Anti-Tumor Agents. 136.[1] Cumingianosides A–F, Potent Antileukemic New Triterpene Glucosides, and Cumindysosides A and B, Trisnor- and Tetranortriterpene Glucosides with a 14,18-Cycloapoeuphane-Type Skeleton from *Dysoxylum cumingianum*

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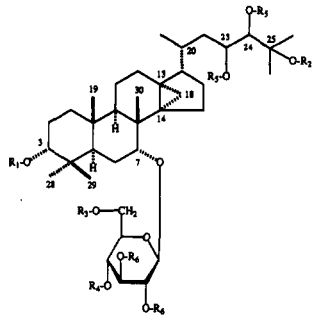
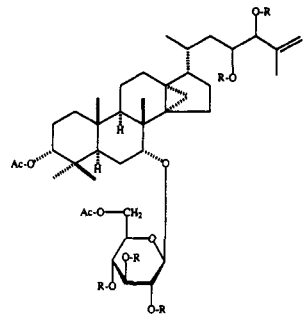
Six new triterpene glucosides, cumingianosides A–F (1–6) as well as a trisnor- and a tetranortriterpene glucoside, cumindysosides A (7) and B (8), respectively, with a 14,18-cycloapoeuphane-type skeleton have been isolated from *Dysoxylum cumingianum* as antileukemic principles. The structures were established on the basis of chemical and spectroscopic evidence. Among them, compounds 1 and 3 exhibited potent selective cytotoxicity against MOLT-4 human leukemia cell with ED_{50} of <0.006 25 and <0.0045 μg/mL, respectively.

As a result of our continuing search for novel plant antitumor agents active against human tumor cell lines, the MeOH extract of the leaves of *Dysoxylum cumingianum* (Meliaceae) were found to show significant ($ED_{50} \leq$ 20 μg/mL) cytotoxicity in RPMI-7951 and TE-671 tissue culture tumor cells. Subsequent bioassay-guided fractionation with these tumor cell lines in vitro has resulted in the isolation of new triterpene glucosides, cumingianosides A–F (1–6), and a trisnor- and a tetranortriterpene glucoside, cumindysosides A (7) and B (8), respectively, as cytotoxic principles. We report herein on the isolation and characterization of these compounds and their cytotoxic activity.



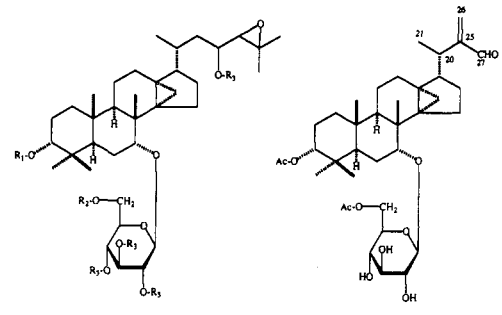
	R_1	R_2	R_3	R_4	R_5	R_6
1	Ac	H	Ac	H	H	H
1a	Ac	H	Ac	Ac	Ac	Ac
1b	H	H	H	H	H	H
1c	H	H	$C(CH_3)_2$		$C(CH_3)_2$	H
2	Ac	H	H	H	H	H
3	Ac	CH_3	Ac	H	H	H
3a	Ac	CH_3	Ac	Ac	Ac	Ac
3b	H	CH_3	H	H	H	H



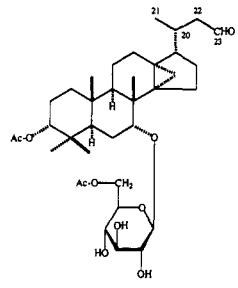
	R
4	H
4a	Ac

The MeOH extract of the leaves of *D. cumingianum* was fractionated by solvent partition to give four fractions: fractions A–D (see Experimental Section). Among these, the fraction B was found to show the most potent cytotoxicity. Repeated chromatography of this fraction on silica gel, Sephadex LH-20, MCI-gel CHP 20P, prep-PAK 500/C_{18}, and Fuji-gel ODSQ3 prepacked columns, afforded 1–8.

Cumingianoside A (1), one of the major constituents of the fraction B, was obtained as a white amorphous powder



	R_1	R_2	R_3
5	Ac	Ac	H
5a	Ac	Ac	Ac
5d	H	H	H
6	H	Ac	H



and was positive to a Liebermann–Burchard reaction, giving a purple color. The HRFABMS established the molecular formula $C_{40}H_{66}O_{12}$. The glycosidic nature of 1 was indicated by anomeric resonances [δ 4.76 (1 H, d, J = 7.5 Hz); δ 100.1] and was confirmed by acid hydrolysis to liberate D-glucose. The ^1H NMR spectrum revealed the presence of a cyclopropyl methylene group [δ 0.50 and 0.63 (each 1 H, d, J = 5.5 Hz)], six tertiary methyl groups (δ 0.83, 0.90, 1.08, 1.19, 1.60, and 1.65), a secondary methyl group [δ 1.11 (d, J = 6.5 Hz)], and two acetoxyl groups (δ 1.93 and 2.05). It also showed signals due to four oxygen-bearing methine groups [δ 3.58 (s), 4.00 (br s), 4.53 (br t, J = 6.5 Hz), and 4.94 (br s)], together with signals arising from the glucosyl moiety. The ^{13}C NMR spectrum of 1 exhibited the presence of 40 carbon atoms with no double bonds except for the ester carbonyl carbons of the acetyl

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(1) For part 135 see: Shi, Q.; Chen, K.; Fujioka, T.; Kashiwada, Y.; Chang, J. J.; Kozuka, M.; Estes, J. R.; McPhail, A. T.; McPhail, D. R.; Lee, K. H. *J. Nat. Prod.* **1992**, *55*, 1488.

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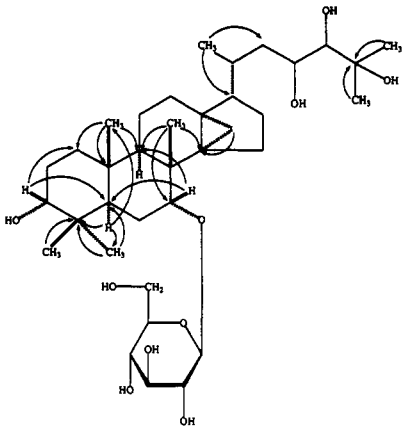


Figure 1. ^1H–^{13}C long-range correlations in **1b**.

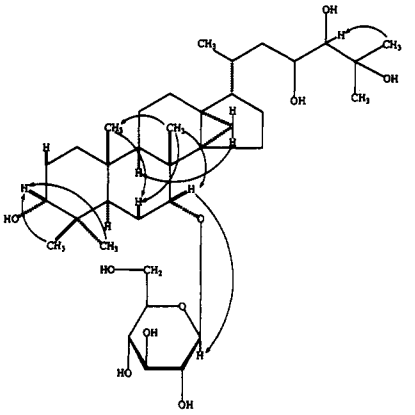
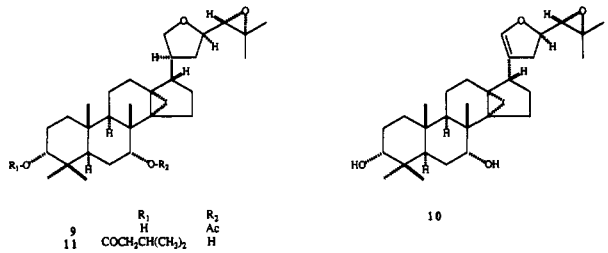


Figure 2. NOE correlations in **1b**.

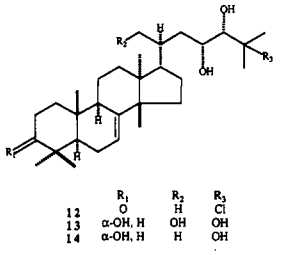
groups. Taking the molecular formula into account, **1** was considered to be a pentacyclic triterpene glucoside. In order to obtain an aglycon of **1**, various conditions of acid hydrolysis were attempted, yielding complicated products, but the genuine aglycon could not be acquired.

