaikhan, 2004). Consequently, their constituents and biological activities have been studied extensively (Ou-Yang et al., 2011; Xia et al., 2012). *A. sibirica* is a coniferous plant of the family Pinaceae distributed in Eurasia including Russia and Northern Europe. A previous study on the constituents of this plant mainly resulted in isolation of triterpenes (Yang et al., 2008).

In the present study, seven new triterpenes were isolated together with eleven known triterpenes, a known sesquiterpene, and a known monoterpene. Four of the new triterpenes had a γ-lactone ring which is typical of the genus *Abies*. The other three had a carboxylic acid, and their absolute configuration at the connected carbon was determined using phenylglycine methyl ester (PGME). Lipase inhibitory activity using the 2,3-dimercapto-1-propanol tributyrate (BALB) method and LDL anti-oxidant activity, both preventative factors for arteriosclerosis, of these constituents and the extract of *A. sibirica* were examined.

2. Results and discussion

A MeOH extract of *A. sibirica* had an inhibitory effect on lipase activity in isolated mouse plasma *in vitro* (IC_{50}, 0.39 mg/mL). The extract was dissolved in water and extracted with diethyl ether. The latter extract was found to exhibit an inhibitory effect (73.3% inhibition, 0.3 mg/mL), whereas the aqueous extract had low activity (1.6–26% inhibition, 0.3 mg/mL). The diethyl ether layer extract was purified on a silica gel column, and yielded 9 fractions (frs. 1A-1I, 5.7–89.7% inhibition, 0.3 mg/mL). Fr. 1B (89.7% inhibition, 0.3 mg/mL) was further fractionated using both an ODS column and HPLC, in accordance with the guide to monitoring inhibitory activity. Similarly, the diethyl ether layer obtained from another MeOH extract was passed through a column of silica gel ($CHCl_3$-MeOH) to yield 7 fractions (frs. 2A-2G). Frs. 2D-2F were

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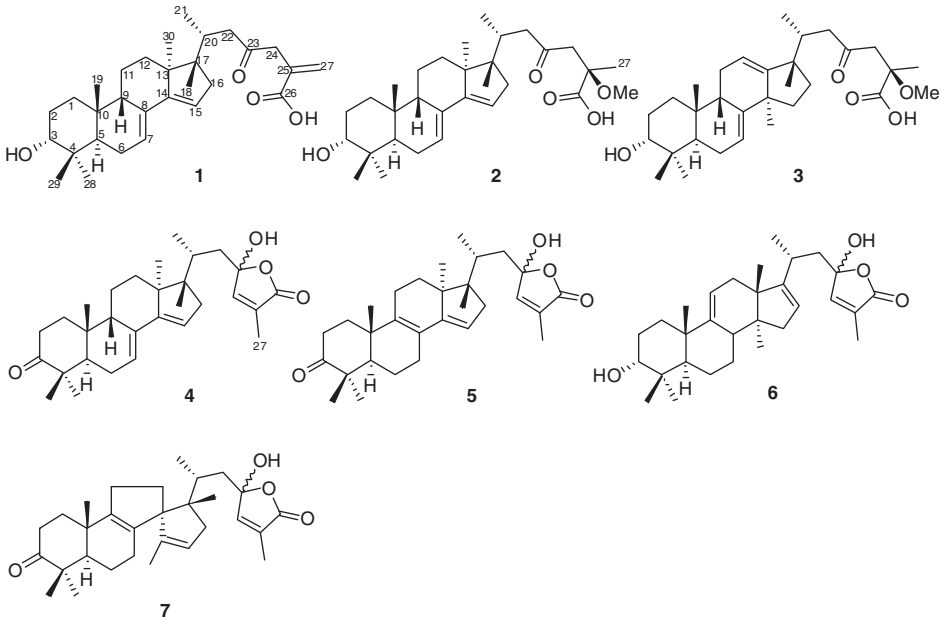

Fig. 1. Structures of **1**–**7**.

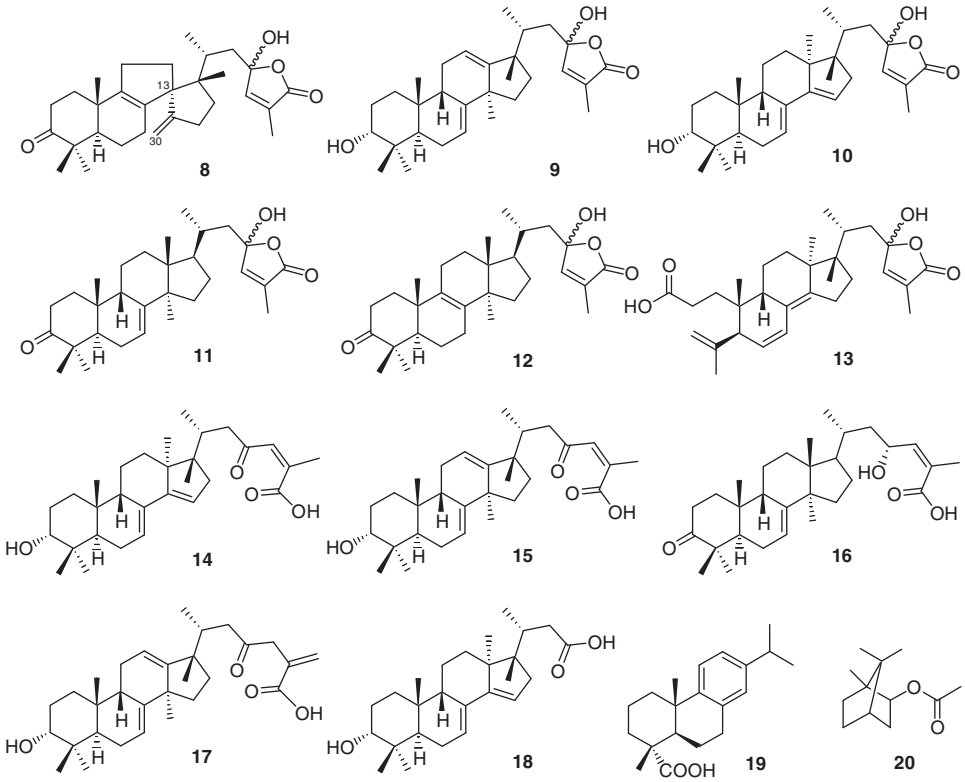

Fig. 2. Structures of **8**–**20**.

separated further using an ODS column and HPLC. Major components in the active fractions were terpenoids such as **1**–**20**, including seven new triterpenes (**1**–**7**, Fig. 1).

