Lactonecembranoids from *Croton laevigatus*

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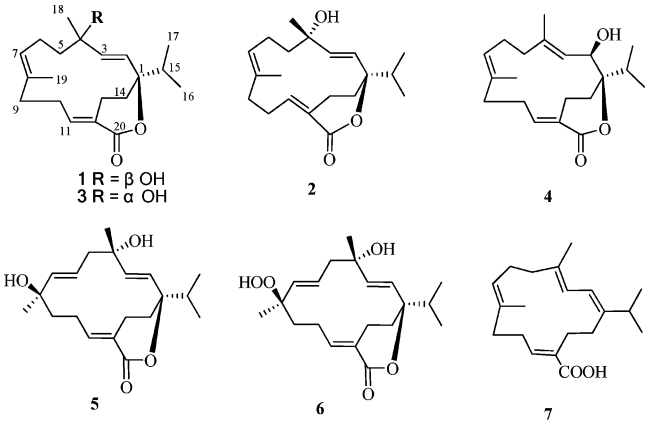
Six new cembranoids, laevigatlactones A−F (**1**−**6**), and the known compound neocrotocembraneic acid (**7**) were isolated from leaves of *Croton laevigatus*. Their structures were elucidated on the basis of spectroscopic analysis, and that of **1** was confirmed by X-ray crystallography. Compound **2** exhibited modest cytotoxicity against HeLa cells, with an IC_{50} value of 38.4 μM.

Croton laevigatus Vahl. (Euphorbiaceae) is an arbor that is found mainly in Yunnan, Guangdong, and Hainan Provinces of China. Its roots and leaves have been commonly used as a folk medicine in the Dai nationality of China for the treatment of injury from fall and fracture, malaria, and stomachache.[1] However, to the best of our knowledge, there have been no previous reports on phytochemical investigations of this species. In our search for new bioactive constituents from medicinal plants used by Dai nationalistic people in China,[2] we initiated chemical studies of the leaves of *C. laevigatus*.

The air-dried and smashed leaves of *C. laevigatus* Vahl. (20 kg) were extracted with MeOH (3 × 80 L). After removal of solvent, the aqueous residue was partitioned successively with petroleum ether, CH_2Cl_2, and *n*-BuOH. Fractionation of the petroleum ether extract led to the isolation of six new cembranoids (**1**−**6**) and the known compound neocrotocembraneic acid (**7**).[3] Details of the isolation, structure elucidation, and cytotoxicity of these compounds are reported herein.



Compound **1**, colorless prisms, had the molecular formula $C_{20}H_{30}O_3$, as determined by analysis of its HRESIMS (*m/z* 341.2078 $[M + Na]^+$), which indicated the presence of six degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 3468 (OH), 1695 (C═O), and 1636 (C═C) cm^{-1}. Analysis of the ^1H, ^{13}C, and HMQC NMR spectroscopic data of **1** (Tables 1 and 2) revealed four methyl groups, six methylene units, one methine, six olefinic carbons (four of which are protonated), two oxygenated quaternary carbons, and one carboxylic carbon. These data accounted for all of the ^1H and ^{13}C NMR resonances except for one exchangeable proton and required compound **1** to have a bicyclic structure.

Interpretation of the $^1H-^1H$ COSY NMR data led to the identification of five isolated proton spin-systems corresponding to C-2−C-3, C-5−C-7, C-9−C-11, C-13−C-14, and C-15−C-17 subunits. The remaining fragment connections were determined by HMBC data (Figure 1) and established a 14-membered-ring diterpene skeleton similar to that of crotocembraneic acid.[2] Considering the chemical shift value of C-1 (δ_C 85.8) as well as the unsaturation requirement for **1**, C-1 and C-20 could be connected to the same oxygen atom to form a lactone. The structure of **1** was confirmed by X-ray crystallography (Figure 2).

