Antitumor Agents. 169.[1] *Dysoxylum cumingianum.* V.[1,2] Cumingianosides P and Q, New Cytotoxic Triterpene Glucosides with an Apotirucallane-Type Skeleton from *Dysoxylum cumingianum*

Toshihiro FUJIOKA,[a] Akio SAKURAI,[a] Kunihide MIHASHI,*[,a] Yoshiki KASHIWADA,[b] Ih-Sheng CHEN,[c] and Kuo-Hsiung LEE*[,d]

Faculty of Pharmaceutical Sciences, Fukuoka University,[a] Nanakuma 8–19–1, Jonan-ku, Fukuoka 814–80, Japan, Niigata College of Pharmacy,[b] Kamishin'ei-cho 5–13–2, Niigata 950–21, Japan, Natural Products Research Center, Kaohsiung Medical College,[c] Kaoshiung, Taiwan, and Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina,[d] Chapel Hill, North Carolina 27599, U.S.A. Received August 9, 1996; accepted September 26, 1996

Detailed chemical studies on the cytotoxic fraction from the leaves of *Dysoxylum cumingianum* have resulted in the isolation of two new triterpene glucosides, cumingianosides P (18) and Q (19), with an apotirucallane-type skeleton. The structures of 18 and 19 were determined by spectral examinations, and by conversion of cumingianosides C (3) and A (1) into 18 and 19, respectively. The cytotoxicities of cumingianosides P and Q against over 50 human cancer cell lines were evaluated. Cumingianoside P exhibited significant (EC_{50} <4 μM) cytotoxicity against 37 human cancer cell lines. Among them, the UO-31 (renal cancer) cell line was the most sensitive to this compound (EC_{50} 0.267 μM). In contrast, cumingianoside Q showed selective cytotoxicity against NCI-H522 (non-small cell lung cancer) cells with an EC_{50} value of 1.67 μM, and exhibited no cytotoxicity (EC_{50} >10 μM) against most of the remaining cancer cell lines.

Key words *Dysoxylum cumingianum*; apotirucallane glucoside; cytotoxicity; Meliaceae; triterpene; triterpene glucoside

In the preceding paper, we reported the isolation and characterization of cumingianosides G—O, triterpene glucosides with a 14,18-cycloapotirucallane-type skeleton from the cytotoxic fraction of the leaves of *Dysoxylum cumingianum* (Meliaceae), as well as the evaluation of the cytotoxicities of these cumingianosides.[1] As a continuation of that work, the present paper deals with the structure elucidation of two additional triterpene glucosides, named cumingianosides P and Q, with an apotirucallane-type skeleton and the evaluation of their cytotoxicity against human cancer cell lines. These new compounds also were isolated from the cytotoxic fraction of *D. cumingianum.*

Cumingianoside P (**18**) was obtained as a white amorphous powder and gave the $(M-H)^-$ ion peak at *m/z* 751 in its negative FAB-MS. The molecular formula ($C_{41}H_{68}O_{12}$) was confirmed by high-resolution (HR) FAB-MS. The existence of a sugar moiety was indicated by an anomeric proton resonance [δ 4.73 (1H, d, J=8 Hz)]

Table 1. ^{1}H-NMR Data (δ, J in Hz) for Compounds **18**, **3**, **19**, and **1b** in Pyridine-d_5+D_2O (400 MHz)

