Coleifolides A and B, Two New Sesterterpenoids from the Aerial Parts of *Scutellaria coleifolia* H.Lév.

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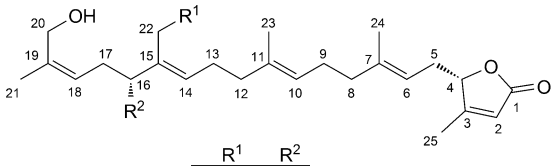
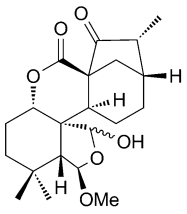
Coleifolides A and B (**1** and **2**), two new sesterterpenoids with a β-methyl-α,β-unsaturated-γ-lactone moiety, were isolated from the aerial parts of *Scutellaria coleifolia* H.Lév. (Lamiaceae), together with three known compounds. Their structures were elucidated by NMR and MS examinations. Coleifolides A and B were concluded to be partially racemic compounds by the HPLC analysis using a chiral column or introduction of chiral derivatizing agents. The absolute configuration of the major isomer was determined by analyses of the CD spectrum as well as NMR data of (*R*)- and (*S*)-2-NMA derivatives. Coleifolides A and B are structurally similar to manoalide derivatives, previously isolated from marine sponges, and appear to be the first examples of this type of compounds being isolated from higher plants.

Introduction. – *Scutellaria* plants, belonging to the Lamiaceae family, include about 350 species, and are widely distributed in template zones and tropical zones of Europe, North America, and East Asia. They have been used as traditional medicines in many countries [1]. For example, roots of *S. baicalensis* have been used for removing fever and anti-inflammation in Chinese Traditional Medicines, while aerial parts of *S. galericulata* have been used as sedative and antispasmodic in United States [1]. Flavonoids, flavonoid glycosides, and *neo*-clerodane-type diterpenoids were shown to be major constituents of the *Scutellaria* plants by previous studies. Biological activities including anticancer, anti-inflammatory, and anti-feedant activity of the isolated compounds were also reported [1]. *Scutellaria coleifolia* H.Lév. is distributed in high altitude regions of Yunnan and Sichuan Provinces. However, there are no reports of chemical investigations on this plant. As part of our search for new drug seeds, we have studied the constituents of the aerial parts of *S. coleifolia*. We describe herein the isolation, structure elucidation, and biological activities of new compounds.

Results and Discussion. – Dried aerial parts of *S. coleifolia*, collected in Sichuan Province in August 2011, were extracted with 70% aqueous acetone (3×15 l) at room temperature. The extract was partitioned between AcOEt and H_2O three times. The AcOEt-soluble fraction (130 g) was repeatedly subjected to column chromatography (silica gel, *MCI-gel CHP 20P*, *Sephadex LH-20*, MPLC on *RP-18* and reversed-phase HPLC) to give coleifolides A (**1**; 5 mg) and B (**2**; 30 mg), irroratin A (**3**; 2 mg) [2], teucvin (**4**; 34 mg) [3] and 12-epiteucvin [3] (**5**; 5 mg).



	R^1	R^2
1	OH	H
2	H	OH



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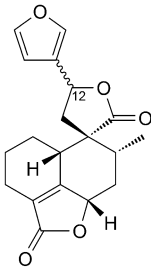


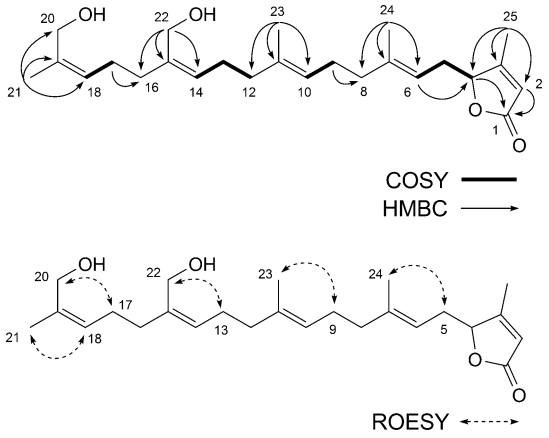
	C(12)
4	(*S*)
5	(*R*)