The configuration of the Δ^2 olefin was assigned as *E* on the basis of the coupling constant ($J = 16$ Hz) between H-2 and H-3, whereas that of the Δ^7 olefin was deduced to be *E* using NOESY cross-peaks of H-7 with H-9a and H-9b, which was also supported by comparison of the ^{13}C NMR chemical shifts for C-19 with that of the *trans* methyl group in the cembranoid skeleton.[3−5] The *E* configuration of the Δ^{11} olefin was established on the basis of the chemical shift of H-11 (δ_H 6.85), which was consistent with an olefinic proton *cis* to a carboxylic group in a trisubstituted olefin (calcd for δ_H, *cis* = δ 6.83),[6] together with the absence of the NOESY correlation between H-11 and H-13. In addition, the distinct cross-peaks from H-2 to H-15, CH_3-16, CH_3-17, and CH_3-18 in the NOESY spectrum suggested that H-2, the isopropyl group at C-1, and CH_3-18 were located on the same face of the cembranoid ring. This was consistent with the result of the single-crystal X-ray diffraction study of **1**. Thus, compound **1** was (1*R**,2*E*,4*R**,7*E*,1*E*)-1-isopropyl-4-hydroxy-4,8-dimethyl-21-oxa-bicyclo[10.2.2]hexadeca-2,7,11-trien-20-one, named laevigatlactone A (**1**).

Compound **2** had the molecular formula $C_{20}H_{30}O_3$ by HRESIMS, the same as **1**. The ^1H and ^{13}C NMR data of **2** revealed structural features similar to those of **1**, except that the chemical shift for H-10a was 1.24 ppm downfield (δ_H 3.53) and H-11 was 1.07 ppm upfield (δ_H 5.78). Significant differences were also observed for the chemical shifts of C-13 and C-18, which were 4.0 and 3.4 ppm downfield, respectively. These data indicated that **2** and **1** possessed opposite configurations at C-4 and the Δ^{11} olefin.

The Δ^2 and Δ^7 double bonds were both assigned an *E* configuration on the basis of the $J_{2,3}$, NOESY correlations, and chemical shift of H_3-19. The Δ^{11} olefin was determined to be *Z* by NOESY correlation of H-11 with H-13a and the chemical shift of H-11 (δ_H 5.78; calcd for $\delta_{H\ trans} = 6.19$).[6] The proton at C-2 showed NOESY correlations to H_3-16 and H_3-17, while H-3 displayed correlation to H_3-18, suggesting different orientations of the C-1 isopropyl group and the C-18 methyl group on the cembranoid ring. Thus, compound **2** was characterized as (1*R**,2*E*,4*S**,7*E*,11*Z*)-1-isopropyl-

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Table 1. ^{13}C NMR (125 MHz) Data of Laevigatlactones A−F (**1**−**6**)