	18	3	19	1b
H-3	4.93 (br s)	4.92 (br s)	4.93 (br s)	4.08 (br s)
H-5	2.45 (br d, 13)	2.38 (br d, 12)	2.45 (br d, 13)	2.63 (dd, 2.5, 12)
H-7	4.45 (br s)	4.01 (br s)	4.21 (br s)	3.62 (br s)
H-15	5.60 (br d, 2)		5.60 (br d, 2)	5.43 (br d, 2.5)
H-18	0.98 (s)	0.50 (d, 6)	0.99 (s)	0.96 (s)
		0.62 (d, 6)		
H-19	0.93 (s)	0.90 (s)	0.93 (s)	0.93 (s)
H-21	1.14 (d, 6.5)	1.11 (d, 6)	1.13 (d, 6.5)	1.14 (d, 6.5)
H-23	4.37 (br t, 7)	4.34 (br t, 7)	4.52 (br t, 7)	4.54 (br t, 8)
H-24	3.56 (br s)	3.55 (br s)	3.57 (br s)	3.63 (br s)
H-26	1.38 (s)	1.40 (s)	1.59 (s)	1.60 (s)
H-27	1.40 (s)	1.42 (s)	1.62 (s)	1.63 (s)
H-28	1.11 (s)	1.11 (s)	1.11 (s)	1.13 (s)
H-29	0.91 (s)	0.88 (s)	0.91 (s)	1.05 (s)
H-30	1.18 (s)	1.08 (s)	1.18 (s)	1.23 (s)
Glc-1	4.73 (d, 8)	4.72 (d, 7.5)	4.73 (d, 7.5)	
2	3.77 (dd, 8, 9)	3.87 (dd, 7.5, 9)	3.78 (dd, 7.5, 9)	
3	4.13 (t, 9)	4.16 (t, 9)	4.13 (t, 9)	
4	3.93 (t, 9)	4.00 (t, 9)	3.94 (t, 9)	
5	3.88 (ddd, 2, 5, 9)	3.94 (ddd, 2, 5.5, 9)	3.88 (ddd, 2, 5, 9)	
6	4.62 (dd, 5, 11.5)	4.68 (dd, 5.5, 12)	4.62 (dd, 5, 11.5)	
	4.92 (dd, 2, 11.5)	4.90 (dd, 2, 12)	4.92 (dd, 2, 11.5)	
$-COCH_3$	1.85 (s)	1.94 (s)	1.85 (s)	
	2.07 (s)	2.04 (s)	2.07 (s)	
$-OCH_3$	3.22 (s)	3.24 (s)		

* To whom correspondence should be addressed.

Table 2. ^{13}C-NMR Data (δ) for Compounds **18**, **3**, **19**, and **1b** in Pyridine-d_5 + D_2O (100 MHz)

	18	**3**	**19**	**1b**
1	34.1	34.5	34.2	33.6
2	23.4	23.4	23.4	26.4
3	78.2	78.1	78.2	75.5
4	36.9	37.0	37.0	37.7
5	42.0	41.4	42.1	40.9
6	21.1	20.7	21.1	25.2
7	77.6	78.1	77.6	73.0
8	43.3	35.4	43.3	44.6
9	43.5	45.3	43.5	42.5
10	37.8	37.7	37.8	38.1
11	17.5	17.4	17.5	17.1
12	36.3	28.1	36.4	35.2
13	46.9	27.1	46.9	47.3
14	158.7	39.4	158.7	162.4
15	120.4	25.5	120.4	119.5
16	36.1	26.1	36.1	35.7
17	62.1	53.3	62.1	61.9
18	19.4	17.3	19.4	18.9
19	16.1	16.3	16.1	15.8
20	32.3	33.1	32.2	32.1
21	20.2	19.8	20.1	20.1
22	42.6	40.2	42.0	41.9
23	68.3	68.4	69.4	69.4
24	76.8	76.9	76.9	77.1
25	78.7	78.7	73.8	73.8
26	22.6	22.6	27.7	27.7
27	20.9	21.1	27.1	27.1
28	27.7	27.7	27.7	28.2
29	22.2	22.2	22.3	22.7
30	28.5	20.3	28.5	29.2
Glc-1	100.3	100.2	100.3	
2	74.7	74.9	74.7	
3	78.4	78.2	78.5	
4	71.3	71.6	71.3	
5	74.6	74.6	74.6	
6	64.5	64.7	64.5	
$-COCH_3$	20.9	20.9	21.0	
	21.1	20.9	21.1	
$-COCH_3$	171.0	170.9	171.1	
	170.8	170.8	170.9	
$-OCH_3$	49.3	49.3		