Acetylation of **1** with pyridine and acetic anhydride gave a heptaacetate (**1a**) [negative FABMS m/z: 947 $(M-H)^-$], while on treatment with 2% NaOMe–MeOH compound **1** yielded a hydrolysate (**1b**). The characteristic signals for the cyclopropyl methylene group in the ^1H NMR spectrum of **1b** suggested the possibility that **1b** could be a 9,19-cyloartane-type triterpene as well as a 14,18-cycloapoeuphane-type triterpene, both of which have been isolated from the plants of the members of Meliaceae.[2-4] The ^1H–^1H COSY spectral examination of **1b** established the proton connections of the following four segments: C-1–C-3, C-5–C-7, C-15–C-17, C-20–C-24. The assignment of the carbon resonances were achieved by the ^1H–^{13}C COSY spectroscopy of **1b**, except for the quaternary carbons at δ 27.2, 35.5, 37.7, 37.9, 39.4, and 73.7. Furthermore, the ^1H–^{13}C long-range COSY spectrum clearly indicated the correlation of these quaternary carbons with the tertiary methyl, methylene, and/or methine carbons through a three- or a two-bond coupling (Figure 1). This spectroscopic evidence, coupled with the NOE examinations (Figure 2), indicated the occurrence of the 14,18-cycloapoeuphane-type skeleton in **1b**. The comparison of the carbon resonances for C-1–C-19, C-28–C-30 with those of the compounds with the 14,18-cycloapoeuphane-type skeleton described in the literature[6] also supported the presence of similar ring systems in **1b**, such as glabretal (**9**),[3,4] ailanthol (**10**),[5] and shimmiarepin A (**11**).[6]



	R_1	R_2
9	H	Ac
11	$COCH_2CH(CH_3)_2$	H

10



	R_1	R_2	R_3
12	O	H	Cl
13	α-OH, H	OH	OH
14	α-OH, H	H	OH

The locations of the hydroxyl groups were concluded to be at C-3, -7, -23, -24, and -25 on the basis of the spectroscopic examinations. The small coupling constant of H-3 [δ 3.64 (br s)] and H-7 [δ 4.15 (br s)] in the ^1H NMR spectrum indicated that both hydroxyl groups possess α-configurations, which were supported by the observation of the NOE between H-3 and 4-$(CH_3)_2$ and H-7 and 8-CH_3, respectively. The coupling constant of H-24 in **1b**, heptaacetate **1a**, and the diacetonide **1c**, derived from **1b** (J = 0, 1.5, and 7.5 Hz, respectively), were similar to those observed in the corresponding derivative of bourjotinolone C (**12**)[7] (J = 0, small, 7 Hz, respectively), sapelin F (**13**)[8,9] (J = 0, 1.6, 8 Hz, respectively), and hispidol A (**14**)[10] (J = 0, 1.6 Hz, respectively), which possess a similar side-chain group in each molecule. The proton as well as carbon resonances for the side-chain group at C-17 were consistent with those found in hispidol A (**14**),[10] which possesses the same side-chain group at C-17. From these spectral observations, the stereochemistry of C-20, -23, and -24 in **1b** was presumed to be similar to those found in **12**–**14**. The X-ray crystallographic analysis is in progress in order to determine the absolute stereostructure of **1**.

The glucosyl moiety was determined to be attached at C-7 hydroxyl group based upon the observation of the NOE between H-7 and anomeric proton signals. The β-linkage of the glucosyl moiety was confirmed by the coupling constant (d, J = 7.5 Hz) of the anomeric proton signal.

The locations of the acetoxyl groups were concluded to be at C-3 and glucosyl C-6 positions judging the downfield shifts of each signal in ^1H [H-3: δ 4.94 (1 H, br s); glucosyl H-6: δ 4.72 (1 H, dd, J = 5.5, 11.5 Hz) and 4.94 (1 H, dd, J = 2, 11.5 Hz)] and ^{13}C [C-3: δ 77.9; glucosyl C-6: δ 64.6]

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Table I. ^{1}H NMR Data (δ, J in Hz) for Compounds 1, 1b, 2–8 in Pyridine-d_5 (300 MHz)

	1	1b	2	3	4
H-3	4.94 (br s)	3.64 (br s)	4.94 (br s)	4.94 (br s)	4.94 (br s)
H-5	2.41 (d, 12 Hz)	2.56 (d, 12.5 Hz)	2.39 (d, 12 Hz)	2.39 (d, 12 Hz)	2.42 (d, 12 Hz)
H-7	4.00 (br s)	4.15 (br s)	4.13 (br s)	4.13 (br s)	4.02 (br s)
H-18	0.50, 0.63 (d, 5.5 Hz)	0.58, 0.82 (d, 5.5 Hz)	0.47, 0.62 (d, 5.5 Hz)	0.47, 0.62 (d, 5.5 Hz)	0.54, 0.69 (d, 5.5 Hz)
H-23	4.53 (br t, 6.5 Hz)	4.54 (m)	4.52 (br t, 6.5 Hz)	4.37 (br t, 6.5 Hz)	4.11 (m)
H-24	3.58 (s)	3.61 (s)	3.60 (s)	3.57 (s)	4.26 (d, 5.5 Hz)
4α-CH_3	1.19	1.33	1.11	1.13	1.13
4β-CH_3	0.83	0.91	0.89	0.88	0.89
8-CH_3	1.08	1.06	1.03	1.08	1.10
10-CH_3	0.90	0.94	0.89	0.90	0.91
20-CH_3	1.11 (d, 6.5 Hz)	1.09 (d, 6 Hz)	1.08 (d, 6 Hz)	1.11 (d, 7.5 Hz)	1.19 (d, 6 Hz)
25-CH_3 or H-26 and 27	1.60, 1.65	1.61, 1.66	1.61, 1.64	1.42, 1.44	5.00, 5.26 (br s), 1.90 (s)
glucosyl					
H-1	4.76 (d, 7.5 Hz)	4.87 (d, 7.5 Hz)	4.82 (d, 7.5 Hz)	4.75 (d, 7.5 Hz)	4.76 (d, 7.5 Hz)
H-6	4.72 (dd, 5.5, 11.5 Hz)	4.33 (dd, 5.5, 11 Hz)	4.32 (dd, 5, 11.5 Hz)	4.72 (dd, 5.5, 11.5 Hz)	4.72 (dd, 5, 11.5 Hz)
	4.94 (dd, 2, 11.5 Hz)	4.57 (dd, 2, 11 Hz)	4.57 (d, 11.5 Hz)	4.94 (dd, 2, 11.5 Hz)	4.93 (d, 11.5 Hz)
Ac	1.93, 2.05		2.03	1.94, 2.05	1.96, 2.06
Me				3.25	

	5	6	7	8
H-3	4.96 (br s)	3.65 (br s)	4.94 (br s)	4.93 (br s)
H-5	2.44 (d, 12 Hz)	2.59 (d, 12 Hz)	2.40 (d, 12 Hz)	2.41 (d, 12 Hz)
H-7	4.01 (br s)	4.00 (br s)	3.99 (br s)	4.03 (br s)
H-18	0.55, 0.64 (d, 5.5 Hz)	0.66, 0.84 (d, 5.5 Hz)	0.27, 0.52 (d, 5.5 Hz)	0.38, 0.55 (d, 6 Hz)
H-23	3.90 (m)	3.94 (m)		9.79 (br s)
H-24	2.99 (d, 8 Hz)	2.99 (d, 8 Hz)		
4α-CH_3	1.14	1.35	1.13	1.13
4β-CH_3	0.89	0.93	0.88	0.87
8-CH_3	1.07	1.15	1.08	1.08
10-CH_3	0.92	0.97	0.90	0.90
20-CH_3	1.13 (d, 6 Hz)	1.12 (d, 6.5 Hz)	1.07 (d, 7 Hz)	0.93 (d, 7 Hz)
25-CH_3 or H-26 and 27	1.34, 1.36	1.34, 1.38	5.90, 6.08 (br s), 9.56 (s)	
glucosyl				
H-1	4.78 (d, 7.5 Hz)	4.78 (d, 7.5 Hz)	4.71 (d, 7.5 Hz)	4.76 (d, 7.5 Hz)
H-6	4.73 (dd, 5.5, 11.5 Hz)	4.72 (dd, 5.5, 11.5 Hz)	4.69 (dd, 5, 11.5 Hz)	4.75 (dd, 5, 11 Hz)
	4.95 (dd, 2, 11.5 Hz)	4.94 (d, 11.5 Hz)	4.89 (d, 11.5 Hz)	4.90 (d, 11 Hz)
Ac	1.91, 2.05		1.96, 2.06	1.93, 2.05

NMR spectra of 1 as compared to those of 1b.

On the basis of the spectral and chemical evidence described above, the structure of cumingianoside A is suggested as 3-*O*-acetyl-3α,7α,23,24,25-pentahydroxy-14,18-cycloapoeuphanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (1).

The ^{1}H NMR spectrum of cumingianoside B (2) was similar to that of 1, exhibiting the occurrence of a cyclopropyl methylene group, six tertiary methyl groups, a secondary methyl group, and four oxygen-bearing methine groups, together with sugar signals. However, the absence of one of the acetoxyl signals was observed in the ^{1}H NMR spectrum of 2. The $(M - H)^-$ ion peak (m/z 695) of 2 in the negative FABMS was 42 mass units less than that (m/z 737) of 1, which was consistent with this finding.