position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[b]
1	85.8, qC	86.1, qC	88.9, qC	89.2, qC	87.9, qC	88.2, qC
2	127.5, CH	125.3, CH	127.7, CH	72.3, CH	126.4, CH	126.8, CH
3	138.0, CH	138.1, CH	138.9, CH	124.1, CH	140.4, CH	140.7, CH
4	73.0, qC	73.9, qC	74.5, qC	140.0, qC	74.4, qC	74.6, qC
5	42.2, CH_2	43.5, CH_2	43.5, CH_2	40.5, CH_2	47.7, CH_2	48.1, CH_2
6	23.0, CH_2	22.6, CH_2	23.2, CH_2	25.4, CH_2	125.9, CH	130.1, CH
7	126.1, CH	125.7, CH	130.4, CH	125.5, CH	138.5, CH	135.0, CH
8	132.5, qC	133.3, qC	133.4, qC	132.3, qC	73.2, qC	85.3, qC
9	36.4, CH_2	37.2, CH_2	38.3, CH_2	38.2, CH_2	42.7, CH_2	38.2, CH_2
10	25.8, CH_2	26.6, CH_2	27.5, CH_2	25.3, CH_2	26.1, CH_2	25.8, CH_2
11	145.9, CH	148.5, CH	147.9, CH	143.5, CH	148.7, CH	148.7, CH
12	125.7, qC	124.6, qC	128.1, qC	126.9, qC	125.7, qC	126.2, qC
13	20.6, CH_2	24.6, CH_2	22.0, CH_2	21.4, CH_2	22.2, CH_2	22.4, CH_2
14	27.3, CH_2	28.7, CH_2	30.0, CH_2	22.7, CH_2	29.1, CH_2	29.4, CH_2
15	37.5, CH	37.9, CH	39.2, CH	33.2, CH	38.3, CH	38.6, CH
16	17.3, CH_3	17.5, CH_3	18.2, CH_3	17.0, CH_3	17.9, CH_3	18.2, CH_3
17	16.7, CH_3	16.6, CH_3	17.4, CH_3	17.0, CH_3	17.0, CH_3	17.3, CH_3
18	26.9, CH_3	30.3, CH_3	32.6, CH_3	16.1, CH_3	31.1, CH_3	31.4, CH_3
19	17.1, CH_3	17.2, CH_3	17.4, CH_3	15.7, CH_3	24.8, CH_3	20.4, CH_3
20	167.8, qC	166.8, qC	170.5, qC	168.6, qC	170.9, qC	171.1, qC

[a] ^{13}C NMR data recorded in $CDCl_3$. [b] Recorded in CD_3OD.

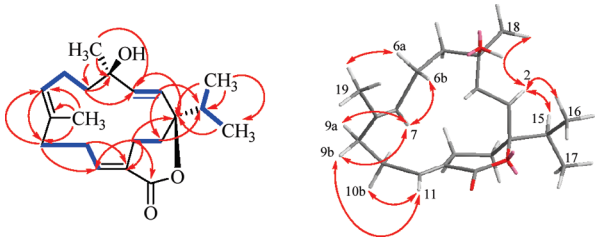

Figure 1. Key H−H COSY (−), HMBC (↷), and NOESY correlations (↔) for laevigatlactone A (**1**).

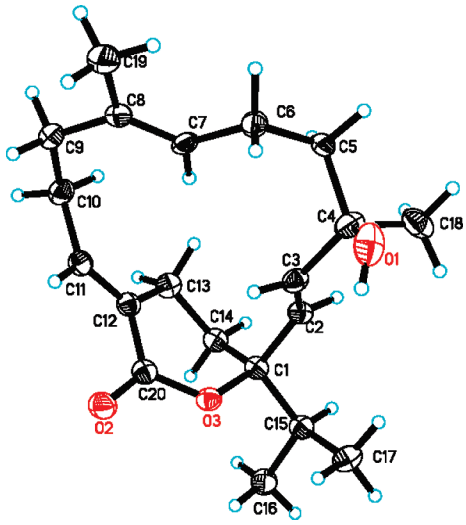

Figure 2. Thermal ellipsoid representation of laevigatlactone A (**1**).

4-hydroxy-4,8-dimethyl-21-oxa-bicyclo[10.2.2]hexadeca-2,7,11-trien-20-one, named laevigatlactone B (**2**).

Compound **3** was obtained as a yellow oil. The HRESIMS of **3** gave a pseudomolecular ion $[M + Na]^+$ peak at *m/z* 341.2070, indicating the same molecular formula as **1** and **2**. Analysis of the ^1H and ^{13}C NMR data revealed that **3** could be a stereoisomer of **1**, which was confirmed by $^1H-^1H$ COSY and HMBC data. NOESY data were used to establish the relative configuration of **3**. Most cross-peaks observed in its NOESY spectrum were the same as those of **1**, except for the correlation of H-3 with H_3-18 observed in **3** instead of H-2 with H_3-18 in **1**, indicating that **3** had a different configuration at C-4. Thus, compound **3** was characterized as (1*R**,2*E*,4S*,7*E*,11*E*)-1-isopropyl-4-hydroxy-4,8-dimethyl-21-oxabicyclo[10.2.2]hexadeca-2,7,11-trien-20-one, named laevigatlactone C (**3**).

