A New Megastigmane Palmitate and a New Oleanane Triterpenoid from *Aster yomena* MAKINO

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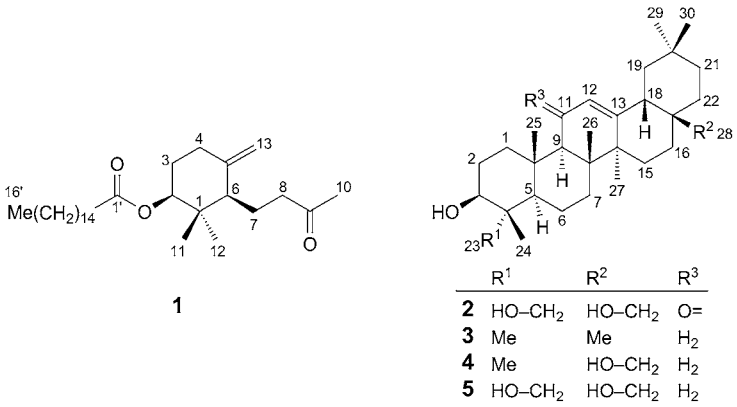
A new megastigmane palmitate, 9-oxomegastigm-5(13)-ene-2β-palmitate (**1**), and a new oleanane triterpenoid, (3β)-3,23,28-trihydroxyolean-12-en-11-one (**2**), together with three known oleanane-type triterpenoids, β-amyrin (**3**), erythrodiol (**4**), and (3β)-olean-12-ene-3,23,28-triol (**5**), were isolated from the aerial parts of *Aster yomena* (Asteraceae). Their structures were identified based on 1D- and 2D-NMR analysis, including ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY techniques.

Introduction. – *Aster yomena* MAKINO (Asteraceae) is a perennial herb that is distributed throughout Korea and Japan. The whole plant is used in Korean traditional medicine for the treatment of bronchial asthma, inflammation, and colds [1][2]. In our ongoing investigation on biologically active compounds from natural sources, we studied the aerial parts of *A. yomena.* As a result, a new megastigmane palmitate, 9-oxomegastigm-5(13)-ene-2β-palmitate (**1**) and a new oleanane-type triterpenoid, (3β)-3,23,28-trihydroxyolean-12-en-11-one (**2**), together with three known oleanane-type triterpenoids, β-amyrin (**3**), erythrodiol (**4**), and (3β)-olean-12-ene-3,23,28-triol (**5**) were isolated (see *Fig. 1*). Of these compounds, the oleanane-type triterpenoids **3**–**5** were obtained from this plant for the first time. Here, the isolation and structure elucidation of the new compounds were reported.



1

	R^1	R^2	R^3
2	HO–CH_2	HO–CH_2	O=
3	Me	Me	H_2
4	Me	HO–CH_2	H_2
5	HO–CH_2	HO–CH_2	H_2

Fig. 1. *Structures of compounds* **1**–**5**

Results and Discussion. – Compound **1** was obtained as colorless oil. The molecular formula was determined as $C_{29}H_{52}O_3$ by HR-FAB-MS ($[M+Na]^+$ peak at *m/z* 471.3803) and ^{13}C-NMR spectra. The ^{1}H- and ^{13}C-NMR spectra, in combination with a HSQC spectrum (*Table 1*), showed signals for a COO group (δ(C) 173.4), 14 CH_2 groups (δ(H) 2.30–2.33 (*m*, 2 H), 1.60–1.64 (*m*, 2 H) 1.35–1.25 (*m*, 24 H); δ(C) 34.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 25.1, 22.7), and a primary Me group (δ(H) 0.88 (*t*, *J* = 7.0); δ(C) 14.1), indicating the presence of a palmitate group, as evidenced by the intense mass fragment-ion peak observed at *m/z* 255 [$Me(CH_2)_{14}COO^-$] in the ESI mass spectrum. In addition, signals, which were assignable to three tertiary Me groups (δ(H) 2.12, 0.97, 0.85; δ(C) 30.1, 26.4, 18.7), one C=O group (δ(C) 209.1), one exocyclic CH_2 group (δ(H) 4.88 and 4.57 (2 br. *s*; δ(C) 109.8), two CH groups (δ(H) 4.68 (*dd*, *J* = 3.9, 7.6), 1.72 (*dd*, *J* = 2.0, 11.7); δ(C) 77.7, 51.8), four CH_2 groups (δ(C) 42.7, 30.4, 28.4, 19.9), and two quaternary C-atoms (δ(C) 146.7, 39.0) evidenced the presence of a megastigmane skeleton moiety in **1** [3]. The ^{1}H,^{1}H-COSY spectrum showed connectivities of C(2) with C(3), C(3) with C(4), C(6) with C(7), and C(7) with C(8). In the HMBC spectrum, correlations H–C(2)/C(1′); CH_2(3)/CH_2(4)/H–C(6) and C(5); CH_2(7)/CH_2(8)/Me(12)/CH_2(13) and C(6) were observed. These findings indicated that the palmitate group was at C(2). Besides, the megastigmane moiety of **1** was similar to that of 3β-*O*-(β-D-glucopyranosyloxy)megastigm-9-one, which was isolated from *Laggera alata* [4], except for the presence of an exocyclic CH_2 group (δ(H) 4.88 and 4.57 (2 br. *s*); δ(C) 109.8), and the palmitate substituent at C(2).