The partial hydrolysis of 1 with 2% NaOMe–MeOH yielded a product, which was found to be identical to 2 by the physical and spectral comparisons. In the ^{1}H NMR spectrum of 2, the glucosyl C-6 methylene proton signals were shifted further upfield [δ 4.32 (1 H, dd, J = 5, 11.5 Hz) and 4.57 (1 H, d, J = 11.5 Hz)] as compared with those [δ 4.72 (1 H, dd, J = 5.5, 11.5 Hz) and 4.94 (1 H, dd, J = 2, 11.5 Hz)] of 1, while the signal due to H-3 was observed at δ 4.94 (1 H, br s), whose chemical shift was identical to that of 1. The ^{13}C NMR spectrum also showed an upfield shift (−1.1 ppm) of the glucosyl C-6 signal as well as a downfield shift (+3.6 ppm) of the glucosyl C-5 signal as compared to those of 1. From these spectral observations, the acetoxyl group at glucosyl C-6 was concluded to be absent in 2, thus confirming the structure of 2 to be 3-*O*-acetyl-3α,7α,23,24,25-pentahydroxy-14,18-cycloapoeuphanyl 7-*O*-β-D-glucopyranoside (2).

Cumingianoside C (3), contained in relatively large amounts in the fraction B, showed a $(M - H)^-$ ion peak at m/z 751 in the negative FABMS. The ^{1}H NMR spectrum of 3 was analogous to that of 1 except for the appearance of a methoxyl signal (δ 3.25) and an upfield shift of two tertiary methyl groups assignable to 25-$(CH_3)_2$. The ^{13}C NMR spectrum of 3 also resembled that of 1. The six carbon resonances in the region from δ 64.8 to 100.3 indicated the occurrence of a 6-acetylglucoside moiety. The other carbon resonances were also consistent with those found in 1. However, a downfield shift of the hydroxy-bearing quaternary carbon (+4.6 ppm), assignable to the C-25, as well as an upfield shift of two methyl groups (−5.0 and −5.9 ppm), suggested that the methyl group was located at C-25 hydroxy group. In addition, acetylation of 3 with acetic anhydride and pyridine to give heptaacetate (3a) [negative FABMS m/z: 961 $(M - H)^-$] indicated that the methoxy group was attached to C-25 hydroxyl group. From the findings described above, the structure of cumingianoside C can be represented by formula 3.

Cumingianoside D (4) exhibited, in the negative FABMS, the $(M - H)^-$ ion peak at m/z 719 that is 18 mass units less than that of 1. On acetylation with acetic anhydride and pyridine, 4 gave a heptaacetate (4a) [negative FABMS m/z: 929 $(M - H)^-$]. A comparison of the ^{1}H NMR spectrum of 4 with that of 1 showed the absence of one of the C-25 methyl signals as well as a downfield shift of the remaining C-25 methyl signal. It also showed the appearance of the signals [δ 5.00 and 5.26 (each br s)] due to an exomethylene group. In the ^{13}C NMR spectrum, a methyl signal and the oxygen-bearing quaternary carbon signal due to C-25 were absent, while the olefinic carbon resonances were observed at δ 112.6 and 147.6. These spectral findings suggest that the hydroxyl group at C-25 in 1 was dehydrated to form an exomethylene group in 4.

Table II. ^{13}C NMR Data (δ) for Compounds 1, 1b, 2–8 in Pyridine-d_5 (75.5 MHz)

	1	1b	2	3	4	5	6	7	8
1	34.4	33.9	34.6	34.5	34.6	34.5	34.1	34.6	34.5
2	23.3	26.4	23.6	23.5	23.5	23.4	26.2	23.5	23.4
3	77.9	75.6	77.4	78.0	78.3	78.1	75.8	78.0	78.0
4	36.9	37.9	37.2	37.0	37.8	37.0	37.9	37.1	37.0
5	41.4	40.2	41.4	41.5	41.6	41.4	40.4	41.5	41.4
6	20.6	20.9	20.4	20.9	20.9	20.7	21.6	20.9	20.7
7	78.6	78.1	78.6	78.7	78.3	78.1	78.3	78.0	78.1
8	35.4	35.5	35.7	35.4	35.6	35.1	35.3	36.5	35.6
9	45.3	45.1	45.4	45.4	45.4	45.4	45.4	45.3	45.3
10	37.6	37.7	37.9	37.7	38.4	37.7	38.0	37.8	37.4
11	17.3	17.4	17.3	17.4	17.3	17.4	17.6	17.4	17.4
12	28.1	28.1	28.2	28.1	28.3	27.9	28.2	28.4	27.6
13	30.5	27.2	27.9	27.8	27.3	27.0	27.1	28.1	27.0
14	39.3	39.4	39.8	39.4	39.6	39.3	39.4	39.5	39.4
15	25.4	25.5	25.8	25.5	25.8	24.8	25.0	25.8	24.6
16	26.0	26.4	26.5	26.1	26.2	26.1	26.5	26.0	26.0
17	53.4	53.5	53.6	53.4	53.1	53.0	53.2	52.0	52.4
18	17.4	17.5	17.5	17.5	17.6	17.4	17.9	16.7	17.0
19	16.2	15.5	16.4	16.3	16.4	16.3	16.8	16.5	16.3
20	33.0	33.1	33.3	33.2	34.2	32.4	32.5	34.4	30.6
21	19.7	19.7	19.8	19.8	19.0	20.2	20.4	19.8	20.0
22	39.6	39.6	39.6	40.3	38.4	38.7	39.0		47.8
23	69.5	69.5	69.7	68.5	72.5	69.7	69.8		203.5
24	77.0	77.0	77.3	77.0	79.4	70.1	70.0		
25	73.6	73.7	74.0	78.2	147.6	58.7	59.0	133.9	
26	27.7	27.8	27.9	22.7	112.6	25.2	25.3	154.9	
27	27.1	27.1	27.4	21.2	21.2	20.8	21.0	194.9	
28	27.1	30.0	27.2	27.1	27.9	27.7	29.1	27.9	27.8
29	22.2	22.9	22.3	22.3	22.4	22.2	23.1	22.4	22.3
30	20.3	20.4	20.5	20.4	20.5	20.3	20.6	20.6	20.4
glucosyl									
1′	100.1	100.4	99.8	100.3	100.5	100.3	101.3	100.4	100.3
2′	74.9	75.4	75.4	75.0	75.0	75.0	75.2	75.0	75.0
3′	78.2	78.4	78.4	78.2	78.4	78.3	78.3	78.3	78.3
4′	71.5	72.4	72.5	71.6	71.7	71.6	71.7	71.7	71.7
5′	74.6	78.0	78.2	74.7	74.7	74.6	74.6	74.7	74.7
6′	64.6	63.4	63.5	64.8	64.8	64.7	65.0	64.9	64.9
Ac									
	20.8		21.4	20.9	20.6	20.9	21.6	21.4	20.9
	21.0			20.9	20.9	21.1		21.3	21.2
	169.4		171.2	170.9	170.9	170.7	171.1	170.7	170.8
	169.2			171.0	171.0	170.8		170.8	171.0
Me					49.4				

In order to confirm the structure of **4**, an attempt was made to prepare **4a**. Treatment of the heptaacetate of **1** (**1a**) with $SOCl_2$ in pyridine gave a product, identical to **4a**. From the chemical and spectral evidence described above, the structure of cumingianoside D was established as 3-*O*-acetyl-3α,7α,23,24-tetrahydroxy-14,18-cycloapoeuph-25-enyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (**4**).

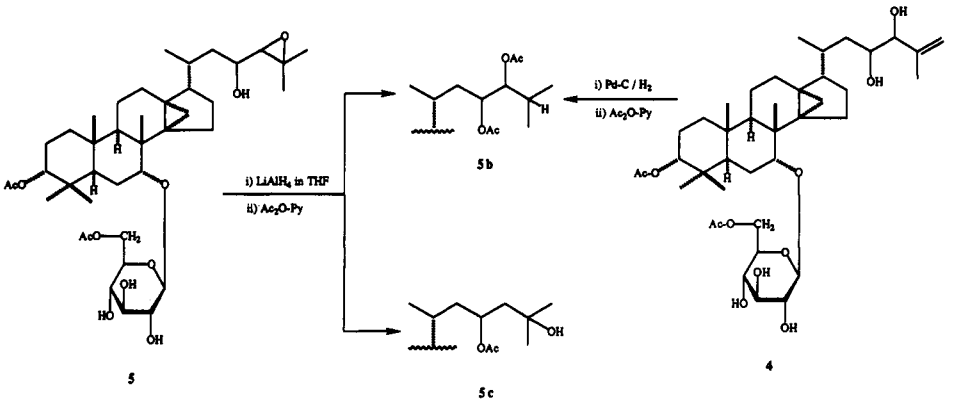
Cumingianoside E (**5**) was also accumulated in a large amount in the fraction B. The negative FABMS of **5** showed the $(M - H)^-$ ion peak at m/z 719, which is 18 mass units less than that of **1**. The ^1H NMR spectrum of **5** resembled that of **1**, showing signals due to a cyclopropyl methylene group [δ 0.55 and 0.64 (each 1 H, d, J = 5.5 Hz)], six tertiary methyl groups (δ 0.89, 0.92, 1.07, 1.14, 1.34, and 1.36), a secondary methyl group [δ 1.13 (d, J = 6 Hz)], two acetoxyl group (δ 1.91 and 2.05), and glucosyl signals. It also showed a characteristic one-proton doublet at δ 2.99 (J = 8 Hz). The ^{13}C NMR spectrum was also analogous to that of **1**, except for the upfield shift of oxygen-bearing methine (δ 70.1) and quaternary (δ 58.7) carbons and two methyl groups.