Compound **4** had the same molecular formula ($C_{20}H_{30}O_3$) as **1**−**3** by HRESIMS. The ^1H and ^{13}C NMR data of **4** indicated a structure similar to that of **1**−**3**. However, a significant difference was observed for the ^{13}C NMR chemical shift of the C-18 methyl group in **4**, which was 10.8 ppm upfield compared to that in **1**, implying connection to a double bond. In addition, one oxymethine proton was present (δ_H 4.20), whereas the *trans* olefinic protons of Δ^2 in **1** were absent in the ^1H NMR spectrum of **4**. These observations suggested that the double bond was at Δ^3 in **4** and that the OH group was attached to C-2. Analysis of the $^1H-^1H$ COSY and HMBC data established the planar structure of **4**, and the relative configuration was determined by analysis of the NMR chemical shifts and NOESY data. The ^{13}C NMR chemical shifts of the C-18 and C-19 methyl groups (δ_C 16.1 and 15.7, respectively) assigned the *E* configuration for Δ^3 and Δ^7.[4,5] NOESY cross-peaks of H-2 with H_3-16, H_3-17, and H_3-18 indicated that these protons were on the same face of the cembranoid ring. The configuration of the Δ^{11} olefin was assigned as *E* on the basis of the chemical shift of H-11 (δ_H 6.65), the same as that of **1**.[6] Thus, **4** was established as (1*R**,2*R**,3*E*,7*E*,11*E*)-1-isopropyl-2-hydroxy-4,8-dimethyl-21-oxabicyclo[10.2.2]hexadeca-3,7,11-trien-20-one, named laevigatlactone D (**4**).

The modified Mosher method is often used to determine the absolute configuration of secondary alcohols in natural products, but our experimental results of the *R*/*S*-MTPA products of **4** were not consistent with the rule. In addition, results from CD analysis of the in situ formed complex with $[Rh_2(OCOCF_3)_4]$[7] were not correlated to the stereochemistry of the C-2 secondary alcohol by applying the bulkiness rule (Supporting Information). Therefore, the absolute configuration of **4** was not determined.

Compound **5** was assigned the molecular formula $C_{20}H_{30}O_4$, one more oxygen atom than that of **3**. The ^1H NMR and ^{13}C NMR data of **5** revealed the same skeleton as **3**, except that the Δ^7 double bond was changed to Δ^6 in **5** and another OH group was connected at C-8, which was supported by $^1H-^1H$ COSY and HMBC correlations.

The configurations of Δ^2, Δ^6, and Δ^{11} were all assigned as *E* by analysis of the coupling constants of $J_{2,3}$ and $J_{6,7}$ (both 15.6 Hz) and the chemical shift of H-11 (δ_H 6.82). NOESY correlations of H-2 with CH_3-16 and CH_3-17 and those of H-3 with CH_3-18 indicated the same relative configurations of C-1 and C-4 as those found in **3**. The correlations of H-7 with H-3 and H-5b, and H-6

Table 2. ^{1}H NMR (500 MHz, *J* in Hz) Data of Laevigatlactones A−F (**1**−**6**)