In the inhibitory assay with mouse plasma, compounds **10** and **11** showed concentration-dependent inhibition and IC_{50} values of 0.51 and 0.27 mM, respectively (Table 1). Although IC_{50} values were not estimated for their small yields, compounds **6**, **9**, **12**, and **15** showed inhibitory activity (24.9–63.4%, Table 1) at 0.1 mg/mL. Orlistat (IC_{50}: 0.09 mM) was used as a positive control. These results suggested that some triterpenes from *A. sibirica* including **10** and **11** have the potential to affect lipid metabolism as a lipase inhibitor.

The methanol extract of *A. sibirica* also had LDL anti-oxidative activity (Table 1). Five constituents (**5**, **6**, **8**–**10**) were found to have moderate activity (IC_{50}: 39.42–198.62 μM, Table 1).

The compounds **8**–**20** (Fig. 2) were identified based on spectroscopic data as (5*R*,20*R*)-23-hydroxy-8(14→13*R*)-abeo-17,13-friedo-3-oxolanosta-8,14(30),24-triene-26,23-olide (**8**) (Ou-Yang et al.,

2011), (3α,9β,17α)-3,23,23-trihydroxy-17-methyl-γ-lactone-18-nordammara-7,12,24-trien-26-oic acid (**9**) (Hasegawa et al., 1987b; Gao et al., 2008), 7,14,24-mariesatrien-26,23-olide-3α,23-diol (**10**) (Gao et al., 2008), 23-hydroxy-3-oxo-9β-lanosta-7,24-dien-26,23-olide (**11**) (Raldugin et al., 1989), 23-hydroxy-3-oxolanosta-8,24-dien-26,23-olide (**12**) (Grishko et al., 1998), 3,4-seco-4(28),6,8(14),24-mariesatetraene-26,23-olide-23-hydroxy-3-oic acid (**13**) (Gao et al., 2008), 23-oxo-mariesiic acid A (**14**) (Hasegawa et al., 1987b), 23-oxo-mariesiic acid B (**15**) (Hasegawa et al., 1987b), (23*R*,24*Z*)3-oxo-9β-lanosta-7,24-dien-23-hydroxy-27-oic acid (**16**) (Yang et al., 2010), 13,17-*friedo*-3α-hydroxy-9β-lanosta-7,12,25(27)-trien-23-oxo-26-oic acid (**17**) (Yang et al., 2010), isopseudolarifuroic acid B (**18**) (Yang and Yue, 2001), dehydroabietic acid (**19**) (Shitara et al., 2007), and bornyl acetate (**20**) (Mino et al., 2007).

The novel triterpenes **1**–**7** were isolated as amorphous powders with the ^{1}H and ^{13}C NMR spectroscopic data (measured in $CDCl_3$ at 30 °C) shown in Table 2 and the Section 4.

Compound **1** was deduced to have the molecular formula $C_{30}H_{44}O_4$ based on HREIMS (*m*/*z* 468.3243, calcd for $C_{30}H_{44}O_4$, 468.3241). The ^{13}C NMR spectrum of its A–D ring was similar to that of 7,14,22*Z*,24-mariesatetraen-26,23-olide-3-α-ol (Gao et al., 2008). Typical signals in the ^{1}H NMR spectrum, five singlet methyl proton resonances (δ 0.85, 0.86, 0.94, 0.97, 0.99), a doublet methyl proton signal at δ 0.85 (3H, d, *J* = 7.0 Hz, H-21), and four olefinic proton resonances at δ 5.18 (1H, dd, *J* = 3.0, 1.5 Hz, H-15), 5.57 (1H, dd, *J* = 6.0, 2.0 Hz, H-7), 5.75 (1H, br s, H-27), and 6.45 (1H, br s, H-27), were detected. These singlet methyl proton signals were assigned to the methyls in the A–D ring (Table 1) based on analysis of 2D-NMR spectra. In the HMBC spectrum, the H-7 proton was long-range coupled with δ 38.0 (C-5), 53.0 (C-9), and 152.9 (C-14), and the H-15 proton was long-range coupled with δ 45.0 (C-16), 50.5 (C-17), 51.7 (C-13), and 136.7 (C-8). Moreover, two methyl proton resonances at δ 0.94 and 0.99 (H-29 and 28) correlated with an oxygenated carbon C-3 (δ 76.8), a quaternary carbon C-4 (δ 37.2), and a methine carbon C-5. These results established that the A–D ring moiety was a 7,14-dien-3-ol. Signals of the side-chain were very similar to those of methyl isofirmanoate (Hasegawa et al., 1987a) and **17**. Two carbonyl carbons at δ 170.4 (carboxyl, C-26) and 207.2 (ketone, C-23) were correlated with H-24 methylene proton resonances at δ 3.34 (1H, d, *J* = 17.0 Hz) and 3.43 (1H, d, *J* = 17.0 Hz), the terminal olefinic methylene protons [H-27, δ 5.75 (br s) and 6.45 (br s)] were correlated with C-26, and the H-21 methyl proton signal was correlated with C-17 (δ 50.5), C-20 (δ 33.6), and C-22 (δ 46.7), in the HMBC spectrum. The NOE experiment showed that the hydroxy group at C-3 has the α-configuration (H-3 correlated with H-28 and -29) (Gao et al., 2008). These results suggested that the structure of **1** was as shown in Fig. 1. H-7 was correlated with H-15 and H-24 correlated with H-27 correlated with each other in the NOE spectra supporting this conclusion.

The ^{1}H NMR, ^{13}C NMR, and MS spectra of **2** and **3** resembled each other. The molecular formula $C_{31}H_{48}O_5$ for **2** and **3** was determined by HRFABMS (**2**, *m*/*z* 523.3390; **3**, 523.3412, calcd for $C_{31}H_{48}O_5Na$, 523.3400).