Coleifolide A (**1**) was obtained as colorless oil, and its molecular formula was determined as $C_{25}H_{38}O_4$ from a *pseudo*-molecular-ion peak observed at *m*/*z* 425.2667 ($[M+Na]^+$; calc. 425.2665) in the HR-ESI-MS. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed the presence of four quaternary Me groups (δ(H) 1.56 (*s*), 1.60 (*s*), 1.87 (*s*), 2.00 (*s*)), two HO–CH_2 units (δ(H) 4.46 (*s*), 4.49 (*s*)), one O-bearing CH group (δ(H) 4.95 (*t*, *J* = 5.0)), and five olefinic H-atoms (δ(H) 5.14–5.16 (*m*), 5.15–5.19 (*m*), 5.39 (*t*, *J* = 7.1), 5.44 (br. *s*), 5.91 (br. *s*)). The ^{13}C-NMR spectrum (*Table 1*) with DEPT experiment displayed 25 C-atom resonances, including seven sp^3 CH_2 C-atoms (δ(C) 26.6, 26.8, 27.1, 30.6, 36.1, 40.0, 40.8), five trisubstituted C=C bonds (δ(C) 117.4 (*d*), 169.1 (*s*), 117.2 (*d*), 139.6 (*s*), 124.6 (*d*), 135.5 (*s*), 126.9 (*d*), 140.2 (*s*), 126.9 (*d*), 136.6 (*s*)), and one carboxyl C-atom (δ(C) 173.1). The presence of a β-methyl-α,β-unsaturated-γ-lactone moiety was deduced from the HMBC data of H–C(2) (δ(H) 5.91 (br. *s*)) with C(1) (δ(C) 173.1), H–C(4) (δ(H) 4.95 (*t*, *J* = 5.0)) with C(1), and of Me(25) (δ(H) 1.87 (*s*)) with C(2) (δ(C) 117.4), C(3) (δ(C) 169.1), and C(4) (δ(C) 84.4) (*Fig. 1*). In contrast, the ^{1}H,^{1}H-COSY correlations of CH_2(5) (δ(H) 2.25–2.31 (*m*), 2.59–2.66 (*m*))/H–C(6) (δ(H) 5.14–5.16 (*m*)), CH_2(9) (δ(H) 2.07–2.10 (*m*))/H–C(10) (δ(H) 5.15–5.19 (*m*)), CH_2(12) (δ(H) 2.05–2.08 (*m*))/CH_2(13) (δ(H) 2.28–2.33 (*m*))/H–C(14) (δ(H) 5.39 (*t*, *J* = 7.1)), and of CH_2(17) (δ(H) 2.47–2.49 (*m*))/H–C(18) (δ(H) 5.44 (br. *s*)), coupled with the HMBC cross-peaks of Me(21) (δ(H) 2.00 (*s*)) with C(18) (δ(C) 126.9), C(19) (δ(C) 136.6), and C(20) (δ(C) 61.0), CH_2(22) (δ(H) 4.49 (*s*)) with C(14) (δ(C) 126.9), C(15) (δ(C) 140.2), and C(16) (δ(C) 36.1), Me(23) (δ(H) 1.56 (*s*)) with C(10) (δ(C) 124.6), C(11) (δ(C) 135.5), and C(12) (δ(C) 40.8), and of Me(24) (δ(H) 1.60 (*s*)) with C(6) (δ(C) 117.2), C(7) (δ(C) 139.6), and C(8) (δ(C) 40.0) (*Fig. 1*), revealed that the presence of 16,18-dihydroxygeranylgeranyl moiety. Furthermore, the ^{1}H,^{1}H-COSY correlations of H–C(4)/CH_2(5), as well as the HMBC cross-peak of H–C(6) with C(4) (*Fig. 1*) indicated that the dihydroxy