Table 1. *^{1}H-* (600 MHz) *and ^{13}C-NMR* (150 MHz) *Data of Compound* **1**. Recorded in $CDCl_3$; δ in ppm, *J* in Hz. Atom numbering as indicated in *Fig. 1*

Position	δ(H)	δ(C)
C(1)		39.0
H–C(2)	4.68 (*dd*, *J* = 3.9, 7.6)	77.7
CH_2(3)	1.82–1.84 (*m*), 1.57–1.61 (*m*)	28.4
CH_2(4)	2.26–2.30 (*m*), 1.99–2.03 (*m*)	30.4
C(5)		146.7
H–C(6)	1.72 (*dd*, *J* = 2.0, 11.7)	51.8
CH_2(7)	1.85–1.89 (*m*), 1.76–1.80 (*m*)	19.9
CH_2(8)	2.48–2.53 (*m*), 2.27–2.34 (*m*)	42.7
C(9)		209.1
Me(10)	2.12 (*s*)	30.1
Me(11)	0.85 (*s*)	18.7
Me(12)	0.97 (*s*)	26.4
CH_2(13)	4.88 (br. *s*), 4.57 (br. *s*)	109.8
C(1′)		173.4
CH_2(2′)	2.30–2.33 (*m*)[a])	34.8
CH_2(3′)	1.60–1.64 (*m*)	25.1
CH_2(4′ to 13′)	1.35–1.25 (*m*, 20 H)[a])	29.2–29.7
CH_2(14′)	1.35–1.25 (*m*, 2 H)[a])	31.9
CH_2(15′)	1.35–1.25 (*m*, 2 H)[a])	22.7
Me(16′)	0.88 (*t*, *J* = 7.0)	14.1

[a]) Signals overlapped.

The relative configuration of **1** was confirmed by a NOESY experiment (*Fig. 3*), and a comparison with pertinent reference values [5–7]. The configuration of the palmitate group at C(2) could be determined as β on the basis of the coupling pattern of H–C(2) (δ(H) 4.68 (*dd*, $J = 3.9, 7.6$)). In the NOESY spectrum, distinct NOE between H–C(2) and Me(12), and H–C(2) and H–C(6) indicated that all of these are located on the same side (α). On the basis of the above results, the structure of **1** is determined as 9-oxomegastigm-5(13)-ene-2β-palmitate.

Compound **2** was obtained as amorphous powder. It gave rise to a positive *Libermann–Burchard* color reaction, indicating a triterpenoid structure. The molecular formula was determined as $C_{30}H_{48}O_4$ by HR-FAB-MS ($[M-H]^-$ peak at *m/z* 471.3491) and ^{13}C-NMR spectra (*Table 2*). The ^{1}H- and ^{13}C-NMR and HSQC spectra of **2** exhibited 30 C-atom resonances, six Me groups (δ(H) 1.43, 1.16, 1.13, 0.92, 0.92, 0.69; δ(C) 33.5, 24.1, 24.0, 19.3, 17.4, 12.9), one C=O group (δ(C) 202.8), one olefinic H-atom