and by six aliphatic carbon resonances (Table 2), and was confirmed by acid hydrolysis to yield D-glucose. The ^{1}H-NMR spectrum of cumingianoside P (**18**) (Table 1) revealed the presence of seven tertiary methyl groups (δ 0.91, 0.93, 0.98, 1.11, 1.18, 1.38, 1.40) and an olefinic group [δ 5.60 (1H, br d, J=2 Hz)], along with a secondary methyl group [δ 1.14 (d, J=6.5 Hz)]; in contrast, 14,18-cycloapotirucallane-type triterpenes generally contain only six tertiary methyl groups and no double bonds. The ^{1}H-NMR spectrum also showed the absence of cyclopropyl methylene signals, which are characteristic of 14,18-cycloapotirucallane-type triterpenes. These spectral features were similar to those found in the hydrolysate (**1a**)[2c] of cumingianoside A (**1**), previously obtained by the treatment of **1** with *p*-toluenesulfonic acid in dry acetone at reflux. This hydrolysate contained an apotirucallane-type skeleton. The ^{1}H-NMR spectrum of **18** showed, together with two acetoxy (δ 1.85, 2.07), a methoxy (δ 3.22), and the sugar signals, signals due to four oxygen-bearing methine groups [δ 3.56 (br s), 4.37 (br t, J=7 Hz), 4.45 (br s), 4.93 (br s)], whose chemical

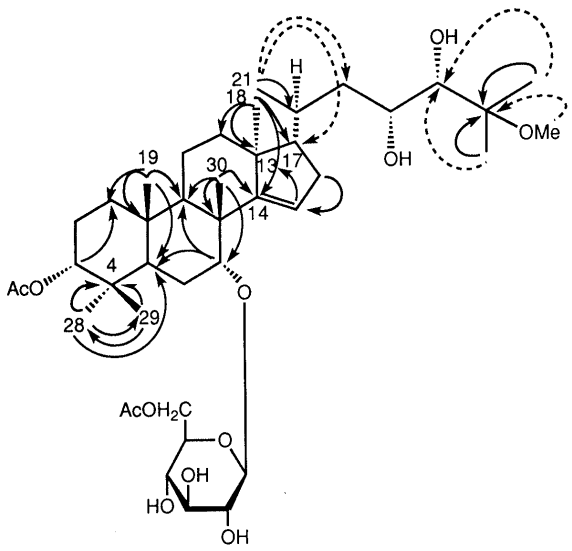


Fig. 1. ^{1}H–^{13}C Long-Range Correlations in **18**
(H⟶C; J_{C-H}=10 Hz; H---→C: J_{C-H}=5 Hz).

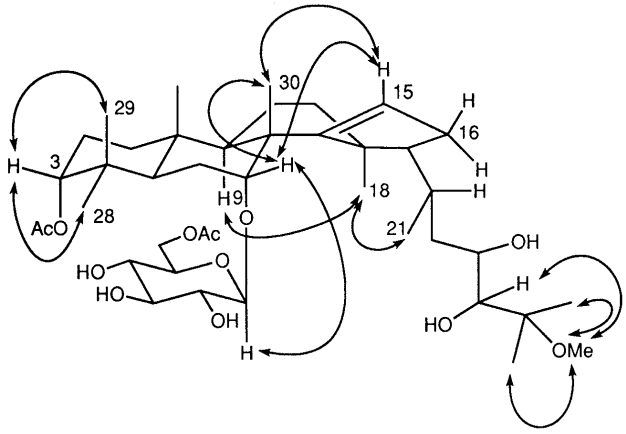


Fig. 2. NOE Correlations in **18**

shifts and coupling patterns correlated closely with those for H-24, H-23, H-7, and H-3, respectively, in cumingianoside C (**3**). The presence of the apotirucallane-type skeleton as well as the locations of the hydroxy groups at C-3, 7, 23, 24, and 25 in **18** were confirmed by ^{1}H–^{13}C long-range correlation spectroscopy (COSY) (Fig. 1) and nuclear Overhauser effect (NOE) spectroscopy (NOESY) (Fig. 2) examinations.