On acetylation with acetic anhydride and pyridine, **5** yielded a hexaacetate (**5a**) [negative FABMS m/z: 887]. The ^1H NMR spectrum of **5a** showed, together with signals arising from the glucosyl moiety, methine signals at δ 3.84 (br s) and 4.66 (br s) at relatively lower field, being assignable to H-7 and H-3, respectively, based on their coupling patterns. It also showed methine signals at δ 4.84 (m) and 2.74 (d, J = 9 Hz), which were found to be assignable to H-23 and H-24, respectively, on the basis of ^1H–^1H COSY spectrum. Taking into account the fact that the chemical shift of H-24 was almost identical to that of **5**, and upfield shift of oxygen-bearing methine and quaternary carbons observed in the ^{13}C NMR spectrum of **5**, the presence of an epoxy group was argued at C-24 and C-25. In order to confirm the structure of **5**, the following reaction was attempted. Reduction of **5** with $LiAlH_4$ followed by acetylation with acetic anhydride and pyridine yielded a heptaacetate (**5b**) [negative FABMS m/z: 932 $(M - H)^-$] and a hexaacetate (**5c**) [negative FABMS m/z: 890 $(M - H)^-$], which were considered to be formed by opening the epoxy ring in a different direction. On the other hand, hydrogenation of **4** with Pd–C/H_2 followed by acetylation afforded a product, which was shown to be identical with **5b**.

The locations of acetyl groups were concluded to be at C-3 and glucosyl C-6 hydroxyl groups, since in the ^{13}C NMR spectrum of **3**, the carbon resonances due to C-3 and glucosyl C-6 were in good agreement with those of **1**. On the basis of the chemical and spectral observation described above, the structure of **5** was established as 3-*O*-acetyl-3α,7α,23-trihydroxy-24,25-epoxy-14,18-cycloapoeuphanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (**5**).

The negative FABMS of **6** showed $(M - H)^-$ ion peak at m/z 677, which is 42 mass units less than that of **5**. The ^1H NMR spectrum was similar to that of **5**, but differed

Scheme I



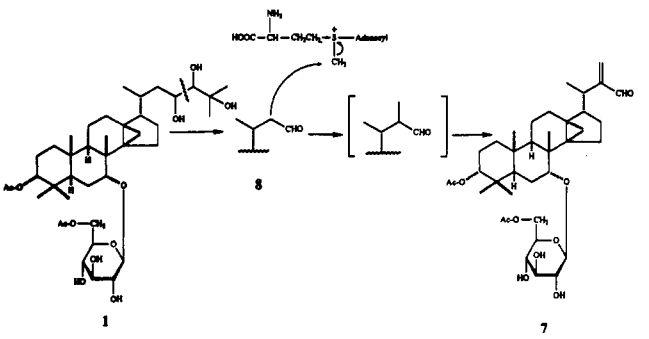
in the absence of one of the acetoxyl signals. Alkaline hydrolysis of **6** yielded a hydrolysate, which was shown to be identical with the product (**5d**) obtained by similar hydrolysis of **5**.

The location of the acetoxyl group was concluded to be at the C-6 position in the glucosyl moiety, since in the ^1H NMR spectrum two proton signals [δ 4.72 (1 H, dd, J = 5.5, 11.5 Hz) and 4.94 (1 H, d, J = 11.5 Hz)], which were assignable to the glucosyl C-6 methylene protons in view of the large coupling constant, were shifted downfield. From these findings, the structure of cumingianoside F was concluded to be represented by formula **6**.

Since cumindysosides A (**7**) and B (**8**) exhibited similar chromatographic properties, they were initially regarded as homogeneous. However, HPLC analysis as well as ^1H and ^{13}C NMR spectra showed the presence of two compounds. The mixture was separated by chromatography over a Fuji-gel ODSQ3 prepacked column, with monitoring by HPLC.

The molecular formula of **7** was confirmed as $C_{37}H_{56}O_{10}$ by HRFABMS. The ^1H NMR spectrum of **7** was similar to those of foregoing compounds, showing signals due to a cyclopropyl methylene group [δ 0.27 and 0.52 (each 1 H, d, J = 5.5 Hz)], four tertiary methyl groups (δ 0.88, 0.90, 1.08, 1.13), assignable to 4β-CH_3, 10-CH_3, 8-CH_3, and 4α-CH_3, respectively, a secondary methyl [δ 1.07 (d, J = 7 Hz)], ascribable to 20-CH_3, two acetoxyl groups (δ 1.96 and 2.06), and two oxygen-bearing methine groups [δ 3.99 (br s), and 4.94 (br s)], assignable to H-7 and H-3, respectively, together with signals arising from the sugar moiety. It also showed two one-proton singlets at δ 5.90 and 6.08 and a one-proton singlet at δ 9.56, indicating the presence of an exomethylene and an aldehyde group. The carbon NMR spectrum of **7** showed the occurrence of 37 carbons, among which six carbon resonances in the region from δ 64.9 to 100.4 indicated the presence of the 6-acetylglucoside moiety. In addition, the carbon resonances due to C-1–19, -28, -29, and -30, being in good accord with those of compounds **1–5**, indicated the same partial structure, but differed only in the substituents at C-17. This was confirmed by the ^1H–^1H COSY and NOESY, as well as ^1H-^{13}C COSY and long-range COSY spectra. The remaining carbon signals, including methyl (δ 19.8), methine (δ 34.4), two olefinic [δ 133.9 (t) and 154.9 (s)], and aldehyde (δ 194.9) carbons, were considered to compose the side-chain group at C-17. The ^1H–^{13}C long-range COSY spectrum showed the correlation between the exomethylene proton signals at δ 5.90 and 6.08, and the aldehyde carbon signal at δ 194.9, as well as the methine carbon signal at δ 34.4 through a three-bond coupling. It also showed coupling between the aldehyde proton at δ 9.56 and the quaternary olefinic carbon at δ 154.9. The NOE was observed between

Scheme II



the aldehyde proton and one (δ 5.90) of the exomethylene protons. On the basis of these spectral observations, the structure of cumindysoside A was concluded to be represented by formula **7**.

The negative FABMS of **8** showed the $(M - H)^-$ ion peak at m/z 647, which is 12 mass units less than that of **7**. The ^1H NMR spectrum of **8** resembled that of **7**, except for the absence of the signals due to the exomethylene group. The ^{13}C NMR spectrum showed 36 carbon signals. The carbon resonances due to C-1–19, -28, -28, -29, and -30, as well as the glucosyl carbons indicated the presence of the same partial structure in **8** as that of **7**, thus suggesting the occurrence of the C_4 side chain at C-17. The ^1H–^1H COSY spectrum of **8** showed the coupling between the aldehyde proton [δ 9.79 (br s)] and methylene protons [δ 2.49 (dd, J = 3, 15 Hz) and ca. 2.1]. Furthermore, these methylene protons were shown to be coupled with the methine proton assignable to H-20, which was further coupled with the methyl signal at δ 0.93 (d, J = 7 Hz). From these spectral findings, the structure of cumindysoside B was represented by formula **8**.

Cumingianosides A–F (**1–6**) appear to be the first examples of the glucoside for 14,18-cycloapoeuphane-type triterpene. Cumindysosides A (**7**) and B (**8**) are novel trisnor- and tetranortriterpene glucosides with a 14,18-cycloapoeuphane skeleton, respectively. Compound **8** was considered to be formed from **1** by oxidative cleavage between C-23 and C-24. On the other hand, compound **7** possesses a biogenetically irregular side-chain group at C-17. The cooccurrences of **1**, **7**, and **8** from the same plant are suggestive of the possible biogenetic pathway for **7** to be derived from **1**, being accumulated in large amounts by this plant, as shown in Scheme II.