For **2**, the ^{1}H and ^{13}C NMR spectra were similar to those of **1** except for the signals of the side-chain. The 2D-NMR and NOE spectra showed that the A–D ring was the same as that of **1**. A singlet methyl (δ 1.47, 3H, H-27), a doublet methyl (δ 0.83, 3H, *J* = 7.0 Hz, H-21), a methoxy (δ 3.32, 3H, s), and two sets of methylene proton [δ 2.19 (1H, overlapped, H-22), 2.46 (1H, d, *J* = 16.5 Hz, H-22), 2.93 (1H, d, *J* = 16.5 Hz, H-24), and 2.98 (d, *J* = 16.5 Hz, H-24)] resonances were observed in the ^{1}H NMR spectrum. A carboxyl carbon (δ 174.9, C-26) and a ketone (δ 207.5, C-23) signal were detected in the ^{13}C NMR spectrum. In the spectra, **2** had an oxygenated quaternary carbon (δ 77.9, C-25) signal and the methyl signals (H-27) instead of the exomethylene signals of **1**. In the HMBC spectrum, the methoxy protons and the singlet methyl protons were long-range coupled with the oxygenated carbon (C-25). Moreover, the H-24 methylene protons were correlated with C-23, 25, 26, and 27. Another methylene proton (H-22) correlated with C-20, 21, 23, and 24. These results established that the side-chain of **2** was as shown in Fig. 1. Absolute stereochemistry at C-25 was determined by the PGME method (Yabuuchi and Kusumi, 2000). Compounds **2a** and **2b** were (*S*)- and (*R*)-PGME amides of **2**, and ^{1}H NMR values (**2a**–**2b**) suggested that C-25 has the *R*-configuration (Fig. 3). The NOE experiment showed that the C-3 hydroxy group had the α-configuration (H-3 correlated with H-28, 29). H-7 correlated with H-15 and H-27 correlated with H-24 in the NOE spectra, supporting this conclusion.

For **3**, the ^{1}H and ^{13}C NMR spectra were similar to those of **15** and **17** (Hasegawa et al., 1987b; Yang et al., 2010) except for the signals of the side-chain. In the HMBC spectrum, the methyl proton at δ 1.17 (3H, s, H-30) was correlated with olefinic carbon resonances at δ 145.9 (C-8) and 156.1 (C-13). These results suggested that the A–D ring moiety was 7,12-dien-3-ol. Signals of the side-chain in the spectra were similar to those of **2**. In the HMBC spectrum, the methoxy proton resonance (δ 3.30, 3H, s) and the singlet methyl proton signal (δ 1.45, s, H-27) were long-range coupled with an oxygenated carbon (δ 77.7, C-25) as in the case of **2**. Compound **3** was an isomer of **2** as shown in Fig. 1.

Compounds **4**–**7** have the same side-chain moiety, a γ-lactone ring with a lactol as shown in Fig. 1. Compounds **8**–**13** also have the side chain moiety. Some signals in the ^{1}H NMR spectra of these compounds showed broad weak peaks or divided peaks. Moreover, more resonances were observed in the ^{13}C NMR spectra than expected from the MS and analyses. These spectra were attributed to tautomeric mixtures from the γ-lactone moiety (Gao et al., 2008). Main signals are listed in Table. 1 and minor tautomeric peaks are shown in Section 4. The chemical structure was determined using the main signals.

The molecular formula $C_{30}H_{42}O_4$ for **4** and **5** was determined by HREIMS (**4**: *m*/*z* 466.3087, **5**: *m*/*z* 466.3076, calcd for $C_{30}H_{42}O_4$, 466.3084).

For **4**, the resonances of the A–D ring in the ^{13}C NMR spectrum were almost superimposable onto those of methyl 3,23-dioxo-mariesiate A (Hasegawa et al., 1987b), except for the side chain signals. A carbonyl carbon resonance at δ 216.9 was assigned to C-3 by HMBC correlations with H-1, 2, 28, and 29. An olefinic proton at δ 5.61 (1H, br s, H-7) and H-28, 29 were correlated with C-5 (δ 45.0) in the HMBC spectrum, and H-7 was correlated with H-15 (δ 5.22, 1H, br s) in the NOE spectrum. These results suggested that the A–D ring moiety of **4** was the same as that of methyl 3,23-dioxo-mariesiates A. Signals of ^{1}H, ^{13}C NMR spectra for the side-chain moiety were almost superimposable onto those of 7,14,24-mariesatrien-26,23-olide-3α,23-diol (Gao et al., 2008). Oxygenated carbon (δ 106.3, C-23), olefinic carbon (δ 147.9 and 131.6, C-24 and 25), carbonyl carbon (δ 171.7, C-26), and methyl proton (δ 1.92, s, H-27) resonances were typical for a γ-lactone ring

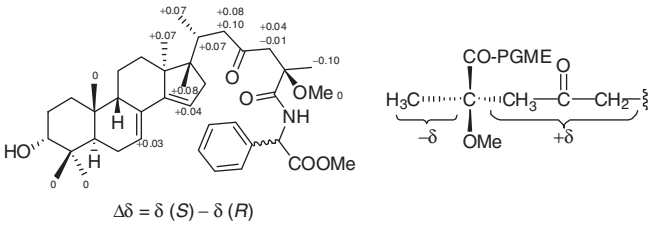

Fig. 3. Differences in chemical shifts of ^{1}H NMR (**2a**–**2b**) used to the determine absolute stereochemistry of C-25 for **2**.

Table 1
Lipase inhibitory activity and LDL anti-oxidative activity of methanol extract and constituents of *Abies sibirica*.