Table 2. *^{1}H-* (600 MHz) *and ^{13}C-NMR* (150 MHz) *Data of Compound* **2**. Recorded in CD_3OD; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)
CH_2(1)	2.70 (*dt*, $J = 3.4, 13.2$), 0.96–1.01 (*m*)	40.1
CH_2(2)	1.68–1.75 (*m*), 1.54–1.60 (*m*)	27.6
H–C(3)	3.60 (*dd*, $J = 5, 11$)	73.2
C(4)		43.9
H–C(5)	1.16–1.18 (*m*)[a])	48.3
CH_2(6)	1.50–1.54 (*m*), 1.40–1.48 (*m*)	18.3
CH_2(7)	1.79–1.84 (*m*)[a]), 1.36–1.41 (*m*)[a])	33.3
C(8)		45.0
H–C(9)	2.47 (*s*)	63.2
C(10)		38.2
C(11)		202.8
H–C(12)	5.51 (*s*)	128.8
C(13)		173.5
C(14)		46.9
CH_2(15)	1.79–1.85 (*m*)[a]), 1.17–1.21 (*m*)[a])	27.0
CH_2(16)	1.97–2.02 (*m*), 1.29–1.33 (*m*)	22.7
C(17)		38.4
H–C(18)	2.22 (*dd*, $J = 4.2, 13.7$)	44.2
CH_2(19)	1.79–1.84 (*m*)[a]), 1.07–1.12 (*m*)	46.4
C(20)		32.1
CH_2(21)	1.35–1.41 (*m*), 1.19–1.24 (*m*)	35.1
CH_2(22)	1.59–1.65 (*m*), 1.39–1.43 (*m*)[a])	31.9
CH_2(23)	3.52 (*d*, $J = 11.0$), 3.29 (*d*, $J = 11.0$)	66.6
Me(24)	0.69 (*s*)	12.9
Me(25)	1.16 (*s*)	17.4
Me(26)	1.13 (*s*)	19.3
Me(27)	1.43 (*s*)	24.1
CH_2(28)	3.38 (*d*, $J = 11.0$), 3.16 (*d*, $J = 11.0$)	69.8
Me(29)	0.92 (*s*)[a])	33.5
Me(30)	0.92 (*s*)[a])	24.0

[a]) Signals overlapped.

(δ(H) 5.51 (*s*); δ(C) 128.8), two O-bearing CH_2 groups (δ(H) 3.38, 3.16 (2*d*, $J = 11.0$), 3.52, 3.29 (2*d*, $J = 11.0$); δ(C) 69.8, 66.6), as well as one O-bearing CH group (δ(H) 3.60 (*dd*, $J = 5.0$, 11.0); δ(C) 73.2). The above mentioned data and the molecular formula pointed to a triterpenoid with an olean-12-ene skeleton [7]. Furthermore, ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and HMBC data (*Fig. 2*) revealed that the structure of **2** was very similar to that of 3β,28-dihydroxy-12-oleanene-11-one, which was isolated from *Viburnum awabuki* [8], except for the presence of an additional O-bearing CH_2 group at C(4). The signals at δ(H) 3.52, 3.29 (2*d*, $J = 11.0$), which were coupled with that at δ(C) 66.6, were assigned to the CH_2(23) moiety bearing an OH group. In the HMBC spectrum, correlations between H–C(23) and C(3)/Me(24) indicated that this OH group might be located at C(23). In addition, the OH group at C(3) could be determined to be β-oriented on the basis of the coupling pattern of H–C(3) (δ(H) 3.60 (*dd*, $J = 5.0$, 11.0)) [5–7]. The relative configuration of **2** was deduced from NOESY experiments (*Fig. 3*). Correlations between H–C(5), tentatively assigned in an α-orientation, and H–C(23), H–C(5), and H–C(9); H–C(5), and H–C(23), H–C(23), and H–C(3); H–C(9) and Me(27) indicated that they could be on the same side (α), while correlations between Me(24) and Me(25), Me(25) and H–C(18), H–C(18) and H–C(28), and H–C(18) and Me(30) indicated that they should be on the opposite side (β). On the basis of the above results, the structure of **2** is identified as (3β)-3,23,28-trihydroxyolean-12-en-11-one.

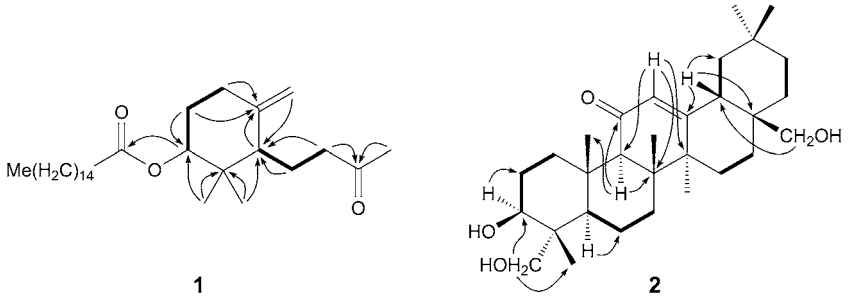


Fig. 2. *Key ^{1}H,^{1}H-COSY correlations* (—) *and HMBCs* (H → C) *of* **1** *and* **2**