no.	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[b]
2	5.40 (1H, d, 16.0)	5.62 (1H, d, 15.5)	5.58 (1H, d, 15.5)	4.20 (1H, d, 9.5)	5.53 (1H, d, 15.6)	5.54 (1H, d, 15.5)
3	5.50 (1H, d, 16.0)	5.68 (1H, d, 15.5)	5.29 (1H, d, 15.5)	4.99 (1H, d, 9.5)	5.22 (1H, d, 15.6)	5.26 (1H, d, 15.5)
5a	1.83 (1H, br.d, 14.5)	1.84 (1H, dd, 13.5, 1.0)	1.88 (1H, dd, 14.0, 1.0)	2.23 (1H, m)	2.31 (1H, dt, 15.0, 3.0)	2.34 (1H, dt, 15.0, 3.0)
5b	1.68 (1H, ddd, 14.5, 9.5, 2.5)	1.51 (1H, ddd, 13.5, 9.0, 1.0)	1.53 (1H, ddd, 14.0, 9.0, 1.0)	1.97 (1H, m)	2.18 (1H, m)	2.18 (1H, dt, 15.0, 4.0)
6a	2.20 (1H, m,)	2.18 (1H, m)	2.11 (1H, m)	2.21 (1H, m)	5.64 (1H, ddd, 15.6, 10.8, 3.0)	5.73 (1H, ddd, 15.5, 11.0, 3.0)
6b	1.78 (1H, m)	2.03 (1H, dd, 17.0, 9.0)	1.99 (1H, m)	2.08 (1H, m)		
7	4.73 (1H, t, 5.0)	4.82 (1H, td, 5.5, 1.5)	4.72 (1H, t, 5.0)	4.84 (1H, t, 7.5)	5.33 (1H, dd, 15.6, 1.8)	5.29 (1H, dd, 15.5, 2.0)
9a	2.19 (1H, brd, 13.0)	2.25 (1H, brd, 13.5)	2.20 (1H, dd, 13.0, 6.5)	2.28 (1H, dd, 12.0, 3.0)	1.90−1.86 (2H, m)	1.88 (1H, dd, 13.5, 2.5)
9b	2.02 (1H, brt, 13.0)	1.95 (1H, brt, 13.5)	1.98 (1H, brt, 13.0)	2.16 (1H, dd, 12.0, 3.0)		1.87 (1H, dd, 13.5, 2.5)
10a	2.29 (1H, td, 13.0, 11.0)	3.53 (1H, m)	2.35 (1H, td, 13.0, 11.5)	2.42 (1H, td, 12.0, 3.0)	2.21 (1H, td, 10.2, 3.6)	2.25−2.3 (1H, m)
10b	2.14 (1H, m)	2.21 (1H, m)	2.18 (1H, d, 13.5)	2.16 (1H, qd, 12.0, 3.0)	2.15 (1H, m)	2.23−2.21 (1H, m)
11	6.85 (1H, ddt, 11.0, 4.0, 2.5)	5.78 (1H, ddt, 11.5, 3.5, 2.0)	6.88 (1H, ddt, 11.5, 5.0, 2.5)	6.65 (1H, brd, 11.0)	6.82 (1H, dt, 11.0, 2.0)	6.83 (1H, dt, 11.0, 2.0)
13a	2.40 (1H, dddd, 17.0, 6.5, 4.0, 2.5)	2.56−2.45 (2H, m)	2.44 (1H, dddd, 17.0 5.5, 3.5, 1.5)	2.57 (1H, m)	2.43 (1H, dddd, 16.8, 6.6, 4.2, 1.8)	2.45 (1H, dd, 17.0, 4.5)
13b	2.24 (1H, m)		2.14 (1H, m)	2.13 (1H, m)	2.25 (1H, m, ddd, 16.8, 6.6, 3.0)	2.28 (1H, ddd, 17.0, 11.5, 2.5)
14a	1.94 (1H, ddd, 13.5, 6.5, 2.5)	1.89 (1H, ddd,13.0, 5.5, 2.0)	1.96 (1H, ddd,14.0, 5.5, 1.5)	1.94 (1H, dt, 12.0, 6.0)	2.01 (1H, dd, 13.8, 6.0)	2.01 (1H, dd, 14.0, 6.0)
14b	1.81 (1H, td, 13.5, 7.5)	1.78 (1H, td, 13.0, 7.5)	1.76 (1H, td, 14.0, 5.5)	1.38 (1H, td, 12.0, 6.0)	1.75 (1H, td, 13.8, 6.0)	1.75 (1H, td, 14.0, 6.0)
15	1.87 (1H, sept, 7.0)	1.86 (1H, sept, 6.5)	1.82 (1H, sept, 7.0)	2.57 (1H, sept, 7.0)	1.84 (1H, sept, 7.0)	1.85 (1H, sept, 7.0)
16	0.96 (3H, d, 7.0)	0.95 (3H, d, 6.5)	0.98 (3H, d, 7.0)	1.00 (3H, d, 7.0)	0.96 (3H, d, 7.0)	0.96 (3H, d, 7.0)
17	0.97 (3H, d, 7.0)	0.96 (3H, d, 6.5)	0.98 (3H, d, 7.0)	1.01 (3H, d, 7.0)	0.96 (3H, d, 7.0)	0.97 (3H, d, 7.0)
18	1.32 (3H, s)	1.29 (3H, s)	1.22 (3H, s)	1.68 (3H, s)	1.24 (3H, s)	1.25 (3H, s)
19	1.55 (3H, s)	1.57 (3H, s)	1.57 (3H, d, 1.0)	1.52 (3H, s)	1.31 (3H, s)	1.33 (3H, s)