Table 1. *^{1}H- and ^{13}C-NMR* (400 and 125 MHz, (D_5)Pyridine) *Data of Coleifolides A and B* (**1** and **2**, resp.). δ in ppm, J in Hz. Atom numbering as indicated in the Formulae.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	173.1	–	173.1
2	5.91 (br. *s*)	117.4	5.91 (br. *s*)	117.2
3	–	169.1	–	169.2
4	4.95 (*t*, *J* = 5.0)	84.4	4.96 (*t*, *J* = 5.0)	84.3
5	2.59–2.66 (*m*), 2.25–2.31 (*m*)	30.6	2.60–2.67 (*m*), 2.25–2.34 (*m*)	30.4
6	5.14–5.16 (*m*)	117.2	5.13–5.19 (*m*)	117.0
7	–	139.6	–	139.5
8	1.98–2.02 (*m*)	40.0	1.98–2.04 (*m*)	39.8
9	2.07–2.10 (*m*)	26.6	2.07–2.14 (*m*)	26.6
10	5.15–5.19 (*m*)	124.6	5.16–5.21 (*m*)	124.3
11	–	135.5	–	135.1
12	2.05–2.08 (*m*)	40.8	2.04–2.10 (*m*)	39.7
13	2.28–2.33 (*m*)	26.8	2.16–2.25 (*m*)	26.5
14	5.39 (*t*, *J* = 7.1)	126.9	5.58–5.66 (*m*)	124.9
15	–	140.2	–	138.7
16	2.47–2.49 (*m*)	36.1	4.34 (br. *t*, *J* = 5.5)	76.9
17	2.47–2.49 (*m*)	27.1	2.68–2.75 (*m*), 2.53–2.61 (*m*)	34.6
18	5.44 (br. *s*)	126.9	5.58–5.66 (*m*)	124.2
19	–	136.6	–	137.8
20	4.46 (*s*)	61.0	4.50 (*d*, *J* = 12.1), 4.43 (*d*, *J* = 12.1)	61.0
21	2.00 (*s*)	21.8	2.01 (*s*)	22.2
22	4.49 (*s*)	59.7	1.81 (*s*)	11.9
23	1.56 (*s*)	16.1	1.59 (*s*)	16.0
24	1.60 (*s*)	16.4	1.60 (*s*)	16.3
25	1.87 (*s*)	13.6	1.88 (*s*)	13.5

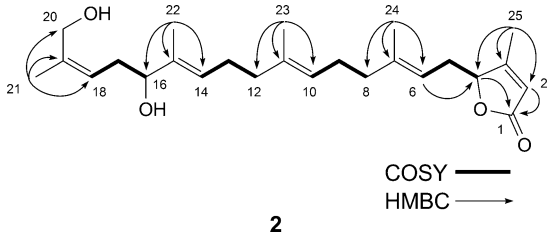
geranylgeranyl moiety was connected to C(4) of the β-methyl-α,β-unsaturated-γ-lactone moiety, and thus the planar structure of **1** was elucidated. The geometries of C=C bonds were elucidated as (6*E*), (10*E*), (14*Z*), and (18*Z*) from the ROESY correlations of CH_2(5) with Me(24), CH_2(9) with Me(23), CH_2(13) with Me(22), CH_2(17) with CH_2(20), and of CH_2(18) with Me(21) (*Fig. 1*). Since the small optical rotation value of **1** ($[\alpha]_D^{17} = +7.4$ ($c = 0.50$, $CHCl_3$)) suggested **1** to be a partial racemate, HPLC analysis of **1** using a chiral column was performed. The analysis showed two peaks corresponding to (+)-isomer (t_R: 14.2 min, OR(+)) and (−)-isomer (t_R: 17.8 min, OR(−)), and their ratio was determined to be 5 : 4 from each peak area. The absolute configuration at C(4) of the (+)-isomer was determined as (*S*) by comparison of the CD spectrum of **1** with those of manoalide derivatives structurally related to **1**. The CD spectrum displayed a positive *Cotton* effect at 209 nm (π–π^*), while manoalide derivatives with (4*R*)-configuration were reported to show negative *Cotton* effect at *ca.* 212 nm [4][5]. Thus, the structure of the major isomer of **1** was concluded as (2*Z*,4*S*,6*E*,10*E*,14*Z*,18*Z*)-20-hydroxy-15-(hydroxymethyl)-3,7,11,19-tetramethylicosa-2,6,10,14,18-pentaen-1,4-olide (=(5*S*)-5-[(2*E*,6*E*,10*Z*,14*Z*)-16-hydroxy-11-(hydroxymethyl)-3,7,15-trimethylhexadeca-2,6,10,14-tetraen-1-yl]-4-methylfuran-2(5*H*)-one).