The α configurations of the C-3 and C-7 hydroxy groups were also indicated from the NOE examination. The *threo* relationship between C-23 and C-24 in **18** was deduced from the proton coupling patterns of H-24, which had a small coupling constant in **18** (J=0 Hz) and in its heptaacetate (**18a**) (J=2 Hz), but a large coupling constant in its acetonide (**18b**) (J=8 Hz); these coupling constants were in good accord with those found in the corresponding derivatives of cumingianoside G (**9**) (J=0, 1.5, 6.5 Hz, respectively). The observation of NOE correlations between the methoxy signal and H-24 and 25-$(CH_3)_2$ as well as the ^{1}H–^{13}C long-range correlation of the methoxy signal and C-25 carbon resonance indicated that the methoxy group was located at C-25.

The position of the glucosyl moiety was determined to be at the C-7 hydroxy group, based upon observation of

NOE between the H-7 and anomeric proton signals. The glucosyl linkage was determined to be β from the coupling constant value (d, $J=8$ Hz) of the anomeric proton signal.

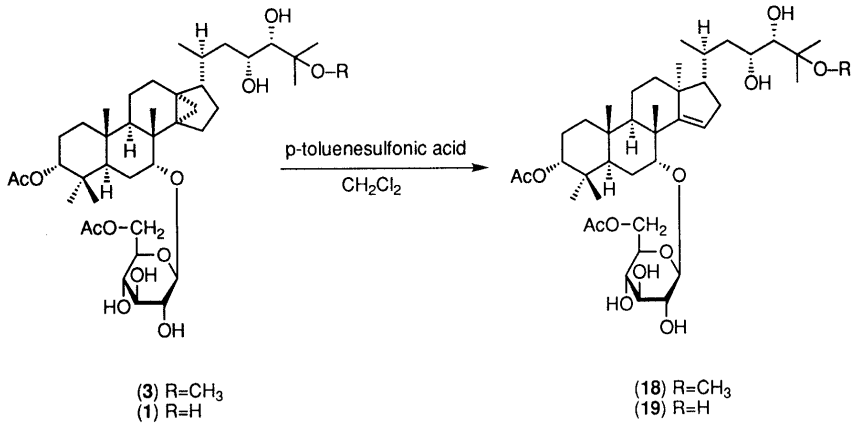
The locations of the acetyl groups were assigned at C-3 and glucosyl C-6 hydroxy groups, since in the ^{13}C-NMR spectrum of **18**, the C-3 and glucosyl C-6 carbon resonances were in good accord with those found in **3**. Consequently, cumingianoside P was concluded to be an apotirucallane-type triterpene glucoside as shown by formula **18**.

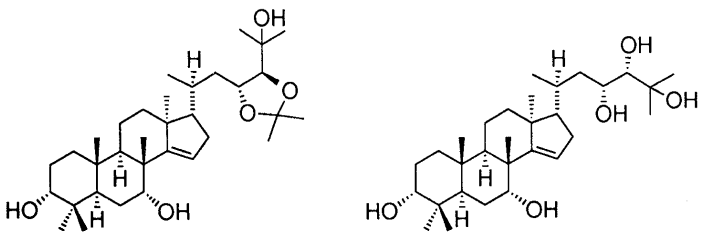
The negative FAB-MS of cumingianoside Q (**19**) gave the $(M-H)^-$ ion peak at m/z 737, which was 14 mass units less than that of **18**. The ^{1}H-NMR spectrum of **19** correlated closely with that of **18** and also showed the absence of a cyclopropyl methylene group and the presence of seven tertiary methyl groups and the olefinic group (see Table 1). These observations, combined with the absence of methoxy groups in **19**, suggested that cumingianoside Q was the apotirucallane-type glucoside represented by formula **19**. Comparison of the ^{13}C-NMR resonances of **19** with those of **18** and those of **1b**, which was prepared from **1a** by treatment with 50% acetic acid, also supported these conclusions.