[a] ^{1}H NMR data recorded in $CDCl_3$. [b] Recorded in CD_3OD.

with CH_3-19, revealed that the C-8 hydroxy group should be β-oriented. Thus, compound **5** was established as (1*R**,2*E*,4*S**,6*E*,8*R**,11*E*)-1-isopropyl-4,8-dihydroxy-4,8-dimethyl-21-oxabicyclo[10.2.2]hexadeca-2,6,11-trien-20-one, named laevigatlactone E (**5**).

Compound **6** had the molecular formula $C_{20}H_{30}O_5$ on the basis of its HRESIMS, one more oxygen atom than that of **5**. The ^{1}H NMR and ^{13}C NMR data of compound **6** were nearly the same as those of **5** except that the chemical shift value of C-8 was 12.1 ppm downfield in the ^{13}C NMR spectrum of **6**, which suggested that a hydroperoxyl group could be attached at C-8 in **6** instead of the hydroxyl group in **5**. This conclusion was further confirmed by the molecular formula and the fragment ions at *m*/*z* 333 and 301 in the EIMS. The relative configuration of **6** was the same as that of **5** on the basis of NOESY experimental data. Therefore, compound **6** was identified as (1*R**,2*E*,4*S**,6*E*,8*R**,11*E*)-1-isopropyl-4-hydroxy-8-hydroperoxyl-4,8-dimethyl-21-oxabicyclo[10.2.2]hexadeca-2,6,11-trien-20-one, named laevigatlactone F (**6**).

Compounds **1**−**6**, which we named laevigatlactones A−F, respectively, are unique cembranoids possessing a six-membered lactone moiety attached to C-1 and C-20, which has not been encountered previously in natural products. Most natural cembranoids have been isolated from lower marine creatures,[8,9] although there are a few reports from terrestrial plants.[10−12] Compound **7** was also isolated in this study, and its high concentration (about 0.7%) suggests that it could be the biosynthetic precursor of compounds **1**−**6** by oxidation of C-1 and then esterification of the OH at C-1 with the C-20 carboxy group.

The cembranoids previously reported always showed cytotoxicity.[4,5,8] Thus, the cytotoxicities of compounds **1**−**7** were evaluated against HeLa cells using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay.[13] However, only compound **2** showed a modest antiproliferative effect against HeLa cells (IC_{50} = 38.4 μM). The remaining compounds were all inactive.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. X-ray data were acquired with a Saturn724 single-crystal X-ray diffractometer. UV absorptions were carried out on a Shimadzu UV-2550 spectrophotometer. IR spectra were obtained using a Shimadzu FTIR-8400S spectrophotometer. NMR spectra were recorded on a Bruker AM 500 spectrometer using the residual $CDCl_3$ (δ_H 7.26/δ_C 77.2) and CD_3OD (δ_H 3.30/δ_C 49.5) signals as references. The 2D NMR experiments (^{1}H−^{1}H COSY, HSQC, HMBC, NOESY) were performed using standard Bruker microprograms. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. EIMS data were obtained on a GCMS-QP 2010 Shimadzu spectrometer, and HRESIMS data were acquired using a LTQ Orbitrap XL mass spectrometer.