Compound	Lipase inhibitory activity		LDL anti-oxidative activity	
	%[a]	IC_{50} (mM)	%[b]	IC_{50} (μM)
1	NO			
2	NO			
4	NO			
5	NO		25.2 ± 4.9	198.62
6	43.0 ± 1.5		60.3 ± 5.0	55.32
7	NO		79.0 ± 0.6	64.11
8	NO			
9	24.9 ± 3.4		81.3 ± 0.7	49.14
10	65.6 ± 2.0	0.51	92.2 ± 2.1	39.42
11	75.6 ± 0.9	0.27	NO	
12	63.4 ± 5.2	ND		
13	NO			
14	NO		NO	
15	32.0 ± 2.2			
16	NO			
19	NO			
20	NO			
methanol ext.	39.9 ± 1.0[c]	0.39 mg/mL	64.1 ± 5.9[d]	0.02 mg/mL
orlistat		0.09		
probucol				10.53

Concentration of test samples in reaction solution, [a]0.1 mg/mL, [b]0.05 mg/mL, [c]0.3 mg/mL, [d]0.025 mg/mL. Each value represents the mean ($n = 3$). Data are expressed as means ± standard error (SE). NO, not observed. ND, not determined.

having a lactol structure (Gao et al., 2008; Li et al., 2009). In the HMBC spectrum, an olefinic proton at δ 6.86 (br s, H-24) was long-range coupled with C-23, 25–27. In the NOE spectrum, a methyl proton (H-27) was correlated with the olefinic proton (H-24). A doublet methyl proton (δ 0.96, 3H, $J = 7.0$ Hz, H-21) and correlated carbon signals [δ 51.7 (C-17), 33.3 (C-20), 41.2 (C-22)] in the HMBC spectrum were resonances of the side-chain moiety. These results suggested that the side-chain moiety was an aliphatic chain with a lactone ring as shown in Fig. 1.

In the ^{1}H and ^{13}C NMR spectra of **5**, signals for the A–D ring, four olefinic carbon resonances, and only one olefinic proton at δ 5.33 (br s, H-15) were observed. Signals for the A and D ring and side-chain moieties were similar to those of **4**, and the 2D-NMR spectra suggested the same partial structure as **4**. In the HMBC spectrum, H-15 was long-range coupled with the olefinic carbon at δ 123.8 (C-8), and H-19 (δ 1.12, s) correlated with another olefinic carbon at δ 140.1. These correlations suggested that C-8,9 was a double bond. Thus, the chemical structures of **4** and **5** were determined as shown in Fig. 1.

Compound **6** was deduced to have the molecular formula $C_{30}H_{44}O_4$ by HREIMS (m/z 468.3237, calcd for $C_{30}H_{44}O_4$, 468.3241). In the ^{1}H and ^{13}C NMR spectra, signals of the side chain showed that **6** has a γ-lactone ring the same as **4** and **5**. The molecular formula suggested that **6** was an isomer of **9** and **10**. However, the olefinic and singlet protons of the A–D ring in **6** differed from those in **9** and **10**. A singlet proton resonance at δ 0.88 (s, H-18) was long-range coupled with carbons at δ 30.8 (C-12), 51.6 (C-13), 46.8 (C-14), and 157.1 (C-17). Another singlet proton signal at δ 0.82 (s, H-30) was long-range coupled with C-13, 14, and 15 (δ 40.8). So these methyl groups face each other across the C-13 and 14 of the C and D rings. A doublet methyl proton signal of the side-chain at δ 1.10 (d, $J = 7.0$ Hz, H-21) and an olefinic proton signal at δ 5.44 (m, H-16) correlated with C-17 in the HMBC spectrum, and H-16 correlated with H-15 (δ 1.70–2.30, overlapped) in the NOE spectrum. These correlations suggested that C16–17 of the D-ring is a double bond. Another olefinic proton resonance at δ 5.33 was assigned to H-11, because it was long-range coupled with C-10, 12, 13 in the HMBC spectrum, and correlated with H-12 proton signals at δ 1.50–1.90 (overlapped) and a H-19 methyl singlet proton resonance in the NOE spectrum. The singlet proton signal at δ 1.09 (s, H-19) correlated with C-1 (δ 30.1), C-9 (δ 149.6), and C-10 (δ 40.1). These results showed that C-9–11 was a double bond. In the NOE spectrum, the H-3 (δ 3.44, m) correlated with H-28 (δ 0.99, s) and H-29 (δ 0.90, s), suggesting that the C-3 hydroxy group has an α-configuration. Thus, the structure of **6** was determined as shown in Fig. 1.

The molecular formula $C_{30}H_{42}O_4$ for **7** was determined by HRE-IMS (m/z 466.3070, calcd for $C_{30}H_{42}O_4$, 466.3084), and **7** is an isomer of **8**. The ^{1}H and ^{13}C NMR spectra of **8** were similar to those of (24*Z*)-8(14–13)-abeo-17,13-friedolanosta-8,14(30),24-triene-3,23-dion-26-oic acids (Raldugin et al., 1992) except for signals of the side chain moiety. For **7**, resonances of the side chain were typical of a γ-lactone ring the same as in **4**–**6**. Its NMR spectra were similar to those of **8** except for resonances of the D-ring. Compound **7** has a vinylic methyl group (δ 1.46, s, H-30) and an olefinic proton (δ 5.23, br s, H-15) instead of the C14–30 exomethylene group of **8**. In the HMBC spectrum, the H-30 signal correlated with a quaternary carbon resonance at δ 71.1 (C-13), two olefinic carbon signals at δ 144.7 (C-14) and 123.0 (C-15), and a methylene carbon resonance at δ 40.5. These results suggested that C14–15 was a double bond in **7**, instead of C14–30 in **8**, as shown in Fig. 1.

The A–D ring skeletons of **1**–**7** were considered to be the results of biosynthesis of the lanostane skeleton by enzymic dehydrogenation of H-17 (Hasegawa et al., 1987a,b; Kuroyanagi et al., 2000). The configurations at C-17, C-20 (**1**–**5**, **7**) and C-13, C-20 (**6**) were supposed to be as shown in Fig. 1.

3. Conclusion

From the leaves of *A. sibirica*, seven new triterpenes (**1**–**7**) and 13 known terpenoids (**8**–**20**) were isolated. Compounds **4**–**7** have a γ-lactone ring with a lactol. Compounds **10** and **11** had IC_{50} values of 0.51 and 0.27 mM respectively for the inhibition of lipase activity in isolated mouse plasma *in vitro* using the BALB method. Some other triterpenes (**6**, **9**, **12**, and **15**) also showed inhibitory activity. Five of these triterpenes (**5**, **6**, **8**–**10**) were LDL oxidation inhibitors.