The cytotoxic activity of these compounds against human cell lines in vitro is summarized in Table III. These compounds were shown to be more sensitive against the leukemia tumor cell lines than those of other cell lines, except for cumingianoside B (**2**). Cumingianosides A (**1**)

Table III. Cytotoxicity (ED_{50} in μg/mL) of Compounds 1–8 against Human Cancer Cell Lines in Vitro

	1	2	3	4	5	6	7	8
disease type and cell line								
leukemia								
MOLT-4	<0.006 25	11.3	<0.004 50	2.34	0.189	0.701	0.860	NT[a]
RPMI8226	0.264	10.3	0.173	1.50	1.95	0.712	0.860	NT[a]
HL60TB	1.35	11.6	3.50	1.62	1.18	0.974	0.643	NT[a]
K-562	2.34	15.4	5.55	2.42	1.13	1.85	1.44	NT[a]
non-small cell lung cancer								
A549/ATCC	2.94	>50	8.01	6.21	2.10	1.06	6.34	NT[a]
colon cancer								
DLD-1	1.83	1.55	7.41	5.52	1.79	8.09	1.38	NT[a]
HCT-15	1.22	>50	7.36	4.87	1.54	8.45	1.56	NT[a]
HCT-8	>10	>10	>10	>10	>10	>10	>10	>10
melanoma								
LOXIMV1	2.80	8.62	6.95	4.86	1.63	5.20	0.807	NT[a]
SK-MEL-28	4.26	9.77	7.40	7.19	1.84	7.41	0.846	NT[a]
RPMI-7951	3.28	5.45	5.13	4.71	4.83	4.16	0.34	>10
ovarian								
IGROV1	2.56	11.5	7.36	3.79	2.42	5.91	2.15	NT[a]
CNS								
TE-671	4.00	4.44	4.29	1.51	4.44	4.23	2.74	0.61
SF-268	8.12	18.4	3.47	5.31	2.44	6.38	5.44	NT[a]

[a] NT: not tested.

and C (3) especially showed potent selective cytotoxicity against MOLT-4 leukemia cells with ED_{50} of <0.006 25 and <0.004 50 μg/mL, respectively, which were found to be 1000 times more sensitive than those of other tumor cell lines. They also showed antileukemic activity against RPMI 8226 with ED_{50} of 0.264 and 0.173 μg/mL, respectively. The other compounds (4–7) also showed relatively potent cytotoxicity (ED_{50} 0.6–2.5 μg/mL) against leukemia cells, except for 2. Generally, triterpene glycosides show none or weak cytotoxicity as seen in 2. Since only 2 has no acetyl group at glucosyl C-6, the 6-*O*-acetylglucosyl moiety might enhance the cytotoxicity.

Experimental Section

General. NMR spectra were obtained at 300 MHz for ^1H and 75 MHz for ^{13}C, with tetramethylsilane as an internal standard, and chemical shift values are given in δ (ppm).

Extraction and Isolation. The air dried leaves of *D. cumingianum* (8.7 kg), collected in Taiwan, were extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure to give the extracts (1980 g), a part of which (580 g) was partitioned with $CHCl_3$ and H_2O. The $CHCl_3$ layer, after removal of the solvent by evaporation, was further partitioned with hexane (C_6H_{14}) and 90% aqueous MeOH to give fraction A (50 g) and fraction B (280 g). The aqueous layer was subsequently extracted with *n*-BuOH yielding fractions C (40 g) and D (210 g).

A part (20 g) of the fraction B was subjected to chromatography over silica gel with $CHCl_3$ containing increasing amounts of MeOH to give 11 fractions: frs 1 (4.47 g), 2 (0.10 g), 3 (0.84 g), 4 (1.71 g), 5 (0.94 g), 6 (3.57 g), 7 (1.46 g), 8 (0.82 g), 9 (2.80 g), 10 (0.72 g), 11 (0.48 g), and 12 (2.18 g). Fraction 3 was further chromatographed over silica gel [C_6H_{14}–Me_2CO (2:1)] to give the mixture of 7 and 8, which was successively separated by Fuji-gel ODSQ3 prepacked column chromatography with monitoring HPLC to yield pure samples of 7 (43 mg) and 8 (24 mg). Fraction 4 was further chromatographed over Sephadex LH-20 [MeOH–H_2O (3:2 → 1:0) and silica gel [benzene (C_6H_6)–Me_2CO (2:1 → 3:2)] to furnish compound 3 (620 mg). Fractions 6 and 7 consisted mainly of 5 and 1, respectively, which were purified by MCI-gel CHP 20P [MeOH–H_2O (9:1 → 1:0)] chromatography to yield pure samples (2.75 g and 2.10 g, respectively). Similar chromatography of fraction 8, which was also contained in compound 1, with MCI-gel CHP 20P afforded compound 1 (510 mg). Repeated chromatography of fraction 5 with MCI-gel CHP 20P [MeOH–H_2O (9:1 → 1:0)] and silica gel [C_6H_{14}–Me_2CO (3:2 → 1:2)] to give compound 4 (334 mg). The MCI-gel CHP 20P column chromatography [MeOH–H_2O (9:1 → 1:0)] followed by silica gel column chromatography [EtOAc–MeOH (98:2 → 9:1)] of fraction 10 yielded compound 6 (45 mg), together with compound 1 (105 mg). Repeated chromatography of fraction 11 with MCI-gel CHP 20P [MeOH–H_2O (9:1 → 1:0)] and prep-PAK 500/C_{18} [MeOH–H_2O (4:1 → 1:0)] gave compound 2 (144 mg).

General Procedure for Acetylation. The sample (42–65 mg) was treated with acetic anhydride (Ac_2O) (1 mL) and pyridine (C_6H_5N) (1 mL) at room temperature overnight. Following the usual workup, the mixture was chromatographed on silica gel [C_6H_{14}–Me_2CO (3:1 → 2:1)] to furnish the acetate.

General Procedure for Methanolysis with 2% NaOMe–MeOH. The sample (60–105 mg) in 2% NaOMe–MeOH (5–10 mL) was kept standing at room temperature overnight. The reaction mixture was neutralized by IR-120B resin, filtered, concentrated, and purified by silica gel chromatography.

Cumingianoside A (1): a white amorphous powder; $[\alpha]^{22}_D$ −47.3° (*c* = 1.1, MeOH); negative FABMS *m/z* 737 $(M - H)^-$; positive FABMS *m/z* 761 $(M + Na)^+$; HRFABMS calcd for $C_{40}H_{66}O_{12}Na$ 761.4452, found *m/z* 761.4452; ^1H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.50, 0.74 (each 1 H, d, *J* = 5.5 Hz, H-18), 0.83, 0.90, 0.91, 1.04 (each 3 H, s, *t*-CH_3), 0.95 (3 H, d, *J* = 6.5 Hz, 20-CH_3), 1.31, 1.32 (each 3 H, s, 25-CH_3), 2.08, 2.10 (each 3 H, s, OAc), 3.15 (1 H, s, H-24), 3.3–3.7 (4 H in total, m, glucosyl H-2–5), 3.82 (1 H, br s, H-7), 4.08 (1 H, dd, *J* = 5, 9 Hz, H-23), 4.30 (1 H, d, *J* = 7 Hz, anomeric H), 4.32 (1 H, d, *J* = 10 Hz, glucosyl H-6), 4.41 (1 H, dd, *J* = 1.5, 10 Hz, glucosyl H-6′), 4.66 (1 H, br s, H-3); (C_6H_5N-d_5 + D_2O) Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{40}H_{66}O_{12}·^3/_2H_2O$: C, 62.72; H, 9.08. Found: C, 62.90; H, 9.04.

Acid Hydrolysis of 1. A solution of 1 in 5% H_2SO_4–50% MeOH was refluxed for 20 h. The reaction mixture was neutralized with IRA-400 resin, concentrated, and chromatographed over silica gel. Elution with $CHCl_3$–MeOH–H_2O (7:3:0.5) furnished D-glucose (21 mg): $[\alpha]^{21}_D$ +51.2° (*c* = 0.6, H_2O); R_f 0.30 [cellulose TLC, *n*-BuOH–C_6H_5N–H_2O (6:4:3)].