Since **1a**, which contains the same apotirucallane-type skeleton seen in **18** and **19**, was obtained by the treatment of cumingianoside A with *p*-toluenesulfonic acid in dry acetone at reflux, an attempt was made to prepare **18** and **19** by acid treatment of cumingianosides C (**3**) and A (**1**), respectively. Among the various conditions examined, treating **1** and **3** with *p*-toluenesulfonic acid in CH_2Cl_2 at room temperature overnight did afford a small amount of **19** and **18**, respectively, although unknown compounds were the major products. These conversions provide final structure confirmation of cumingianosides P (**18**) and Q (**19**); thus, on the basis of the chemical and spectral evidence described above, the structures of cumingianosides P and Q were determined to be 3-*O*-acetyl-25-*O*-methyl-3α,7α,23(*R*),24(*S*),25-pentahydroxy-apotirucallanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside and 3-*O*-acetyl-3α,7α,23(*R*),24(*S*),25-pentahydroxy-apotirucallanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside, respectively.

Co-occurrence of 14,18-cycloapotirucallanes and apotirucallanes in the same plant indicates that the 14,18-cycloapotirucallanes are intermediates in the transformation of the tirucallane into the apotirucallane system, as has previously been suggested.

The cytotoxic activities of cumingianosides P (**18**) and Q (**19**) against over 50 human cancer cell lines *in vitro* are summarized in Table 3. Cumingianoside P (**18**) exhibited significant (EC_{50} <4 μM) cytotoxicity against 37 human cancer cell lines, especially against UO-31 (renal cancer) cells (EC_{50} 0.267 μM). In contrast, cumingianoside Q (**19**) showed significant cytotoxicity only against NCI-H522 (non-small cell lung cancer) cells with an EC_{50} value of 1.67 μM, and exhibited no cytotoxicity (EC_{50} >10 μM) against the remaining cancer cell lines.





1a 1b

Chart 1

Table 3. Cytotoxicity (EC_{50} in μM) of Compounds **18** and **19** against Human Cancer Cell Lines *in Vitro*

	18	**19**
Panel/cell line		
Leukemia		
CCRF-CEM	3.00	>10
HL-60 (TB)	2.59	>10
K-562	3.34	>10
MOLT-4	1.93	>10
RPMI-8226	2.06	>10
SR	NT	>10
Non-small cell lung cancer		
A549/ATCC	4.13	NT
EKVX	4.79	>10
HOP-62	3.98	>10
HOP-92	3.59	>10
NCI-H226	5.21	NT
NCI-H23	4.85	>10
NCI-H322M	3.97	NT
NCI-H460	3.24	>10
NCI-H522	2.76	1.67
Colon cancer		
COLO 205	1.99	>10
HCC-2998	2.14	>10
HCT-116	3.00	>10
HCT-15	3.85	>10
HT29	3.41	>10
KM12	2.93	NT
SW-620	5.39	>10
CNS cancer		
SF-268	6.06	>10
SF-295	3.03	>10
SF-539	2.83	>10
SNB-19	5.01	>10
SNB-75	3.70	>10
U251	4.37	>10
Melanoma		
LOX IMVI	2.32	>10
MALME-3M	5.54	>10
M14	3.16	>10
SK-MEL-2	NT	>10
SK-MEL-28	4.57	>10
SK-MEL-5	2.21	NT
UACC-257	3.02	>10
UACC-62	2.76	>10
Ovarian cancer		
IGROV1	2.78	>10
OVCAR-3	2.94	>10
OVCAR-4	5.86	>10
OVCAR-5	2.88	>10
OVCAR-8	3.73	>10
SK-OV-3	>10	>10
Renal cancer		
786-0	4.80	>10
A498	3.31	>10
ACHN	3.87	>10
CAKI-1	>10	>10
RXF-393	3.86	NT
SN12C	4.20	>10
TK-10	4.52	>10
UO-31	0.287	>10
Prostate cancer		
PC-3	3.48	>10
DU-145	7.10	>10
Breast cancer		
MCF7	3.33	>10
MCF7/ADR-RES	>10	>10
MDA-MB-231/ATCC	2.50	>10
HS 578T	4.36	NT
MDA-MB-435	3.24	>10
MDA-N	3.01	>10
BT-549	>10	NT
T-47D	5.12	>10

NT: not tested.