Plant Material. Leaves of *C. laevigatus* were collected from Xishuangbanna County, Yunnan Province, in July 2007. The sample was identified by one of the authors (C.Z.P.), and a voucher specimen (No. 20070716) has been deposited in the herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. The air-dried and crushed leaves of *C. laevigatus* (20 kg) were extracted with MeOH (3 × 80 L) to afford a crude extract (2120 g) after evaporation under vacuum. The extract was suspended in H_2O (8.0 L) and then partitioned successively with petroleum ether, CH_2Cl_2, and EtOAc (3 × 8.0 L). The petroleum ether extract (673 g) was subjected to silica gel column chromatography (CC) (3.0 kg, 100−200 mesh), eluted with petroleum ether−EtOAc (1:0, 9:1, 4:1, 3:2, 0:1), to afford five corresponding fractions (1−5). Fraction 2 (293 g) was further chromatographed over silica gel (3.0 kg, 100−200 mesh), eluted with petroleum ether−EtOAc (100:1, 50:1, 20:1, 9:1), to obtain compound **7** (130 g). Fraction 3 (152 g) was fractionated by silica gel CC (1.5 kg, 100−200 mesh), using petroleum ether−EtOAc (9:1, 3:2) as eluent, to give six subfractions (3a−3f). Fraction 3e (50 g) was further purified over silica gel (1.5 kg, 100−200 mesh), eluted with petroleum ether−Me_2CO (9:1 to 3:2), to yield compounds **2** (50

mg), **3** (100 mg), **5** (30 mg), and **6** (20 mg). Repeated CC of fraction 3f (17 g) over silica gel, using CH_2Cl_2−Me_2CO (100:1 to 4:1) and petroleum ether−Me_2CO (4:0 to 3:2) elution, led to the isolation of compounds **4** (50 mg) and **1** (100 mg).

Laevigatlactone A (1): colorless prisms (petroleum ether−EtOAc); mp 134−136 °C; $[\alpha]^{25}_D$ −4.80 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.7); IR (KBr) ν_{max} 3468, 1695, 1636, 1387, 1433, 1319, 1271, 1124, 978 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 300 (1), 285 (1), 272 (2), 255 (5), 241 (2), 229 (3), 215 (3), 213 (5), 201 (3), 185 (4), 171 (5), 166 (7), 151 (7), 135 (6), 133 (9), 121 (11), 107 (16), 95 (22), 81 (29), 67 (23), 55 (26), 43 (100); HRESIMS *m/z* 341.2078 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2088).

Laevigatlactone B (2): colorless prisms (petroleum ether−Me_2CO); mp 100−102 °C; $[\alpha]^{25}_D$ −1.63 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.8); IR (KBr) ν_{max} 3495, 2964, 2930, 2889, 1690, 1630, 1383, 1470, 1319, 1242, 1109, 970 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 300 (5), 285 (2), 272 (1), 257 (10), 239 (2), 229 (4), 215 (4), 199 (5), 187 (7), 171 (6), 159 (8), 147 (8), 133 (10), 121 (15), 107 (20), 95 (30), 81 (29), 67 (21), 55 (27), 43 (100); HRESIMS *m/z* 341.2079 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2088).