1

Fig. 1. *Key 2D-NMR correlations of* **1**

Coleifolide B (**2**) was shown to have the same molecular formula ($C_{25}H_{38}O_4$) as compound **1** by the HR-ESI-MS experiment. The ^{1}H- and ^{13}C-NMR data (*Table 1*) were quite similar to those of **1**, except for the observation of the signals due to one O-bearing CH group (δ(H) 4.34 (br. *t*, $J=5.5$)) and one quaternary Me group (δ(H) 1.81 (*s*)) instead of one CH_2 and one HO–CH_2 group, respectively, seen in **1**, indicating **2** also to be a sesterterpenoid with a β-methyl-α,β-unsaturated-γ-lactone moiety. The ^{1}H, ^{1}H-COSY cross-peaks of H–C(16) (δ(H) 4.34 (br. *t*, $J=5.5$))/CH_2(17) (δ(H) 2.53–2.61 (*m*), 2.68–2.75 (*m*))/H–C(18) (δ(H) 5.58–5.66 (*m*)), along with the HMBC data of Me(22) (δ(H) 1.81 (*s*)) with C(14) (δ(C) 124.9), C(15) (δ(C) 138.7), and C(16) (δ(C) 76.9) indicated the positions of the O-bearing CH group and the quaternary Me group to be C(16) and C(22), respectively (*Fig. 2*). The configurations of the C=C bonds were assigned as (6*E*), (10*E*), (14*E*), (18*Z*) from the same ROESY correlations as seen in **1**. Considering the biogenetical relationship between **1** and **2**, together with its small optical rotation value ($[\alpha]_D^{17} = +14.7$ ($c=3.04$, $CHCl_3$)), **2** was also suggested to be a partial racemate. Chiral HPLC analysis of **2**, however, gave only single peak. In order to elucidate the optical nature of **2**, the 21-pivaloate derivative **2a** was treated with (2*R*)-methoxy(naphthalen-2-yl)acetic acid ((*R*)-2-NMA) [6], giving two diastereoisomeric products (major/minor 5 : 3). In contrast, the treatment of **2a** with (*S*)-2-NMA also



2

Fig. 2. *Key 2D-NMR correlations of* **2**

furnished two diastereoisomeric products, whose NMR signals arising from two isomers were identical with those seen in the (*R*)-2-NMA derivatives, although the ratio of two products was inverted. This observation indicated that **2** was also a partially racemic mixture. This was further confirmed by the fact that treatment of **2a** with (2*R*)-methoxy(phenyl)acetic acid (MPA) also afforded two diastereoisomers in the same ratio. The absolute configuration at C(16) of the major isomer was determined as (*R*) from the $\Delta\delta$ value ($\delta_R - \delta_S$) of the isolated proton signals of (*R*)- and (*S*)-2-NMA esters (*Fig. 3*). In contrast, (4*S*)-configuration of the predominant isomer was elucidated from the positive *Cotton* effect at 209 nm observed in the CD spectrum. From these observations, the structure for the major isomer was assigned as shown.

Three known compounds were identified as irroratin A (**3**) [2], teucvin (**4**) [3], and 12-epiteucvin (**5**) [3] by comparison of their spectroscopic data with those described in the literature [2][3]. These compounds were isolated from *Scutellaria* plant for the first time.

Several acyclic sesterterpenoids were known to exhibit cytotoxicity against human cancer cell lines [7][8]. Therefore, cytotoxicity against four human cancer cell lines (KB: human epidermoid carcinoma of the nasopharynx; MCF7: breast carcinoma; A549: human lung carcinoma; HeLa: human cervical carcinoma) was evaluated for coleifolides A and B. Both compounds showed moderate cytotoxicity against all the tested cancer cell lines with IC_{50} values ranging from 13.6 to 37.6 μg/ml (*Table 2*).