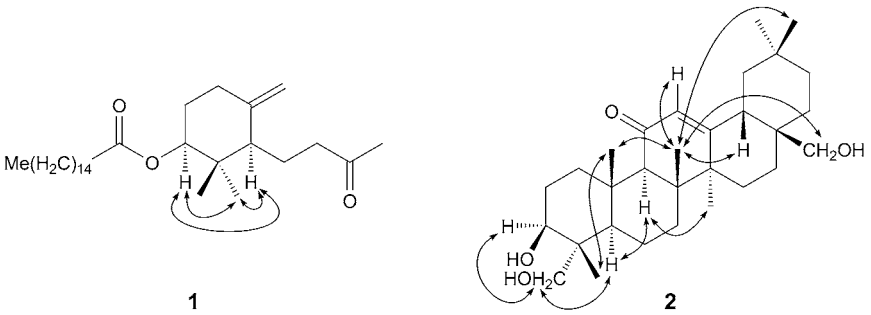


Fig. 3. *Key NOESY correlations of* **1** *and* **2**

Compounds **3**–**5** were identified as β-amyrin [9][10], erythrodiol [8][11], and (3β)-olean-12-ene-3,23,28-triol [12][13], respectively, by comparing their NMR data with those reported in the literature. These compounds were reported for the first time from *A. yomena*.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 40–63 and 63–200 μm, *Merck*), or reversed-phase (RP) silica gel (*LiChroprep® RP-18* (40–63 μm, *Merck*), *MCI* gel *CHP 20P* (70–150 μm, *Mitsubishi Chemical Co.*), and *Sephadex LH-20* (25–100 μm, *Sigma*). TLC: silica gel *60* F_{254} (*5715*, *Merck*) and *RP-18* F_{254s} (*15389*, *Merck*) plates, detected by spraying with 10% H_2SO_4 in H_2O and heating on a hot plate. Sample pretreatment: *YMC Dispo* SPE *ODS* (*C18* 1000 mg/6 ml). Semi-prep. HPLC: *Waters 600* instrument; *YMC-Pack ODS-A* column (250 × 10 mm i.d., 5 μm), flow rate 3.0 ml/min. Optical rotations: *Autopol-IV* polarimeter (*Rudolph Research Flangers*). FT-IR Spectra: *Perkin-Elmer Spectrum 100* FT-IR spectrometer. 1D- and 2D-NMR spectra: *Varian Unity Inova 500* and *Unity Inova 600* spectrometer (KBSI-Gwangju center). HR-FAB-MS: *JEOL JMS 700* mass spectrometer.

Plant Material. The aerial parts of *Aster yomena* MAKINO were collected from the Herbarium of the College of Pharmacy, Chosun University, Korea, in September 2003. Plants were identified by Prof. *Eun-Rhan Woo* of College of Pharmacy, Chosun University, Korea. A voucher specimen was deposited with the Herbarium of the College of Pharmacy, Chosun University (CSU-1029–17).

Extraction and Isolation. The air-dried aerial parts of *A. yomena* (1.9 kg) were extracted with MeOH three times at r.t., and 120 g of residue were obtained. The MeOH extract was suspended in H_2O and partitioned sequentially in CH_2Cl_2 (3 × 4 l), AcOEt (3 × 4 l), and BuOH (3 × 4 l) to afford fractions soluble in CH_2Cl_2 (23.6g), AcOEt (15.3g), and BuOH (48.9g). The CH_2Cl_2 fraction (15 g) was subjected to CC (SiO_2; hexane/acetone 100:0 → 0:100): *Frs. D1 – D12*. *Fr. D3* (320 mg) was subjected to CC (SiO_2; hexane/acetone 30:1 → 1:1): *Frs. D31 – D33*. *Fr. D31* (42 mg) was again subjected to CC (*RP-C_{18}*; 90% aq. MeOH): compound **1** (1.51 mg). *Fr. D11* (2.54 g) was purified by CC (*YMC-ODS*; aq. MeOH 80% – 100%; SiO_2; $CHCl_3$/MeOH 30:1, 20:1): *Frs. D11-131 – D11-136*. *Frs. D11-133* and *D11-134* were purified by semi-prep. HPLC (80% aq. MeOH): compound **5** (t_R 23; 6.1 mg) and **2** (t_R 16; 1.5 mg). *Fr. D5* (800 mg) was submitted to CC (SiO_2; hexane/acetone 10:1): *Frs. D51 – D53*. *Fr. D52* (42 mg) was purified by CC (*RP-C_{18}*; 90% aq. MeOH; SiO_2, hexane/acetone 10:1): compound **3** (4.8 mg). *Fr. D9* (690 mg) was subjected to CC (SiO_2; hexane/AcOEt 5:1): *Frs. D91 – D96*. *Fr. D94* (92 mg) was purified by CC (*RP-C_{18}*; 90% aq. MeOH; SiO_2, hexane/AcOEt 5:1): compound **4** (5.5 mg).