4. Experimental

4.1. General

Optical rotations were measured on a Jasco P-2300 polarimeter. UV spectra were recorded on a Shimadzu MPS-2450. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol JNM-AL400 FT-NMR spectrometer, and chemical shifts were given as δ values with TMS as an internal standard at 30 °C (measured in $CDCl_3$). Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H} = 145$ Hz) and HMBC (optimized for $^nJ_{C-H} = 8$ Hz) pulse sequences with a pulsed field gradient. HRFABMS and HREIMS data were obtained on a Jeol JMS700 mass spectrometer, using either a *m*-nitrobenzyl alcohol or a glycerol matrix. Preparative Yamazen Cartridge column chromatography (CC) and HPLC were performed on a Jasco 2089 with UV at 210 nm, using the following columns: Ultra Pack ODS-SM-50C-M [Yamazen, 37 × 100 mm; mobile phase, CH_3CN–0.2%CF_3CO_2H (80:20)], Cosmosil 5C_{18}–AR II [Nacalai Tesque, 20 × 250 mm; mobile phase, CH_3CN–H_2O (80:20) or (85:15) or (90:10)], Cosmosil 5PE-MS [Nacalai Tesque, 20 × 250 mm; solvent, CH_3CN–0.2%CF_3CO_2H (70:30) or MeOH–H_2O (90:10)], Develosil C30-UG-5 [Nomura Chemical, 20 × 250 mm; solvent, CH_3CN–0.2%CF_3CO_2H (85:15) or (90:10) or (95:5)] and Mightysil RP-18 GP [Kanto Chemical, 10 × 250 mm; solvent, CH_3CN–0.2%CF_3CO_2H (85:15)]. 5,5′-Dithio-bis(2-nitrobenzoic acid) and phenylmethylsulfonyl fluoride were purchased from

Nacalai Tesque Inc. (Kyoto, Japan), while 2,3-dimer-capto-1-propanol tributyrate was purchased from Aldrich Chemical Co. (Milwaukee, WI). Orlistat was obtained from Roshe Products Ltd. (Auckland, NZ). Carvacrol was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

4.2. Plant material

A. sibirica was collected in July 2008 in Mongolia. A voucher specimen has been deposited at the herbarium of Tohoku Pharmaceutical University, No. 20100112. The plant was identified by Dr. C. Sanchir, Institute of Botany, Mongolian Academy of Sciences.

4.3. Extraction and isolation

Dried leaves (150 g) of *A. sibirica* were extracted with MeOH (3 L 2×) at room temperature for a month's duration. The combined extract (37.6 g) was suspended in H_2O (1.5 L), and extracted with Et_2O (1.0 L 3×). The resulting Et_2O extract (12.3 g) was passed through a silica gel column [Wakogel C-200, Wako Pure Chemical Industries Ltd., Osaka, Japan, 30 g; solvent, $CHCl_3$, $CHCl_3$–MeOH (99:1), (98:2), and MeOH] yielding 9 fractions (frs. 1A-1I) and **20** (1.08 g). Fr. 1B [$CHCl_3$–MeOH (99:1) (2.54 g)] was applied to a reversed-phase column using ODS (Cosmosil 140C_{18}-OPN, Nacalai Tesque, Osaka, Japan, 150 g) and eluted with MeOH–water (8:2), MeOH, and EtOAc, respectively. The MeOH–H_2O (8:2) fraction (1.8 g) was next subjected to preparative HPLC, yielding compounds **11** (11.7 mg), **12** (16.1 mg), and **19** (38.0 mg). Subsequently, dried leaves (650 g) of *A. sibirica* were extracted with MeOH (7 L 2×) at room temperature for a month's duration. The resulting extract (164.9 g) was suspended in H_2O (1.5 L), and extracted with Et_2O (1.0 L 3×). The Et_2O extract (68.9 g) was passed through a silica gel column [Wakogel C-200, Wako Pure Chemical Industries Ltd., Osaka, Japan, 200 g; solvent, $CHCl_3$–MeOH (100:0) → (0:100) gradient] yielding 7 fractions (frs. 2A–2G). Frs. 2D–2F [$CHCl_3$–MeOH (99:1)–(9:1) (26.8 g)] were applied to a reversed-phase column using ODS (Cosmosil 140C_{18}-OPN, Nacalai Tesque, Osaka, Japan, 150 g) and eluted with MeOH–H_2O (8:2), MeOH, and EtOAc. The MeOH–H_2O (8:2) fraction (13.9 g) was subjected to preparative HPLC, yielding compounds **1** (7.7 mg), **2** (7.5 mg), **3** (3.1 mg), **4** (37.9 mg), **5** (21.2 mg), **6** (14.6 mg), **7** (11.1 mg), **8** (1.7 mg), **9** (22.0 mg), **10** (88.1 mg), **11** (170.2 mg), **12** (33.0 mg), **13** (50.1 mg), **14** (41.0 mg), **15** (16.8 mg), **16** (27.9 mg), **17** (1.6 mg), and **18** (0.3 mg), repectively.

4.4. 3α-Hydroxymariesia-7,14,25(27)-trien-23-oxo-26-oic acid (*1*)

Colorless amorphous powder; $[\alpha]_D^{23}$ +30.7 (*c* 0.56, MeOH); HREIMS *m*/*z* 468.3243 $[M]^+$ (calcd for $C_{30}H_{44}O_4$, 468.3241); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2.

4.5. (25R)-3α-Hydroxy-25-methoxy-23-oxo-mariesia-7,14-dien-26-oic acid (*2*)

Colorless amorphous powder; $[\alpha]_D^{22}$ +20.0 (*c* 0.66, MeOH); HRFABMS *m*/*z* 523.3390 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3400); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2.

4.6. (25R)-3α-Hydroxy-25-methoxy-23-oxo-mariesia-7,12-dien-26-oic acid (*3*)

Colorless amorphous powder; $[\alpha]_D^{21}$ −103.1 (*c* 0.32, MeOH); HRFABMS *m*/*z* 523.3412 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3400); UV (MeOH) λ_{max} nm ($\log\varepsilon$): 245 (4.75); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2.

4.7. 23-Hydroxy-3-oxomariesia-7,14,24-trien-26,23-olide (*4*)

Colorless amorphous powder; $[\alpha]_D^{21}$ +35.1 (*c* 1.99, MeOH); HREIMS *m*/*z* 466.3087 $[M]^+$ (calcd for $C_{30}H_{42}O_4$, 466.3084); for main peaks of ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2; Observable peaks of another minor tautomer in ^1H-NMR spectrum ($CDCl_3$) δ 6.88 (1H, br s, H-27); Observable minor peaks of ^{13}C NMR spectrum ($CDCl_3$) δ 120.0 (C-7), 33.4 (C-20), 17.5 (C-21), 40.8 (C-22), 106.7 (C-23), 147.7 (C-24), 131.5 (C-25), 171.6 (C-26).