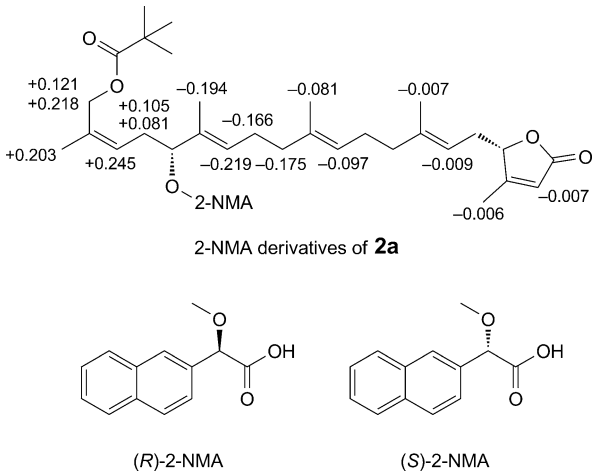


Fig. 3. $\Delta\delta$ *Values* ($\delta_R - \delta_S$) *for 2-NMA ester of* **2a**

Table 2. *Cytotoxicity Data* (IC_{50} in μg/ml) *for Compounds* **1** *and* **2** *against Human Cancer Cell Lines*

	KB	MCF 7	A549	HeLa
1	15.6 ± 0.56	17.4 ± 2.02	37.6 ± 1.75	26.0 ± 0.76
2	14.8 ± 1.01	16.1 ± 1.12	32.5 ± 0.98	13.6 ± 0.29
Daunorubicin	1.26 ± 0.06	1.62 ± 0.13	9.75 ± 0.17	1.80 ± 0.06

Conclusions. – Two new sesterterpenoids, coleifolides A and B, were isolated from the 70% aqueous acetone extract of *S. coleifolia*, along with three known diterpenoids. The structures of the new compounds were elucidated from the extensive spectroscopic analysis. The racemic nature of coleifolaides A and B was elucidated by chiral HPLC analysis or introduction of chiral reagents. In addition, the absolute configurations of the major enantiomers were assigned from the CD data and $\Delta\delta$ value of 2-NMA derivatives. Coleifolides A and B were acyclic sesterterpenoids with a β-methyl-α,β-unsaturated-γ-lactone moiety, structurally similar to acyclic manoalide derivatives, hippolides E and F, isolated from marine sponge [5]. Coleifolides A and B were obtained as partial racemates, whereas hippolides were isolated as optically pure compounds. Our study provided the first example of manoalide derivatives isolated from higher plants.