9-Oxomegastigm-5(13)-ene-2β-palmitate (=(*1S,3R*)*-2,2-Dimethyl-4-methylidene-3-(3-oxobutyl)cyclohexyl Hexadecanoate*; **1**). Colorless oil. $[\alpha]_D^{25} = +11.54$ ($c = 0.07$, $CHCl_3$). IR (KBr): 1732, 1721, 1672, 1376. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-FAB-MS: 471.3803 ($[M+Na]^+$, $C_{29}H_{52}NaO_3^+$; calc. 471.3814).

(3β)-3,23,28-Trihydroxyolean-12-en-11-one (**2**). White powder. $[\alpha]_D^{25} = +52.9$ ($c = 0.10$, $CHCl_3$). IR (KBr): 3406, 1715, 1651. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-FAB-MS: 471.3491 ($[M-H]^-$, $C_{30}H_{47}O_4^-$; calc. 471.3474).

β-Amyrin (**3**). White powder. $[\alpha]_D^{25} = +88.4$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3409, 1645. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.18 (*t*, *J* = 3.5, Me(12)); 3.21 (*dd*, *J* = 5.0, 11.0, H–C(3)); 1.13 (*s*, Me(27)); 1.00 (*s*, Me(23)); 0.97 (*s*, Me(26)); 0.94 (*s*, Me(25)); 0.87 (*s*, Me(29), Me(30)); 0.83 (*s*, Me(28)); 0.79 (*s*, Me(24)). ^{13}C-NMR (75 MHz, $CDCl_3$): 145.4 (C(13)); 121.7 (C(12)); 79.2 (C(3)); 55.4 (C(5)); 47.8 (C(9)); 47.4 (C(18)); 47.0 (C(19)); 41.9 (C(14)); 40.0 (C(8)); 39.0 (C(4)); 38.8 (C(1)); 37.3 (C(22)); 37.1 C (10)); 34.9 (C(21)); 33.6 (C(29)); 32.8 (C(7)); 32.7 (C(17)); 31.3 (C(20)); 28.6 (C(28)); 28.3 (C(23)); 27.4 (C(16)); 27.1 (C(2)); 26.3 (C(15)); 26.2 (C(27)); 23.9 (C(30)); 23.7 (C(11)); 18.6 (C(6)); 17.0 (C(26)); 15.8 (C(25)); 15.7 (C(24)).

Erythrodiol (**4**). White powder. $[\alpha]_D^{25} = +77.0$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3406, 1651. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.19 (*t*, *J* = 3.5, H–C(12)); 3.56 (*d*, 11.0, 1 H of CH_2(28)); 3.22 (*d*, 11.0, 1 H of

CH_2(28)); 3.23 (*dd*, *J* = 5.0, 11.0, H–C(3)); 1.17 (*s*, Me(27)); 1.00 (*s*, Me(23)); 0.94 (*s*, Me(26)); 0.93 (*s*, Me(25)); 0.89 (*s*, Me(29)) 0.87 (*s*, Me(30)); 0.79 (*s*, Me(24)). ^{13}C-NMR (125 MHz, $CDCl_3$): 144.3 (C(13)); 122.4 (C(12)); 79.1 (C(3)); 69.7 (C(28)); 55.2 (C(5)); 47.6 (C(9)); 46.5 (C(19)); 42.4 (C(18)); 41.8 (C(14)); 39.8 (C(8)); 38.8 (C(4)); 38.6 (C(1)); 37.0 (C(10)); 36.9 (C(17)); 34.1 (C(21)); 33.3 (C(29)); 32.6 (C(7)); 31.1 (C(22)); 31.0 (C(20)); 28.1 (C(23)); 27.3 (C(2)); 26.0 (C(27)); 25.6 (C(15)); 23.6 (C(30)); 23.6 (C(11)); 22.0 (C(16)); 18.4 (C(6)); 16.8 (C(26)); 15.6 (C(25)); 15.6 (C(24)).