4.8. 23-Hydroxy-3-oxomariesia-8(9),14,24-trien-26,23-olide (*5*)

Colorless amorphous powder; $[\alpha]_D^{21}$ −2.5 (*c* 1.14, MeOH); HREIMS *m*/*z* 466.3076 $[M]^+$ (calcd for $C_{30}H_{42}O_4$, 466.3084); UV (MeOH) λ_{max} nm ($\log\varepsilon$): 249 (4.88); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2; Observable peaks of another minor tautomer in ^1H NMR spectrum ($CDCl_3$) δ 0.86 (3H, s, H-18), 0.97 (3H, d, *J* = 7.0 Hz, H-21), 6.90 (1H, m, H-24), 1.94 (3H, s, H-27); Observable minor peaks of ^{13}C NMR spectrum ($CDCl_3$) δ 35.0 (C-2), 29.8 (C-10), 45.1 (C-16), 50.8 (C-17), 17.3 (C-21), 40.8 (C-22), 106.7 (C-23), 147.6 (C-24), 131.7 (C-25), 171.5 (C-26), 26.6 (C-28).

4.9. 3α,23-Dihydroxylanosta-9(11),16,24-trien-26,23-olide (*6*)

Colorless amorphous powder; $[\alpha]_D^{21}$ +19.4 (*c* 0.72, MeOH); HREIMS *m*/*z* 468.3237 $[M]^+$ (calcd for $C_{30}H_{44}O_4$, 468.3241); UV (MeOH) λ_{max} nm ($\log\varepsilon$): 246 (4.70); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2; Observable peaks of another minor tautomer in ^1H NMR spectrum ($CDCl_3$) δ 1.33 (1H, m, H-5), 5.31 (1H, m, H-16), 0.71 (3H, s, H-18), 1.08 (3H, s, H-19), 1.11 (3H, d, *J* = 7.0 Hz, H-21), 1.88 (3H, d, *J* = 1.5 Hz, H-27), 0.81 (3H, s, H-30); Observable minor peaks of ^{13}C NMR spectrum ($CDCl_3$) δ 31.3 (C-12), 51.2 (C-13), 46.9 (C-14), 120.5 (C-16), 156.3 (C-17), 19.7 (C-18), 27.8 (C-20), 22.8 (C-21), 43.5 (C-22), 147.1 (C-24), 131.5 (C-25), 10.5 (C-27).

4.10. 23-Hydroxy-8(14→13)-abeo-17,13-fried-3-oxolanosta-8,14(15),24-triene-26,23- olide (*7*)

Colorless amorphous powder; [α] $[\alpha]_D^{21}$ −64.4 (*c* 2.21, MeOH); HREIMS *m*/*z* 466.3070 $[M]^+$ (calcd for $C_{30}H_{42}O_4$, 466.3084); UV (MeOH) λ_{max} nm ($\log\varepsilon$): 246 (4.66); for ^1H NMR and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2; Observable peaks of another minor tautomer in ^1H NMR spectrum ($CDCl_3$) δ 0.93 (3H, s, H-18), 6.84 (1H, br s, H-24); Observable minor peaks of ^{13}C NMR spectrum ($CDCl_3$) δ 144.6 (C-14), 50.7 (C-17), 22.1 (C-18), 37.6 (C-20), 20.9 (C-21), 41.6 (C-22), 107.7 (C-23), 148.5 (C-24), 131.1 (C-25), 172.8 (C-26).

4.11. (S)- and (R)- PGME amides of *2*

To **2** (2.0 mg each) in *N*,*N*-dimethylformamide (0.5 mL) was added either (*S*)-PGME or (*R*)-PGME (5.0 mg). Benzotriazotriazole-1-yl-oxy-tris-pyrrolidinophoniumhexafluorophosphate (10 mg), 1-hydroxybenzotriazole (4.0 mg) and *N*-methylmorpholine (30 μL) were then added. The mixtures were stirred for 15 h at room temperature, and subjected to HPLC [columns, Mightysil RP18GP, 10 × 250 mm; solvents CH_3CN–0.2%CF_3CO_2H (90:10); detector, UV 210 nm], yielding the (*S*)-amide of **2** (0.7 mg) and (*R*)-PGME amide of **2** (0.7 mg), respectively.

4.12. (S)-PGME amide of *2* (*2a*)

Colorless amorphous powder; FAB-MS *m*/*z* 648 $[M+H]^+$, 670 $[M+Na]^+$, ^1H NMR (400 MHz, $CDCl_3$) δ 5.57 (1H, m, H-7), 5.18 (1H, m, H-15), 0.87 (3H, s, H-18), 0.97 (3H, s, H-19), 2.42 (1H, m,

Table 2
NMR spectroscopic data (400 MHz) for compounds **1–7**, **9**.