Experimental Part

General. TLC: silica gel *60 F_{254}* (SiO_2; *Merck*, Germany). Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Marine Chemical, Inc.*, P. R. China), *Sephadex LH-20* (25–100 μm; *GE Health Care*, U.K.), *MCI-gel CHP 20P* (75–150 μm; *Mitsubishi Chemical*, Japan), *Lichroprep RP-18 gel* (40–63 mm; *Merck*, Germany). Chiral anal. HPLC: *JASCO* apparatus consisting of a *PU-2089 plus* pump, *UV-2075* (at 210 nm) and *CO-2065* (at 30°) with *Ceramospher RU-2* column (150 mm × 4.6 mm i.d.; 5 mm; *Shiseido*, solvent: MeOH/H_2O 9:1). Prep. HPLC: *Agilent 1100* liquid chromatograph with *Agilent Zorbax SB-C_{18}* column (250 mm × 9.4 mm i.d.; 5 μm; *Agilent Technologies*) at flow rate 3.0 ml/min. Optical rotations: *JASCO DIP-370* digital polarimeter. CD Spectra: *JASCO CD-J600* spectropolarimeter. 1D- and 2D-NMR spectra: *Bruker AVANCE-500*, *AM-400* and *DRX-500* spectrometer; δ in ppm rel. to Me_4Si (0.03%, *v*/*v*) as internal standard, *J* in Hz. MS: *Applied Biosystems API QSTAR* time-of-flight (TOF) mass spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *S. coleifolia* were collected at Muli County, Sichuan Province, P. R. China, in August 2011. This plant was identified by Prof. *Xi-Wen Li* (Kunming Institute of Botany), and a voucher specimen (KIB 20110811) was deposited with the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The aerial parts of *S. coleifolia* (3 kg, dry weight) were extracted with 70% aq. acetone (15 l) three times at r.t. for 72 h each time. The extract was concentrated *in vacuo*. The concentrate was successively partitioned with AcOEt, BuOH, and H_2O. The AcOEt-soluble fraction (130 g) was subjected to CC (*MCI-gel CHP 20P*; MeOH/H_2O 9:1) to give three fractions (*Frs. 1–3*). *Fr. 2* (94 g) was filtrated, and then, the filtrate (90 g) was subjected to CC (SiO_2; $CHCl_3$/acetone 1:0→0:1)) to yield seven fractions (*Frs. 2.1–2.7*). *Fr. 2.2* (14.1 g) was filtrated to remove flavonoid mixture, and then the filtrate (13.5 g) was applied to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford *Frs. 2.2.1–2.2.4*. *Fr. 2.2.2* (4.5 g) was then fractionated by CC (SiO_2; petroleum ether/acetone 12:1→0:1) to give ten fractions (*Frs. 2.2.2.1–2.2.2.10*). *Fr. 2.2.2.9* (472 mg) was subjected to CC (SiO_2; $CHCl_3$/acetone 1:0→0:1), yielding *Fr. 2.2.2.9.1–2.2.2.9.6*. *Fr. 2.2.2.9.1* (237 mg) was recrystallized from MeOH to give **4** (34 mg), then the filtrate (203 mg) was purified by prep. HPLC (MeCN/H_2O 7:13) to afford **5** (5 mg). *Fr. 2.3* (12.7 g) was further filtrated, and the filtrate (7.8 g) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford *Frs. 2.3.1–2.3.3*. *Fr. 2.3.1* (4.4 g) was applied CC (SiO_2; $CHCl_3$/MeOH 1:0→50:1) to give three fractions (*Frs. 2.3.1.1–2.3.1.3*). *Fr. 2.3.1.1* (2.4 g) was separated by MPLC (*Lichroprep RP-18 gel*; MeOH/H_2O 1:4→1:0) to afford *Frs. 2.3.1.1.1–2.3.1.1.7*. *Fr. 2.3.1.1.2* (341 mg) was separated CC (SiO_2; petroleum ether/acetone 7:1→0:1) to afford six fractions (*Frs. 2.3.1.1.2.1–2.3.1.1.2.6*). *Fr. 2.3.1.1.2.2* (51 mg) was purified by prep. HPLC (MeCN/H_2O 43:57) to give **3** (2 mg). *Fr. 2.3.1.1.2.5* (140 mg) was purified by prep. HPLC (MeCN/H_2O 9:11) to yield **1** (5 mg) and **2** (30 mg).

Coleifolide A (*=(2*Z*,6*E*,10*E*,14*Z*,18*Z*)-20-Hydroxy-15-(hydroxymethyl)-3,7,11,19-tetramethylicosa-2,6,10,14,18-pentaen-1,4-olide*; *=5-[(2*E*,6*E*,10*Z*,14*Z*)-16-hydroxy-11-(hydroxymethyl)-3,7,15-trimethyl-*

*hexadeca-2,6,10,14-tetraen-1-yl]-4-methylfuran-2(5*H*)-one)*; **1**). Pale-yellow oil. $[\alpha]_D^{17} = +7.4$ ($c = 0.50$, $CHCl_3$). CD (EtOH; 1.2×10^{-4} M): 209.4 (+9.0). ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 425.2667 ($[M+Na]^+$, $C_{25}H_{38}NaO_4^+$; calc. 425.2665).

Coleifolide B (*=(2Z,6*E*,10*E*,14*E*,18Z)-16,20-Dihydroxy-3,7,11,15,19-pentamethylicosa-2,6,10,14,18-pentaen-1,4-olide*; *=5-[(2*E*,6*E*,10*E*,14Z)-12,16-Dihydroxy-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-4-methylfuran-2(5*H*)-one*; **2**). Colorless oil. $[\alpha]_D^{17} = +14.7$ ($c = 3.04$, $CHCl_3$). CD (EtOH; 1.2×10^{-4} M): 209.2 (+10.2). ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS (pos.): 425.2667 ($[M+Na]^+$, $C_{25}H_{38}NaO_4^+$; calc. 425.2657).