Experimental[3,4]

Cumingianoside P (18) A white amorphous powder, $[\alpha]_D^{25}$ −82.8° (c=0.50, $CHCl_3$); Positive FAB-MS m/z: 775 $[M+Na]^+$. Negative FAB-MS m/z: 751 $[M-H]^-$; High-resolution FAB-MS Calcd for $C_{41}H_{68}NaO_{12}$ 775.4609. Found m/z: 775.4607; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Acid Hydrolysis of 18 A solution of **18** (50 mg) in 5% H_2SO_4–50% EtOH (2 ml) was refluxed for 20 h. The reaction mixture was neutralized with IR-410 resin, then concentrated, and the residue was chromatographed over MCI gel. Elution with H_2O furnished D-glucose (15 mg): $[\alpha]_D^{25}$ +33.3° (c=0.75, H_2O).

Cumingianoside P Acetate (18a) Compound **18** (10 mg) was treated with acetic anhydride (Ac_2O) (0.5 ml) and pyridine (C_5H_5N) (0.5 ml) at room temperature overnight. The reaction mixture was worked up as usual, and the product was purified by HPLC on a reversed-phase column [YMC-Pack ODS-AM (5 μm, 20 mm i.d. × 250 mm, YMC Co., Ltd.); 100% CH_3CN] to give **18a** (4 mg).

18a: White amorphous powder. Positive FAB-MS m/z 985 $(M+Na)^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.86, 0.89, 0.91, 0.95, 1.07, 1.17, 1.19 (each 3H, s, t-CH_3 × 7), 0.98 (3H, d, J=6.5 Hz, 20-CH_3), 2.00, 2.02, 2.04, 2.05, 2.07, 2.10, 2.16 (each 3H, s, OAc × 7), 3.21 (3H, s, OMe), 3.63 (1H, m, glucosyl H-5), 4.02 (1H, br s, H-7), 4.19 (2H, m, glucosyl H-6), 4.61 (1H, d, J=8 Hz, glucosyl H-1), 4.68 (1H, br s, H-3), 4.92 (1H, dd, J=8, 9 Hz, glucosyl H-2), 4.97 (1H, d, J=2 Hz, H-24), 5.10 (1H, t, J=9 Hz, glucosyl H-4), 5.18 (1H, t, J=9 Hz, glucosyl H-3), 5.22 (1H, br d, J=2 Hz, H-15), 5.41 (1H, ddd, J=2, 8, 10 Hz, H-23).

Acetonide Formation of 18 Followed by Acetylation (18b) A mixture of **18** (20 mg) and $CuSO_4$ (50 mg) in dry Me_2CO (5 ml) was stirred at room temperature for 1 d. The reaction mixture was filtered, then concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel [$CHCl_3$–MeOH (1:0→10:1)] to give an acetonide. The acetonide was subsequently treated with Ac_2O (1 ml) and C_5H_5N (1 ml) at room temperature overnight. After a usual work-up, the mixture was purified by HPLC on a reversed-phase column [YMC-Pack ODS-AM (5 μm, 20 mm i.d. × 250 mm, YMC Co., Ltd.); 100% CH_3CN] to give **18b** (5 mg).

18b: White amorphous powder. Positive FAB-MS m/z 941 $(M+Na)^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87, 0.89, 0.91, 0.95, 1.08, 1.17, 1.22, 1.38, 1.39 (each 3H, s, t-CH_3 × 9), 1.03 (3H, d, J=6.5 Hz, 20-CH_3), 1.99, 2.02, 2.03, 2.08, 2.08 (each 3H, s, OAc × 5), 3.26 (3H, s, OMe), 3.60 (1H, d, J=8 Hz, H-24), 3.62 (1H, m, glucosyl H-5), 3.92 (ddd, J=3, 8, 8 Hz, H-23), 4.06 (1H, br s, H-7), 4.19 (2H, m, glucosyl H-6), 4.61 (1H, d, J=8 Hz, glucosyl H-1), 4.69 (1H, br s, H-3), 4.91 (1H, dd, J=8, 9 Hz, glucosyl H-2), 5.10 (1H, t, J=9 Hz, glucosyl H-4), 5.18 (1H, t, J=9 Hz, glucosyl H-3), 5.22 (1H, br d, J=2 Hz, H-15).