Laevigatlactone C (3): yellow oil; $[\alpha]^{25}_D$ −1.99 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.6); IR (KBr) ν_{max} 3460, 2964, 2922, 1693, 1634, 1269, 1119, 978 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 300 (5), 285 (3), 272 (4), 255 (29), 239 (4), 229 (6), 215 (7), 213 (7), 201 (8), 187 (7), 171 (7), 166 (8), 151 (9), 134 (16), 133 (12), 121 (15), 107 (21), 95 (26), 81 (32), 67 (24), 55 (29), 43 (100); HRESIMS *m/z* 341.2070 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2088).

Laevigatlactone D (4): colorless needles ($CHCl_3$−Me_2CO); mp 179−180 °C; $[\alpha]^{25}_D$ +30.9 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.8); IR (KBr) ν_{max} 3472, 2960, 2943, 2926, 2910, 1688, 1655, 1385, 1472, 1312, 1275, 1132, 960 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 318 $[M]^+$ (3), 300 (2), 285 (1), 257 (4), 242 (3), 235 (26), 217 (5), 208 (12), 189 (6), 165 (2), 149 (8), 121 (20), 105 (15), 95 (35), 83 (39), 81 (30), 71 (68), 67 (29), 55 (38), 43 (100); HRESIMS *m/z* 341.2074 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2088).

Laevigatlactone E (5): colorless powders (petroleum ether−Me_2CO); mp 162−164 °C; $[\alpha]^{25}_D$ +33.1 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 282 (3.7), 239 (4.1), 221 (4.2); IR (KBr) ν_{max} 3431, 2968, 2928, 2910, 1688, 1628, 1380, 1450, 1321, 1269, 1117, 977 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 301 (1), 270 (2), 255 (2), 227 (2), 213 (2), 192 (6), 177 (3), 163 (3), 137 (7), 123 (6), 107 (13), 95 (15), 81 (46), 55 (20), 43 (100); HRESIMS *m/z* 357.2017 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2036).

Laevigatlactone F (6): colorless powders (petroleum ether−Me_2CO); mp 86−88 °C; $[\alpha]^{25}_D$ +1.50 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 233 (3.8); IR (KBr) ν_{max} 3444, 2970, 2928, 2910, 1688, 1630, 1389, 1447, 1290, 1271, 1119, 980 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 333 (1), 317 (5), 301 (5), 255 (6), 227 (8), 213 (8), 192 (16), 177 (13), 161 (16), 137 (38), 123 (28), 107 (55), 95 (75), 81 (100), 67 (45), 55 (71); HRESIMS *m/z* 373.1968 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1985).

X-ray Crystallographic Analysis of Laevigatlactone A (1). [14] Crystallization from petroleum ether−EtOAc (4:1) yielded colorless prisms of **1**. A crystal (0.32 × 0.26 × 0.22 mm) was separated from the sample and mounted on a glass fiber, and data were acquired with a Saturn724 single-crystal X-ray diffractometer with Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator. Structure analysis was made using the SHELXL97 program. Crystal data: $C_{20}H_{30}O_3$ (318 g/mol), space group *C*2/*c*; unit cell dimensions a = 23.345(5) Å, b = 10.440(2) Å, c = 17.519(4) Å, monoclinic, V = 3572.9(15) $Å^3$, D_c = 1.184 mg/m^3, Z = 8. A total of 30 maps and 3144 independent reflections were collected in the range of $0° < \theta < 180°$, of which 2928 were observable reflections [$|F|^2 > 2\sigma|F|^2$)]; completeness to θ_{max} was 99.7%, and non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in Fourier difference maps and refined with idealized geometries and riding constraints. The final indices were R_1 = 0.0603, wR_2 = 0.1425, S = 1.100 [$I > 2\sigma(I)$].

Cytotoxic Assay. The cytotoxic activities of the isolated compounds were evaluated against the HeLa cell line by the MTT colorimetric method with VP-16 and D-24851 as positive controls.[13]

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Supporting Information Available: ^1H and ^{13}C NMR spectra of laevigatlactones A−F (**1**−**6**) and CD spectrum of complex of **4** with $[Rh_2(OCOCF_3)_4]$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 761665). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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