Preparation of **2a**: Compound **2** (6 mg, 14 μmol) was reacted with pivaloyl chloride (50 μl, 0.414 mmol) in pyridine (2 ml) under ice cooling for 10 min. After removal of solvent, the mixture was subjected to CC (SiO_2; hexane/acetone (8:1→5:1) to afford **2a** (4.8 mg, 70%). $[\alpha]_D^{19} = +11.0$ ($c = 0.12$, $CHCl_3$). ^{1}H-NMR ((D_5)pyridine): 6.18 (*d*, *J* = 2.5, HO–C(16)); 5.90 (*quint.*, *J* = 1.5, H–C(2)); 5.71 (*t*, *J* = 7.5, H–C(18)); 5.64 (*t*, *J* = 7.0, H–C(14)); 5.20 (*tq*, *J* = 1.1, 6.9, H–C(10)); 5.17 (*tq*, *J* = 1.1, 7.1, H–C(6)); 4.95 (*t*, *J* = 5.4, H–C(4)); 4.87 (*d*, *J* = 12.3, H_a–C(20)); 4.81 (*d*, *J* = 12.3, H_b–C(20)); 4.35 (*t*, *J* = 6.4, H–C(16)); 2.63–2.71 (*m*, H_a–(17)); 2.59–2.67 (*m*, H_a–C(5)); 2.57–2.64 (*m*, H_b–C(17)); 2.30 (*dt*, *J* = 7.1, 15.0, H_b–C(5)); 2.22 (br. *dd*, *J* = 7.0, 15.0, CH_2(13)); 2.08–2.13 (*m*, CH_2(9)); 2.06–2.09 (*m*, CH_2(8)); 2.00–2.05 (*m*, CH_2(12)); 1.88 (*d*, *J* = 1.5, Me(25)); 1.82 (*s*, Me(22)); 1.80 (*d*, *J* = 1.1, Me(28)); 1.61 (*s*, Me(24)); 1.61 (*s*, Me(23)); 1.21 (*s*, pivaloyl $C(Me)_3$). ^{13}C-NMR ((D_5)pyridine): 178.0 (pivaloyl–O*C*OCMe$_3$); 173.1 (C(1)); 169.1 (C(3)); 139.5 (C(7)); 138.6 (C(15)); 135.2 (C(11)); 131.6 (C(19)); 128.0 (C(18)); 125.2 (C(14)); 124.5 (C(12)); 117.3 (C(2)); 117.2 (C(6)); 84.3 (C(4)); 77.0 (C(16)); 63.5 (C(20)); 39.9 (C(8)); 39.8 (C(12)); 38.9 (pivaloyl–OCO*C*Me$_3$); 34.8 (C(17)); 30.5 (C(5)); 27.3 (3 C, pivaloyl–OCOC*Me*$_3$); 26.8 (C(9)); 26.6 (C(13)); 21.5 (C(21)); 16.4 (C(24)); 16.1 (C(23)); 13.6 (C(25)); 12.0 (C(22)). HR-ESI-MS (pos): 509.3266 ($[M+Na]^+$, $C_{30}H_{46}NaO_5^+$; calc. 509.3243).

*Preparation of (*R*)- or (*S*)-2-NMA Esters of* **2a**: A mixture of **2a** (1 mg, 2.1 μmol), DMAP (1.2 mg, 10 μmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC; 3.0 mg, 15.6 μmol), and (*R*)- or (*S*)-2-NMA (2.1 mg, 10 μmol) in CH_2Cl_2 (2 ml) was stirred at r.t. for 24 h. The mixture was directly purified by prep. TLC (hexane/AcOEt 2:1), giving (*R*)- or (*S*)-2-NMA esters of **2a** (each, 0.8 mg, 57%).