(3β)-Olean-12-ene-3,23,28-triol (**5**). White powder. $[\alpha]_D^{25} = +61.5$ (*c* = 0.60, $CHCl_3$). IR (KBr): 3406, 1651. ^{1}H-NMR (500 MHz, CD_3OD): 5.18 (*t*, *J* = 3.5, H–C(12)); 3.60 (*dd*, *J* = 5.0, 11.0, H–C(3)); 3.52 (*d*, *J* = 11.0, 1 H of CH_2(28)); 3.10 (*d*, *J* = 11.0, 1 H of CH_2(28)); 3.52 (*d*, *J* = 11.0, 1 H of CH_2(23)); 3.30 (*d*, *J* = 11.0, 1 H of CH_2(23)); 1.19 (*s*, Me(27)); 1.00 (*s*, Me(26)); 0.99 (*s*, Me(25)); 0.89 (*s*, Me(29)); 0.88 (*s*, Me(30)); 0.71 (*s*, Me(24)). ^{13}C-NMR (125 MHz, CD_3OD): 145.9 (C(13)); 123.6 (C(12)); 74.0 (C(3)); 69.9 (C(28)); 67.5 (C(23)); 49.0 (C(5)); 48.8 (C(9)); 48.0 (C(19)); 44.0 (C(18)); 43.5 (C(14)); 43.1 (C(4)); 41.2 (C(8)); 39.8 (C(1)); 38.3 (C(10)); 38.0 (C(17)); 35.4 (C(21)); 33.9 (C(29)); 33.4 (C(7)); 32.5 (C(20)); 32.0 (C(22)); 26.8 (C(2)); 26.7 (C(27)); 24.8 (C(15)); 24.2 (C(30)); 23.1 (C(11)); 19.3 (C(16)); 18.4 (C(6)); 17.5 (C(25)); 16.6 (C(26)); 12.9 (C(24)).

REFERENCES

[1] T. B. Lee, 'Illustrated Flora of Korea', Hyang-Moon Publishing, Seoul, 1993, p. 737.
[2] D. K. Ahn, 'Illustrated Book of Korean Medicinal Herbs', Kyo-Hak Publishing, Seoul, 1998, p. 64.
[3] Q.-P. Zhang, B.-F. Zhang, G.-X. Chou, Z.-T. Wang, *Helv. Chim. Acta* **2011**, *94*, 1130.
[4] Q. Zheng, Z. Xu, X. Sun, W. Yao, H. Sun, Christopher H. K. Cheng, Y. Zhao, *Phytochemistry* **2003**, *63*, 835.
[5] P. Liu, P. Hu, R.-X. Deng, R. Li, L. Yang, W.-P. Yin, *Helv. Chim. Acta* **2011**, *94*, 136.
[6] L. T. K. Van, T. M. Hung, S. H. Kim, J. C. Kim, M. Na, H. J. Jung, S. J. Kwack, K. T. Kweon, J, S. Choi, H. K. Lee, K. Bae, B.-S. Min, *Helv. Chim. Acta* **2010**, *93*, 1803.
[7] D.-Q. Luo, H.-Y. Deng, X.-L. Yang, B.-Z. Shi, J.-Z. Zhang, *Helv. Chim. Acta* **2011**, *94*, 1041.
[8] M. Kagawa, H. Minami, M. Nakahara, H. Takahashi, S. Takaoka, Y. Fukuyama, *Phytochemistry* **1998**, *47*, 1101.
[9] M. Shibuya, Y. Katsube, M. Otsuka, H. Zhang, P. Tansakul, T. Xiang, Y. Ebizuka, *Plant Physiol. Biochem.* **2009**, *47*, 26.
[10] J. Bhattacharyya, C. B. Barros, *Phytochemistry* **1985**, *25*, 274.
[11] P. Pant, R. P. Rastogi, *Phytochemistry* **1977**, *16*, 1787.
[12] M. H. Abd El-Razek, *Chin. Pharm. J.* **2006**, *58*, 95.
[13] K. Tori, S. Seo, A. Shimaoka, Y. Tomita, *Tetrahedron Lett.* **1974**, *15* 4227.

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