Position	1				2				3				4 (main)			
	δ_H (J in Hz)	δ_c	HMBC (H to C)	NOE (H to H)	δ_h (J in Hz)	δ_c	HMBC (H to C)	NOE (H to H)	δ_h (J in Hz)	δ_c	HMBC (H to C)	NOE (H to H)	δ_h (J in Hz)	δ_c	HMBC (H to C)	NOE (H to H)
1	0.95[a] 2.04, m	28.8			0.94[a] 1.98[a]	28.7			1.02, m 1.96[a]	29.3			1.60[a] 1.82[a]	35.5	2, 3, 5, 9	2
2	1.63, m 1.98[a]	25.3		3,19	1.62[a] 2.00[a]	25.3		19	1.63, m 1.94[a]	25.3	1, 3	19	2.29, m 2.74, m	35.0	1, 3	1,19
3	3.47, t (3.0)	76.8	1, 2, 5, 28, 29	28,29	3.47, br s	76.4[a]	4, 5, 28, 29	28,29	3.47, t (3.0)	77.2	1, 5	2,28,29		216.9		
4		37.2				37.2				36.8				47.3		
5	1.56, dd (11.5,5.0)	38.0			1.55, dd (11.5,5.0)	38.0	9		1.45[a]	38.1			1.52[a]	45.0	1, 4, 6, 10	
6	1.88–2.01[a]	23.1			1.85–2.05[a]	23.0			1.94[a]	23.2	5, 7	5,7	1.90–2.10[a]	23.9	5, 7, 8	5,7
7	5.57, dd (6.0,2.0)	120.7	5, 6, 9, 14	6,15	5.57, br d (5.0)	120.8	5, 6, 8, 9, 14	6,15	5.63, m	118.8		6	5.61, br s	120.1	5, 6, 9	6,15
8		136.7				136.7				145.9				136.8		
9	1.42	53.0	7, 8, 14		1.41[a]	53.0	8, 11, 14		1.41, m	50.9			5.12[a]	52.4	7, 8, 11	
10		34.8				34.8				34.8				34.6		
11	1.42[a] 1.80–1.90[a]	25.4			1.41[a] 1.80[a]	25.2	9		1.80, m 2.24[a]	28.1			1.36[a] 1.65[a]	25.1		
12	1.44[a] 1.82[a]	33.7			1.45[a] 1.82[a]	33.5			5.45, dd (8.5,2.0)	122.4	11, 14, 17		1.50[a] 2.05[a]	33.1		
13		51.7				51.7				156.1				51.5		
14		152.9				152.8				50.0				152.3		
15	5.18, dd (3.0,1.5)	115.0	8, 13, 14, 16, 17	7,16	5.18, brs	115.0	8, 13, 14, 16, 17	7,16,18	1.40–1.47[a]	37.0			5.22, br s	115.5		7,16
16	1.91, dd (16.0,3.0) 2.20, br d (16.0)	45.0	14, 15		1.89[a] 2.20[a]	45.0			1.40–1.47[a] 1.90[a]	38.3			1.90[a] 2.17, br d (15.5)	45.0		
17		50.5				50.0				46.3				51.7		
18	0.88, s	19.3	13, 16, 17, 20		0.87, s	19.3	16, 20	15,16,20	0.91	24.7	13, 16, 17	12,16	0.88, s	16.7	13, 16, 17	
19	0.97, s	22.3			0.97, s	22.7	1, 9, 10		0.94, s	22.1	1, 5, 9, 10		1.13, s	21.7	1, 5, 9, 10	2
20	2.46, m	33.6			2.42[a]	33.9			2.20[a]	38.4			1.55–1.70[a]	33.3		
21	0.85, d (7.0)	16.6	17, 20, 22		0.83, d (7.0)	16.6	17, 20, 22	20,22	0.87, d (6.0)	15.7	17, 20, 22		0.96, d (7.0)	17.4	17, 20, 22	24
22	2.53, br d (16.5) 2.29 dd (16.5,11.0)	46.7	17, 20, 21, 23		2.19[a] 2.46, d (16.5)	47.6	20, 21, 23, 24		2.18[a] 2.76, d (13.5)	47.2	20, 21, 23		2.04, m	41.2	20, 23, 24	
23		207.2				207.5				208.0				106.3		
24	3.34, d (17.0) 3.43, d (17.0)	46.0	22, 23, 25, 26, 27	27	2.93, d (16.5) 2.98, d (16.5)	49.5	23, 25, 26, 27	27	2.88–2.92 2.98–3.02	49.0	23, 25, 26, 27		6.86, br s	147.9	23, 25, 26, 27	22,27
25		133.8				77.9				77.7				131.6		
26		170.4				174.9				174.3				171.7		
27	5.75, br s 6.45, br s	130.7	24, 25, 26	24	1.47, s	21.3	24, 25, 26	24,OMe	1.45	21.4	24, 25, 26	OMe	1.92, s	10.4	23, 25, 26	24
28	0.99, s	28.2	3, 4, 5, 29	3,5	0.99, s	28.2	3, 4, 5, 29	3,5	0.98, s	28.2	3, 4, 5, 29		1.10, s	25.5	3, 4, 5, 29	5
29	0.94, s	23.0	3, 4, 5, 28	3	0.94, s	23.1	3, 4, 5, 28	3	0.93, s	23.0	3, 4, 5, 28		1.10, s	22.6	3, 4, 5, 28	2
30	0.86, s	17.1	12, 13, 14, 17		0.85, s	17.1	13,17		1.17	25.9	8, 13, 14, 15		0.88, s	19.0	12, 13, 17	
OMe					3.32, s	51.6	25		3.30-	51.5	25					

(*continued on next page*)

Table 2 (*continued*)