Cumingianoside A acetate (1a): a white amorphous powder; $[\alpha]^{24}_D$ −59.4° (*c* = 0.65, $CHCl_3$); negative FABMS *m/z* 947 $(M - H)^-$, 905 $(M - Ac)^-$; positive FABMS *m/z* 971 $(M + Na)^+$; HRFABMS calcd for $C_{50}H_{76}O_{17}Na$ 971.4980, found *m/z* 971.4974; ^1H NMR (300 MHz, $CDCl_3$) δ 0.45, 0.61 (each 1 H, d, *J* = 6 Hz, H-18), 0.85 (3 H, s, *t*-CH_3), 0.89 (6 H, s, 2 × *t*-CH_3), 0.94 (3 H, d, *J* = 6.5 Hz, 20-CH_3), 1.00 (3 H, s, *t*-CH_3), 1.20, 1.24 (each 3 H, s, 25-CH_3), 2.02, 2.03, 2.04, 2.07, 2.09, 2.13, 2.22 (each 3 H, s, OAc), 3.65 (1 H, m, glucosyl H-5), 3.83 (1 H, br s, H-7), 4.19 (2 H, d, *J* = 3 Hz, glucosyl H-6), 4.57 (1 H, d, *J* = 8 Hz, anomeric H), 4.67 (1 H, br s, H-3), 4.88 (1 H, d, *J* = 1.5 Hz, H-24), 5.05 (1 H, dd, *J* = 8, 9 Hz, glucosyl H-2), 5.15 (1 H, t, *J* = 8 Hz, glucosyl H-4), 5.21 (1 H, t, *J* = 8 Hz, glucosyl H-3), 5.38 (1 H, br t, H-23).

Deacetylcumingianoside A (1b): colorless needles (from dilute MeOH); mp 154–157 °C; $[\alpha]^{22}_D$ −46.0° (*c* = 0.81, MeOH); negative FABMS *m/z* 653 $(M - H)^-$; positive FABMS *m/z* 677 $(M + Na)^+$; HRFABMS calcd for $C_{36}H_{62}O_{10}Na$ 677.4240, found

m/z 677.4244; ^{1}H NMR Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{36}H_{62}O_{10}\cdot 2H_2O$: C, 62.58; H, 9.63. Found: C, 62.12; H, 9.64.

Acetonide for 1b with $CuSO_4$ in Dry Me_2CO (1c). A mixture of **1b** (60 mg) and $CuSO_4$ (400 mg) in dry Me_2CO (7 mL) was kept standing at room temperature for 1 day with stirring and was heated at 60 °C for 2 h with stirring. The reaction mixture was filtered, concentrated under reduced pressure, and subjected to chromatography over silica gel. Elution with $CHCl_3$–MeOH (30:1 → 20:1) yielded **1c** (25 mg) as a white amorphous powder: $[\alpha]^{24}_D$ −53.2° (c = 0.3, $CHCl_3$); negative FABMS m/z 733 $(M - H)^-$; positive FABMS m/z 757 $(M + Na)^+$; HRFABMS calcd for $C_{42}H_{70}O_{10}Na$ 757.4866, found m/z 757.4866; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.42, 0.68 (each 1 H, d, J = 6 Hz, H-18), 0.83, 0.88, 0.94, 1.00, 1.18, 1.26, 1.99, 1.40, 1.44, 1.53 (each 3 H, s, t-CH_3), 1.02 (3 H, d, J = 8 Hz, 20-CH_3), 3.22 (1 H, m, glucosyl H-5), 3.36 (1 H, t, J = 8 Hz, glucosyl H-2), 3.43 (1 H, br s, H-3), 3.50 (1 H, d, J = 7.5 Hz, H-24), 3.57 (1 H, t, J = 8 Hz, glucosyl H-4), 3.67 (1 H, t, J = 8 Hz, glucosyl H-3), 3.71 (1 H, d, J = 10.5 Hz, glucosyl H-6), 3.78 (1 H, br s, H-7), 3.89 (1 H, dd, J = 5, 10.5 Hz, glucosyl H-6′), 3.97 (1 H, br t, J = 8 Hz, H-23), 4.31 (1 H, d, J = 7.5 Hz, anomeric H).

Cumingianoside B (2): a white amorphous powder; $[\alpha]^{22}_D$ −54.6° (c = 0.9, MeOH); negative FABMS m/z 695 $(M - H)^-$; positive FABMS m/z 719 $(M + Na)^+$; HRFABMS calcd for $C_{38}H_{64}O_{11}Na$ 719.4346, found m/z 719.4334; ^{1}H NMR Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{38}H_{64}O_{11}\cdot{}^3/_2H_2O$: C, 63.04; H, 9.33. Found: C, 63.35; H, 9.31.

Partial Methanolysis of 1 with 2% NaOMe–MeOH. A solution of 1 (60 mg) in 2% NaOMe–MeOH (1 mL) was kept standing at room temperature for 2 h. The reaction mixture was neutralized by IR-120B resin, filtered, concentrated, and chromatographed over silica gel. Elution with $CHCl_3$–MeOH (20:1 → 10:1) gave a hydrolysate (46 mg) as a white amorphous powder, which was shown to be identical with **2** by physical and spectral comparisons.

Cumingianoside C (3): a white amorphous powder; $[\alpha]^{22}_D$ −46.7° (c = 1.1, MeOH); negative FABMS m/z 751 $(M - H)^-$; positive FABMS m/z 775 $(M + Na)^+$; HRFABMS calcd for $C_{41}H_{68}O_{12}Na$ 775.4609, found m/z 775.4615; ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.52, 0.74 (each 1 H, d, J = 5.5 Hz, H-18), 0.83, 0.90, 0.91, 1.03 (each 3 H, s, t-CH_3), 0.95 (3 H, d, J = 6.5 Hz, 20-CH_3), 1.23, 1.31 (each 3 H, s, 25-CH_3), 2.07, 2.10 (each 3 H, s, OAc), 3.10 (1 H, s, H-24), 3.26 (3 H, s, OCH_3), 3.3–3.7 (4 H in total, m, glucosyl H-2–5), 3.82 (1 H, br s, H-7), 4.06 (1 H, dd, J = 5, 8.5 Hz, H-23), 4.30 (1 H, d, J = 7 Hz, anomeric H), 4.31 (1 H, d, J = 11.5 Hz, glucosyl H-6), 4.42 (1 H, br d, J = 11.5 Hz, glucosyl H-6′), 4.66 (1 H, br s, H-3); (300 MHz, C_6H_5N-d_5 + D_2O) Table I; ^{13}C NMR: Table II. Anal. Calcd for $C_{41}H_{68}O_{12}\cdot{}^1/_2H_2O$: C, 64.62; H, 9.13. Found: C, 64.28; H, 9.12.

Cumingianoside C acetate (3a): a white amorphous powder; $[\alpha]^{24}_D$ −50.7° (c = 0.54, $CHCl_3$); negative FABMS m/z 961 $(M - H)^-$; positive FABMS m/z 985 $(M + Na)^+$; HRFABMS calcd for $C_{51}H_{78}O_{17}Na$ 985.5136, found m/z 985.5131; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.46, 0.61 (each 1 H, d, J = 6 Hz, H-18), 0.85 (3 H, s, t-CH_3), 0.89 (6 H, s, 2 × t-CH_3), 0.96 (3 H, d, J = 6 Hz, 20-CH_3), 1.01 (3 H, s, t-CH_3), 1.17, 1.20 (each 3 H, s, 25-CH_3), 2.03 (3 H, s, OAc), 2.04, 2.08 (each 6 H, s, 2 × OAc), 2.13, 2.19 (each 3 H, s, OAc), 3.22 (3 H, s, OCH_3), 3.65 (1 H, m, glucosyl H-5), 3.83 (1 H, br s, H-7), 4.18 (2 H, d, J = 3 Hz, glucosyl H-6), 4.58 (1 H, d, J = 7.5 Hz, anomeric H), 4.67 (1 H, br s, H-3), 4.99 (1 H, d, J = 1.5 Hz, H-24), 5.05 (1 H, t, J = 8 Hz, glucosyl H-2), 5.15 (1 H, t, J = 8 Hz, glucosyl H-4), 5.22 (1 H, t, J = 8 Hz, glucosyl H-3), 5.37 (1 H, m, H-23).

Cumingianoside D (4): a white amorphous powder; $[\alpha]^{22}_D$ −39.2° (c = 1.05, MeOH); negative FABMS m/z 719 $(M - H)^-$; positive FABMS m/z 743 $(M + Na)^+$; HRFABMS calcd for $C_{40}H_{64}O_{11}Na$ 743.4346, found m/z 743.4347; ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.48, 0.71 (each 1 H, d, J = 5.5 Hz, H-18), 0.83, 0.90, 0.91, 1.03 (each 3 H, s, t-CH_3), 1.00 (3 H, d, J = 6.5 Hz, 20-CH_3), 1.77 (3 H, s, 25-CH_3), 2.07, 2.09 (each 3 H, s, OAc), 3.3–3.6 (4 H in total, m, glucosyl H-2–5), 3.73 (1 H, m, H-23), 3.81 (1 H, br s, H-7), 3.88 (1 H, d, J = 4 Hz, H-24), 4.30 (1 H, d, J = 7 Hz, anomeric H), 4.34 (1 H, d, J = 10 Hz, glucosyl H-6), 4.41 (1 H, dd, J = 1.5, 10 Hz, glucosyl H-6′), 4.67 (1 H, br s, H-3), 5.01, 5.07 (each 1 H, s, H-26), (300 MHz, C_6H_5N-d_5 + D_2O) Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{40}H_{64}O_{11}\cdot 2H_2O$: C, 63.45; H, 9.06. Found: C, 63.87; H, 8.80.