Cumingianoside Q (19) White amorphous powder, $[\alpha]_D^{25}$ −77.5° (c=0.52, $CHCl_3$). Positive FAB-MS m/z: 761 ($[M+Na]^+$). Negative FAB-MS m/z: 737 ($[M-H]^-$). High-resolution FAB-MS Calcd for $C_{40}H_{66}NaO_{12}$: m/z 761.4452. Found m/z: 761.4446. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Preparation of the Derivative (1b) A mixture of **1a** (10 mg) and 50% acetic acid was refluxed for 2 h. The reaction mixture then was concentrated under reduced pressure, and the residue was subjected to silica gel chromatography using $CHCl_3$–MeOH (1:0→10:1) to give **1b** (5 mg).

1b: White amorphous powder. ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Treatment of 1 and 3 with *p*-Toluenesulfonic Acid in CH_2Cl_2 Compounds **1** and **3** (10 mg each) were treated separately with *p*-toluenesulfonic acid (2 mg) in CH_2Cl_2 (2 ml) at room temperature overnight. The reaction mixture was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The products were examined by HPLC and TLC to detect cumingianoside Q (**19**) [HPLC: t_R 6.081 min (Cosmosil C-18AR, 5 μm, 4.6 mm i.d. × 250 mm, Nacarai Tesque Co., Ltd.; 70% CH_3CN, 1 ml/min); and TLC: Rf 0.38 (Silica gel 60F_{254}, Merck; $CHCl_3$:MeOH=10:1)] and cumingianoside P (**18**) [HPLC: t_R 9.783 min (Cosmosil C-18AR, 5 μm, 4.6 mm i.d. × 250 mm, Nacarai Tesque Co., Ltd.; 70% CH_3CN, 1 ml/min); and TLC: Rf 0.20 (Silica gel 60F_{254}, Merck; $CHCl_3$:MeOH=10:1)], respectively. Subsequent large-scale reaction of compound **1** and **3** (100 mg each) with *p*-toluenesulfonic acid (5 mg) in CH_2Cl_2 (10 ml) was carried out separately at room temperature overnight. After work-up as before, each reaction mixture was separated by semi-preparative scale HPLC [column: YMC-Pack ODS-AM (5 μm, 20 mm i.d. × 250 mm) (YMC Co., Ltd.); solvent: 60% CH_3CN; flow rate: 8 ml/min] to yield products (2.8 mg and 4.9 mg, respectively), which were

shown to be identical with cumingianosides Q (**19**) and P (**18**), respectively, by spectral comparisons.

Biological Assay The *in vitro* cytotoxicity assay was carried out using the National Cancer Institute protocol. Details of the assay procedures have been reported.[5)]

Acknowledgment The authors wish to thank Dr. A. Mauger, Drug Synthesis and Chemistry Branch, National Cancer Institute and Dr. B. L. Roberson, Starks C. P., for *in vitro* human tumor cell line assays, and Mr. H. Hanazono for measurement of mass spectra. This work was supported by Grant CA-17625 from the National Cancer Institute awarded to K. H. Lee.

References and Notes

1) For Antitumor Agents part 168 and *Dysoxylum cumingianum*. IV see: Fujioka T., Sakurai A., Mihashi K., Kashiwada Y., Chen I.-S., Lee K.-H., *Chem. Pharm. Bull.*, **45**, 68—74 (1997).
2) *a*) Kashiwada Y., Fujioka T., Chang J. J., Chen I. S., Mihashi K., Lee K.-H., *Bioorganic & Medicinal Chemistry Letters*, **2**, 395—398 (1992); *b*) *Idem*, *J. Org. Chem.*, **57**, 6948—6953 (1992); *c*) Kashiwada Y., Fujioka T., Mihashi K., Marubayashi N., Mizuki K., Chen I.-S., Lee K.-H., *J. Nat. Prod.*, **58**, 495—503 (1995).
3) The isolation of cumingianosides P (**18**) and Q (**19**) was described in the experimental section of the preceding paper.[1)]
4) The instruments and materials used in this work were the same as described in the preceding paper.[1)]
5) Boyd M. B., Paul K. D., *Drug. Development Res.*, **34**, 91—109 (1995).