Position	5 (main)				6 (main)				7 (main)				9 (main)		
	δ_H (J in Hz)	δ_C	HMBC (H to C)	NOE (H to H)	δ_H (J in Hz)	δ_C	HMBC (H to C)	NOE (H to H)	δ_H (J in Hz)	δ_C	HMBC (H to C)	NOE (H to H)	δ_H (J in Hz)	δ_C	HMBC (H to C)
1	1.60[a] 1.90[a]	34.9			1.30–2.40[a]	30.1			1.60[a] 1.98[a]	36.2			1.96[a]	29.3	10, 19
2	2.45[a] 2.57, m	33.4	1, 3		1.70, m 2.03, m	25.7	1, 3		2.56, m	34.6	1, 3, 10	1	1.63[a] 1.96[a]	24.3	
3		217.8			3.44, br s	76.3	1	28,29		219.3			3.47, br s	77.2	1, 4, 29
4		47.3				37.9				47.4				37.0	
5	1.68, m	51.1	4, 6, 7, 10, 28, 29		1.36, m	46.7			1.62[a]	51.3	4		1.48[a]	37.9	
6	2.00–2.20[a]	22.8			1.30–1.50[a]	22.0	5, 7		1.45–1.80[a]	20.3			1.42[a] 1.96[a]	22.9	5, 7, 8 5, 7, 8
7	1.60–1.70[a]	19.5	8, 9		2.20–2.50[a]	28.0			1.73[a]– 2.00[a] 2.00[a]	26.2			5.63, br s	118.7	5, 9
8		123.8			2.38, m	40.0	30			137.5				145.7	
9		140.1				149.6				144.1				50.6	
10		37.7				40.1				36.0				34.8	
11	1.70[a] 2.45[a]	34.4	8, 9		5.33, m	114.0	10, 12, 13	12	2.00[a]	26.2			2.15–2.30[a]	28.4	
12	1.50–1.80[a]	29.9	9, 11, 13, 17		1.50–1.90[a]	30.8			2.05–2.40[a]	29.2			5.57, m	123.0	9, 11, 17
13		47.9				51.6				71.1				156.6	
14		148.1				46.8				144.7				50.2	
15	5.33, br s	116.9	8, 13, 16, 17		1.70–2.30[a]	40.8			5.23, br s	123.0	13, 16, 17, 30	30	1.30–1.70[a]	36.8	
16	1.96, m 2.30, d (16.0)	45.2	14, 15, 17, 18		5.44, m	121.5	13, 15, 17	15	1.90–2.15[a]	40.5			1.30–1.70[a]	38.4	
17		50.7				157.1				50.8				46.8	
18	0.78, s	17.1	13, 16, 17, 20		0.88, s	19.9	12, 13, 14, 15	8,15	0.91, s	21.9	13, 16, 17, 20	24	0.93, s	25.8	13, 16, 17
19	1.12, s	18.7	1, 9, 10	1	1.09, s	21.1	1, 10	1,11	1.06, s	19.5	1, 5, 9, 10		0.93, s	22.1	1, 9, 10
20	1.10[a]	33.1			1.60[a]	27.7			1.62[a]	37.2	23		1.30–1.70[a]	37.9	
21	1.00, d (7.0)	17.5	17, 20, 22	24	1.10, d (7.0)	23.3	17, 20, 22	22	0.83, br s	20.6	17, 20, 22	15,24	1.01, d (7.0)	18.0	17, 20, 22
22	2.17[a]	41.2	17, 20, 21, 23, 24		2.25–2.45[a]	43.0			1.60[a]	41.7	17, 20, 23		1.69, dd (14.0, 8.5)	40.5	17, 20, 21, 23
					1.85–2.10[a]				2.16[a]				2.44, br d (14.0)		17, 20, 21, 23
23		106.2				105.8				107.4				106.8	
24	6.87, m	147.7	23, 25, 26, 27	27	6.79, m	147.4	23, 25, 26, 27	27	6.86, br s	148.4	23, 25, 26, 27	18, 22, 27	6.71, s	147.2	23, 25, 26
25		131.8				131.3				131.2				131.9	
26		171.6				171.8				172.9				171.8	
27	1.94, s	10.5	24, 25, 26	24	1.90, d (6.5)	10.4	24, 25, 26	16,24	1.92, s	10.3	24, 25, 26	24	1.89, s	10.4	24, 25, 26
28	1.11, s	26.7	3, 4, 5, 29		0.99, s	28.4	3, 4, 5, 29	3,5	1.10, s	27.0	3, 5, 29	5	0.98, s	28.1	3, 4, 5, 29
29	1.08, s	21.3	3, 4, 5, 28		0.90, s	22.6	3, 4, 5, 28	3	1.08, s	21.1	3, 5, 28	5	0.93, s	23.2	3, 4, 5, 28
30	0.86, s	15.7	12, 13, 14, 17		0.82, s	19.9	13, 14, 15		1.46, s	13.5	13, 14, 15	15	1.12, s	25.4	8, 14, 15

[a] Overlapped.
[b] Interchangeable.

H-20), 0.83 (3H, d, J = 7.0 Hz, H-21), 2.22 (1H, m, H-22), 2.46 (1H, m, H-22), 2.77 (1H, d, J = 15.5 Hz, H-24), 2.99 (1H, d, J = 15.5 Hz, H-24), 1.36 (3H, s, H-27), 0.99 (3H, s, H-28), 0.93 (3H, s, H-29), 0.84 (3H, s, H-30), 3.30 (3H, s, H-OMe).

4.13. (R)-PGME amide of **2** (**2b**)

Colorless amorphous powder; FAB-MS m/z 648 $[M+H]^+$, 670 $[M+Na]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.54 (1H, m, H-7), 5.14 (1H, m, H-15), 0.79 (3H, s, H-18), 0.97 (3H, s, H-19), 2.36 (1H, m, H-20), 0.76 (3H, d, J = 7.0 Hz, H-21), 2.14 (1H, m, H-22), 2.36 (overlapped, H-22), 2.73 (1H, d, J = 15.5 Hz, H-24), 3.00 (1H, d, J = 15.5 Hz, H-24), 1.46 (3H, s, H-27), 0.99 (3H, s, H-28), 0.93 (3H, s, H-29), 0.77 (3H, s, H-30), 3.30 (3H, s, H-OMe).

4.14. Assay of lipase activity

Lipase activity was determined as described previously (Kobayashi et al., 2004; Yamada et al., 2010). Cardiac blood was collected from mice with a heparin-treated cylinder and centrifuged to prepare plasma. Each test sample was adjusted to the relevant final reaction concentration in ultrapure H_2O, EtOH, or DMSO and added to the mouse plasma prior to the assay. Inhibitory activity (%) was calculated as follows: $(1 - B/A) \times 100$, where A is the activity of the enzyme in the absence of the test solution and B, that in the presence of the test solution. The final concentration for inhibitory activity of the H_2O layer extract, ether layer extract, and fractions obtained by column chromatography was 0.3 mg/mL.

4.15. Human LDL anti-oxidative activity

The assay was carried out according to the method of Hirano et al. (1997). The sample adjusted to each final reaction concentration in EtOH or DMSO was added to a 4.18×10^{-5} mM Human LDL saline solution. After a 20 mM 2,2-azobis (4-methylvaleronitrile) EtOH was added as an initiator, the change in absorbance of 234 nm was measured at 5 min intervals at 37 °C for 200 min. LDL oxidation was shown as the reaction speed (ΔAbs/min) which is the increase in absorbance from 100 min to 200 min after the initiator was added. LDL oxidative inhibition was calculated using the following equation; I (%) = $(\Delta A_{con}/\text{min} - \Delta A_{sam}/\text{min})/\Delta A_{con}/\text{min} \times 100$, where ΔA_{con} is the reaction speed without the test sample and ΔA_{sam} is the reaction speed with the sample. The increasing reaction speed of a blank (without LDL) was subtracted from each reaction speed.

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