Cumingianoside D acetate (4a): a white amorphous powder; $[\alpha]^{20}_D$ −55.3° (c = 0.8, $CHCl_3$); negative FABMS m/z 929 $(M - H)^-$; positive FABMS m/z 953 $(M + Na)^+$; HRFABMS calcd for $C_{50}H_{74}O_{16}Na$ 953.4875, found m/z 953.4874; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.47, 0.62 (each 1 H, d, J = 6 Hz, H-18), 0.85 (3 H, s, t-CH_3), 0.89 (6 H, s, 2 × t-CH_3), 0.95 (3 H, d, J = 6 Hz, 20-CH_3), 1.01 (3 H, s, t-CH_3), 1.85 (3 H, s, 25-CH_3), 2.02, 2.03, 2.04, 2.06, 2.12, 2.14 (21 H in total, each s, OAc), 3.65 (1 H, m, glucosyl H-5), 3.83 (1 H, br s, H-7), 4.19 (2 H, d, J = 3 Hz, glucosyl H-6), 4.58 (1 H, d, J = 7.5 Hz, anomeric H), 4.66 (1 H, br s, H-3), 4.95 (2 H, s, H-26), 5.04 (1 H, t, J = 8 Hz, glucosyl H-2), 5.14 (1 H, t, J = 8 Hz, glucosyl H-4), 5.16 (1 H, d, J = 4 Hz, H-24), 5.21 (1 H, t, J = 8 Hz, glucosyl H-3), 5.37 (1 H, m, H-23). Anal. Calcd for $C_{50}H_{74}O_{16}$: C, 64.49; H, 8.01. Found: C, 64.69; H, 8.14.

Treatment of 1a with $SOCl_2$ in C_6H_5N. A solution of **1a** (80 mg) in C_6H_5N (3 mL) was treated with $SOCl_2$ (2 drops), and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4, concentrated, and subjected to chromatography over silica gel. Elution with C_6H_{14}–Me_2CO (4:1) gave a product (47 mg) as a white amorphous powder, which was shown to be identical with **4a** by spectral comparisons.

Cumingianoside E (5): a white amorphous powder; $[\alpha]^{22}_D$ −39.6° (c = 1.04, MeOH); negative FABMS m/z 719 $(M - H)^-$; positive FABMS m/z 743 $(M + Na)^+$; HRFABMS calcd for $C_{40}H_{64}O_{11}Na$ 743.4346, found m/z 743.4353; ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.52, 0.75 (each 1 H, d, J = 5.5 Hz, H-18), 0.83, 0.91, 0.92, 1.04 (each 3 H, s, t-CH_3), 0.98 (3 H, d, J = 6.5 Hz, 20-CH_3), 1.34, 1.35 (each 3 H, s, 25-CH_3), 2.07, 2.09 (each 3 H, s, OAc), 2.65 (1 H, d, J = 8 Hz, H-24), 3.3–3.6 (4 H in total, m, glucosyl H-2–5), 3.58 (1 H, m, H-23), 3.83 (1 H, br s, H-7), 4.31 (1 H, d, J = 7 Hz, anomeric H), 4.37 (1 H, d, J = 10 Hz, glucosyl H-6), 4.42 (1 H, dd, J = 1.5, 10 Hz, glucosyl H-6′), 4.67 (1 H, br s, H-3); (300 MHz, C_6H_5N-d_5 + D_2O) Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{40}H_{64}O_{11}\cdot H_2O$; C, 65.01; H, 9.00. Found: C, 65.02; H, 8.86.

Cumingianoside E acetate (5a): a white amorphous powder; $[\alpha]^{24}_D$ −60.2° (c = 0.52, $CHCl_3$); negative FABMS m/z 887 $(M - H)^-$; positive FABMS m/z 911 $(M + Na)^+$; HRFABMS calcd for $C_{48}H_{72}O_{15}Na$ 911.4769, found m/z 911.4772; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.51, 0.68 (each 1 H, d, J = 6 Hz, H-18), 0.85 (3 H, s, t-CH_3), 0.89 (6 H, s, 2 × t-CH_3), 0.97 (3 H, d, J = 6 Hz, 20-CH_3), 1.02 (3 H, s, t-CH_3), 1.36, 1.39 (each 3 H, s, 25-CH_3), 2.02, 2.04, 2.06, 2.08, 2.13 (18 H, in total, each s, OAc), 2.74 (1 H, d, J = 9 Hz, H-24), 3.65 (1 H, dt, J = 3, 9 Hz, glucosyl H-5), 3.84 (1 H, br s, H-7), 4.19 (2 H, d, J = 3 Hz, glucosyl H-6), 4.58 (1 H, d, J = 7.5 Hz, anomeric H), 4.66 (1 H, br s, H-3), 4.84 (1 H, m, H-23), 5.05 (1 H, dd, J = 7.5, 9 Hz, glucosyl H-2), 5.14 (1 H, t, J = 9 Hz, glucosyl H-4), 5.21 (1 H, t, J = 9 Hz, glucosyl H-3). Anal. Calcd for $C_{48}H_{72}O_{15}$: C, 64.84; H, 8.16. Found: C, 64.90; H, 8.18.

Deacetylcumingianoside E (5d): a white amorphous powder; $[\alpha]^{21}_D$ −40.0° (c = 0.6, MeOH); negative FABMS m/z 635 $(M - H)^-$; positive FABMS m/z 659 $(M + Na)^+$; HRFABMS calcd for $C_{36}H_{60}O_{19}Na$ 659.4135, found m/z 659.4134; ^{1}H NMR (300 MHz, C_6H_5N-d_5 + D_2O) δ 0.66, 0.88 (each 1 H, d, J = 5.5 Hz, H-18), 0.92 (3 H, s, 4β-CH_3), (3 H, s, 10-CH_3), 1.09 (3 H, d, J = 6 Hz, 20-CH_3), 1.10 (3 H, s, 8-CH_3), 1.34 (6 H, s, 4α- and 25-CH_3), 1.37 (3 H, 25-CH_3), 2.58 (1 H, d, J = 12 Hz, H-5), 2.98 (1 H, d, J = 8.5 Hz, H-24), 3.65 (1 H, br s, H-3), 3.92 (1 H, m, H-23), 3.99 (1 H, m, glucosyl H-5), 4.03 (1 H, dd, J = 7.5, 9 Hz, glucosyl H-2), 4.14 (1 H, t, J = 9 Hz, glucosyl H-4), 4.18 (1 H, br s, H-7), 4.34 (1 H, dd, J = 5.5, 11.5 Hz, glucosyl H-6), 4.59 (1 H, dd, J = 2.5, 11.5 Hz, glucosyl H-6′), 4.88 (1 H, d, J = 7.5 Hz, anomeric H). Anal. Calcd for $C_{36}H_{60}O_9\cdot 2H_2O$: C, 64.26; H, 9.59. Found: C, 64.22; H, 9.56.