*Data of (*R*)-2-NMA Ester of* **2a**: ^{1}H-NMR ((D_5)pyridine): 5.881–5.899 (*m*, H–C(2)); 5.477 (*dd*, *J* = 6.1, 7.6, H–C(16)); 5.406 (*t*, *J* = 7.1, H–C(18)); 5.375 (*t*, *J* = 7.2, H–C(14)); 5.147–5.195 (*m*, H–C(6)); 5.048 (*t*, *J* = 6.9, H–C(10)); 4.925–4.964 (*m*, H–C(4)); 4.838 (*d*, *J* = 12.5, H_a–C(20)); 4.752 (*d*, *J* = 12.5, H_b–C(20)); 2.645–2.725 (*m*, H_a–C(17)); 2.597–2.678 (*m*, H_a–C(5)); 2.507–2.595 (*m*, H_b–C(17)); 2.260–2.333 (*m*, H_b–C(5)); 2.060–2.135 (*m*, CH_2(9)); 1.990–2.040 (*m*, CH_2(8)); 1.905–1.972 (*m*, CH_2(13)); 1.865 (*d*, *J* = 1.4, Me(25)); 1.794 (*t*, *J* = 7.7, CH_2(12)) 1.729 (*s*, Me(21)); 1.615 (*s*, Me(24)); 1.483 (*s*, Me(22)); 1.467 (*s*, Me(23)). HR-ESI-MS (pos.): 707.3959 ($[M+Na]^+$, $C_{43}H_{56}NaO_7^+$; calc. 707.3924).

*Data of (*S*)-2-NMA Ester of* **2a**: ^{1}H-NMR ((D_5)pyridine): 5.888–5.906 (*m*, H–C(2)); 5.594 (*t*, *J* = 7.0, H–C(14)); 5.450 (*dd*, *J* = 5.7, 8.0, H–C(16)); 5.155–5.205 (*m*, H–C(6)); 5.138–5.184 (*m*, H–C(18)); 5.122–5.167 (*m*, H–C(10)); 4.927–4.965 (*m*, H–C(4)); 4.716 (*d*, *J* = 12.3, H_a–C(20)); 4.534 (*d*, *J* = 12.3, H_b–C(20)); 2.599–2.674 (*m*, H_a–C(5)); 2.580 (*dt*, *J* = 8.0, 15.1, H_a–C(17)); 2.470 (*dt*, *J* = 5.7, 15.1, H_b–C(17)); 2.262–2.332 (*m*, H_b–C(5)); 2.072–2.137 (*m*, CH_2(13)); 2.075–2.138 (*m*, CH_2(9)); 2.012–2.067 (*m*, CH_2(8)); 1.969 (*t*, *J* = 7.8, CH_2(12)); 1.871 (*d*, *J* = 1.5, Me(25)); 1.677 (*s*, Me(22)); 1.622 (*s*, Me(24)); 1.548 (*s*, Me(23)); 1.526 (*s*, Me(21)). HR-ESI-MS (pos.): 707.3942 ($[M+Na]^+$, $C_{43}H_{56}NaO_7^+$; calc. 707.3924).

*Preparation of (*R*)-MPA Ester of* **2a**: A mixture of **2a** (2.1 mg, 4.3 μmol), DMAP (1.2 mg, 10 μmol), EDC (3.8 mg, 20 μmol), and (*R*)-MPA (3.3 mg, 20 μmol) in CH_2Cl_2 (2 ml) was stirred at r.t. for 21 h. The mixture was purified by prep. TLC (hexane/AcOEt 2:1) to yield (*R*)-MPA ester of **2a** (0.9 mg, 33%). HR-ESI-MS (pos.): 657.3793 ($[M+Na]^+$, $C_{39}H_{54}NaO_7^+$; calc. 657.3767).

Cell Lines and Cell Culture. KB (human epidermoid carcinoma of the nasopharynx) cells, A549 cells (human lung carcinoma) and HeLa cells (human cervical carcinoma) were cultured in *Dulbecco*'s modified *Eagle*'s medium (DMEM) with 10% fetal bovine serum (FBS). MCF7 (breast carcinoma) cells were cultured in *RPMI1640* supplemented with 10% FBS. All cells were incubated at 37° in a humidified atmosphere with 5% CO_2/95% air.

Biological Assay. Cells were seeded at each density (1×10^5 cells/well for KB, A549 and HeLa, or 5×10^4 cells/well for MCF7) in 96-well plate and pre-incubated for 24 h. Test samples were dissolved in small amount of DMSO and diluted in the appropriate culture medium (final concentration of DMSO $< 0.5\%$). After removal of pre-incubated culture medium, 100 µl of medium containing various concentration (2, 10, 50, and 100 µg/ml) of test compound were added and further incubated for 48 h. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay [9]. IC_{50} Values (concentration in µg/ml required to inhibit cell viability by 50%) were calculated using the concentration-inhibition curve. Cytotoxic activities are shown as mean ± SE from three or four experiments.

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