Reduction with $LiAlH_4$ of 5, Followed by Acetylation (5b and 5c). A mixture of **5** (200 mg) and $LiAlH_4$ (50 mg) in anhydrous tetrahydrofuran (THF, 10 mL) was refluxed for 3 h with stirring. The excess reagent was decomposed by EtOAc, and the reaction mixture was diluted by water and extracted by EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a syrup, which was subsequently treated with Ac_2O (1.5 mL) and C_6H_5N (1.5 mL) at room temperature overnight. The reaction mixture was worked up as usual and

subjected to chromatography over silica gel. Elution with C_6H_{14}–EtOAc (3:1 → 2:3) afforded the heptaacetate (**5b**) (84 mg) and the hexaacetate (**5c**) (41 mg). **5b**: a white amorphous powder; $[\alpha]^{20}_D$ −68.3° (*c* = 0.4, $CHCl_3$); negative FABMS *m/z* 931 $(M - H)^-$; positive FABMS *m/z* 955 $(M + Na)^+$; HRFABMS calcd for $C_{50}H_{76}O_{16}Na$ 955.5031, found *m/z* 955.5030; ^1H NMR (300 MHz, $CDCl_3$) δ 0.46, 0.61 (each 1 H, d, *J* = 6 Hz, H-18), 0.85 (3 H, s, *t*-CH_3), 0.87 (3 H, d, *J* = 7 Hz, 20-CH_3), 0.88 (6 H, s, 2 × *t*-CH_3), 1.02 (3 H, s, *t*-CH_3), 0.90, 0.93 (each 3 H, d, *J* = 6.5 Hz, 25-CH_3), 1.00 (3 H, s, *t*-CH_3), 2.02, 2.03, 2.06, 2.08, 2.12, 2.14 (21 H in total, each s, OAc), 3.67 (1 H, dt, *J* = 3.5, 9 Hz, glucosyl H-5), 3.82 (1 H, br s, H-7), 4.19 (2 H, d, *J* = 3.5 Hz, glucosyl H-6), 4.57 (1 H, d, *J* = 7.5 Hz, anomeric H), 4.66 (1 H, br s, H-3), 4.78 (1 H, dd, *J* = 3, 8 Hz, H-24), 5.04 (1 H, dd, *J* = 7.5, 9 Hz, glucosyl H-2), 5.14 (1 H, t, *J* = 9 Hz, glucosyl H-4), 5.21 (1 H, t, *J* = 9 Hz, glucosyl H-3), 5.21 (1 H, m, H-23). **5c**: a white amorphous powder; $[\alpha]^{20}_D$ −81.2° (*c* = 0.55, $CHCl_3$); negative FABMS *m/z* 889 $(M - H)^-$; positive FABMS *m/z* 913 $(M + Na)^+$; HRFABMS calcd for $C_{48}H_{74}O_{15}Na$ 913.4925, found *m/z* 913.4926; ^1H NMR (300 MHz, $CDCl_3$) δ 0.48, 0.65 (each 1 H, d, *J* = 6 Hz, H-18), 0.85 (3 H, s, *t*-CH_3), 0.89 (6 H, s, 2 × *t*-CH_3), 0.96 (3 H, d, *J* = 7 Hz, 20-CH_3), 1.02 (3 H, s, *t*-CH_3), 0.90, 0.93 (each 3 H, d, *J* = 6.5 Hz, 25-CH_3), 1.02 (3 H, s, *t*-CH_3), 1.31, 1.34 (each 3 H, s, 25-CH_3), 2.01, 2.02, 2.03, 2.05, 2.06, 2.13 (each 3 H, s, OAc), 3.65 (1 H, dt, *J* = 3.5, 9 Hz, glucosyl H-5), 3.84 (1 H, br s, H-7), 4.19 (2 H, d, *J* = 3.5 Hz, glucosyl H-6), 4.58 (1 H, d, *J* = 7.5 Hz, anomeric H), 4.66 (1 H, br s, H-3), 5.04 (1 H, dd, *J* = 7.5, 9 Hz, glucosyl H-2), 5.11 (1 H, m, H-23), 5.13 (1 H, t, *J* = 9 Hz, glucosyl H-4), 5.21 (1 H, t, *J* = 9 Hz, glucosyl H-3).

Hydrogenation with Pd–C/H_2 of 4, Followed by Acetylation. 4 (100 mg) was hydrogenated over 10% Pd–C (20 mg) in EtOH (10 mL) under hydrogen atmosphere overnight. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to a syrup, which was subjected to chromatography over silica gel. Elution with $CHCl_3$–MeOH (20:1 → 15:1) yielded a product (73 mg) as a white amorphous powder: $[\alpha]^{20}_D$ −49.6° (*c* = 0.48, MeOH); negative FABMS *m/z* 721 $(M - H)^-$; positive FABMS *m/z* 745 $(M + Na)^+$; HRFABMS calcd for $C_{40}H_{66}O_{11}Na$ 745.4502, found *m/z* 745.4518; ^1H NMR (300 MHz, C_6H_5N-d_5 + D_2O) δ 0.52, 0.65 (each 1 H, d, *J* = 5.5 Hz, H-18), 0.89 (3 H, s, 4β-CH_3), 0.91 (3 H, s, 10-CH_3), 1.09 (3 H, s, 8-CH_3), 1.13 (3 H, d, *J* = 6 Hz, 20-CH_3), 1.14 (6 H, s, 4α-CH_3), 1.15, 1.21 (each 3 H, d, *J* = 6 Hz, 25-CH_3), 1.91, 2.05 (each 3 H, s, OAc), 2.42 (1 H, d, *J* = 12 Hz, H-5), 3.39 (1 H, dd, *J* = 3.5, 6 Hz, H-24), 3.91 (1 H, dd, *J* = 7.5, 8.5 Hz, glucosyl H-2), 3.99 (1 H, m, glucosyl H-5), 4.03 (1 H, br s, H-7), 4.03 (1 H, t, *J* = 8.5 Hz, glucosyl H-4), 4.14 (1 H, t, *J* = 9 Hz, glucosyl H-4), 4.10 (1 H, m, H-23), 4.20 (1 H, t, *J* = 8.5 Hz, glucosyl H-3), 4.73 (1 H, dd, *J* = 5, 10 Hz, glucosyl H-6), 4.76 (1 H, d, *J* = 7.5 Hz, anomeric H), 4.94 (1 H, d, *J* = 10 Hz, glucosyl H-6′), 4.95 (1 H, br s, H-3). Anal. Calcd for $C_{40}H_{66}O_{11}·H_2O$: C, 64.84; H, 9.25. Found: C, 65.07; H, 9.21. The product was further acetylated as described above to give a heptaacetate, which was shown to be identical with **5b** by physical and spectral comparisons.

Cumingianoside F (6): a white amorphous powder; $[\alpha]^{21}_D$ −35.0° (*c* = 0.62, MeOH); negative FABMS *m/z* 677 $(M - H)^-$; positive FABMS *m/z* 701 $(M + Na)^+$; HRFABMS calcd for $C_{38}H_{62}O_{10}Na$ 701.4240, found *m/z* 701.4241; ^1H NMR (300 MHz, $CDCl_3$ + D_2O) δ 0.52, 0.78 (each 1 H, d, *J* = 5 Hz, H-18), 0.84, 0.88, 0.94, 1.02 (each 3 H, s, *t*-CH_3), 0.95 (3 H, d, *J* = 6.5 Hz, 20-CH_3), 1.34, 1.35 (each 3 H, s, 25-CH_3), 2.07 (3 H, s, OAc), 2.66 (1 H, d, *J* ≈ 8 Hz, H-24), 3.3–3.6 (5 H, in total, m, glucosyl H-2–5 and H-23), 3.42 (1 H, br s, H-3), 3.78 (1 H, br s, H-7), 4.24 (1 H, d, *J* = 7.5 Hz, anomeric H), 4.26 (1 H, br d, *J* = 11.5 Hz, glucosyl H-6), 4.39 (1 H, d, *J* = 11.5 Hz, glucosyl H-6′); (C_6H_5N-d_5 + D_2O) Table I; ^{13}C NMR Table II. Anal. Calcd for $C_{38}H_{62}O_{10}·H_2O$; C, 65.49; H, 9.26. Found: C, 65.99; H, 9.29.

Cumindysoside A (7): a white amorphous powder; $[\alpha]^{21}_D$ −64.5° (*c* = 0.55, MeOH); negative FABMS *m/z* 659 $(M - H)^-$, 617 $(M - Ac - H)^-$; positive FABMS *m/z* 683 $(M + Na)^+$; HRFABMS calcd for $C_{37}H_{56}O_{10}Na$ 683.3771, found *m/z* 683.3777; ^1H NMR Table I; ^{13}C NMR Table II.

Cumindysoside B (8): a white amorphous powder; $[\alpha]^{21}_D$ −57.9° (*c* = 0.42, MeOH); negative FABMS *m/z* 647 $(M - H)^-$, 605 $(M - Ac - H)^-$; positive FABMS *m/z* 671 $(M + Na)^+$: HRFABMS calcd for $C_{36}H_{56}O_{10}Na$ 671.3772, found *m/z* 671.3770; ^1H NMR Table I; ^{13}C NMR Table II.

Acid Hydrolysis of Compounds 2–8. A solution of each sample in 5% H_2SO_4–50% MeOH (5 mg/mL) was refluxed for 1 day. The reaction mixture was concentrated under reduced pressure to give an aqueous solution, which was extracted by $CHCl_3$. The aqueous layer was neutralized by IRA-400 resin, filtered, and concentrated under reduced pressure. The residue was subjected to TLC examination to detect glucose: R_f 0.30 [cellulose TLC, *n*-BuOH–C_6H_5N–H_2O (6:4:3)].

Biological Assay. The in vitro cytotoxicity was carried out according to a National Cancer Institute protocol[11] and is described in ref 12.

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Supplementary Material Available: ^{13}C NMR spectra of 7 and 8 (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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