Antitumor Agents. 168.[1] *Dysoxylum cumingianum.* IV.[2] The Structures of Cumingianosides G—O, New Triterpene Glucosides with a 14,18-Cycloapotirucallane-Type Skeleton from *Dysoxylum cumingianum*, and Their Cytotoxicity against Human Cancer Cell Lines

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Nine new triterpene glucosides, named cumingianosides G—O (4—7, 9, 12—15), containing a 14,18-cycloapotirucallane-type skeleton were isolated from a cytotoxic fraction of the leaves of *Dysoxylum cumingianum*. The structures of the new compounds were established on the basis of chemical and spectral examinations. Evaluation of the cytotoxic activity of cumingianosides G—O showed that cumingianoside M exhibited significant (<4 μM) cytotoxicity, especially against leukemia and melanoma cell lines.

Key words *Dysoxylum cumingianum*; 14,18-cycloapotirucallane glucoside; cytotoxicity; Meliaceae; triterpene; triterpene glucoside

Our previous papers reported the isolation and characterization of six triterpene glucosides, cumingianosides A—F (**1**—**3**, **8**, **10**, **11**), as well as a trisnor- and a tetranortriterpene glucoside, cumindysosides A (**16**) and B (**17**), respectively, with a 14,18-cycloapotirucallane-type skeleton[3,4] from a cytotoxic fraction of the leaves of *Dysoxylum cumingianum* (Meliaceae). Among these compounds, cumingianosides A (**1**) and C (**3**) exhibited potent selective cytotoxicity against MOLT-4 human leukemia cells with EC_{50} values of <0.00625 and <0.0045 μM,[2b,5] respectively. Further detailed investigation of this cytotoxic fraction has resulted in the isolation of nine additional triterpene glucosides, cumingianosides G—O (**4**—**7**, **9**, **12**—**15**), with a 14,18-cycloapotirucallane-type skeleton. The cytotoxicities of **4**, **5**, **9**, **12**, **13** and **15** were evaluated against a panel of 59 human cancer cell lines. This paper describes the isolation and characterization of these compounds and the evaluation of their cytotoxic activity.

Repeated chromatography of the previously obtained fraction B, which exhibited potent cytotoxicity against human cancer cell lines, on silica gel, Sephadex LH-20, MCI-gel CHP 20P, and Cosmosil 140 C18 PREP, as well as semi-preparative scale HPLC afforded cumingianosides G—O (**4**—**7**, **9**, **12**—**15**). Among these compounds, cumingianoside G (**4**) was found to be identical with deacetylcumingianoside A,[2a] which had previously been prepared by 2% NaOMe–MeOH treatment of cumingianoside A (**1**).

The ^{1}H-NMR spectrum of cumingianoside H (**5**) was similar to that of **4**, with signals ascribable to a cyclopropyl methylene group [δ 0.60, 0.83 (each d, J=5.5 Hz)], six tertiary methyl groups (δ 0.91, 0.95, 1.11, 1.31, 1.59, 1.62), a secondary methyl group [δ 1.11 (d, J=6 Hz)], and four oxygen-bearing methine groups [δ 3.58 (br s), 3.62 (br s), 4.01 (br s), 4.50 (br t, J=7 Hz)], together with sugar signals. The negative FAB-MS of **5** showed the $(M-H)^-$ ion peak at m/z 695, which was 42 mass units higher than that (m/z 653) of **4**. This result, together with the observation of an acetyl signal (δ 2.06) in the ^{1}H-NMR spectrum of **5**, indicated that **5** was the monoacetate of **4**. On treatment with 2% NaOMe–MeOH, **5** yielded a product that was identified as **4** by spectral comparison. The acetoxy group in **5** was concluded to be at the glucosyl C-6 position, since the glucosyl C-6 methylene proton signals were shifted downfield [δ 4.67 (1H, dd, J=6, 12 Hz), 4.89 (1H, dd, J=2, 12 Hz)] as compared with those [δ 4.27 (1H, dd, J=6, 11.5 Hz), 4.52 (1H, dd, J=3, 11.5 Hz)] of **4**. On the basis of the evidence described above, the structure of **5** was confirmed to be 3α,7α, 23(*R*),24(*S*),25-pentahydroxy-14,18-cycloapotirucallanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (**5**).

Cumingianoside I (**6**) and J (**7**) gave the same $(M-H)^-$ ion peak (m/z 737) in their negative FAB-MS, and were shown to possess the same molecular formula ($C_{40}H_{66}O_{12}$) as that of cumingianoside A (**1**) using high-resolution (HR) FAB-MS. The ^{1}H-NMR spectra of **6** and **7** closely correlated with that of **1**, showing the presence of 3α,7α,23(*R*),24(*S*),25-pentahydroxy-14,18-cycloapotirucallanyl and sugar moieties (see Table 1). The presence of two acetoxy groups in **6** and **7** was revealed by appropriate ^{1}H-NMR resonances [δ 1.89, 2.08 (each s)]. On treatment of **6** and **7** with 2% NaOMe–MeOH, both gave cumingianoside G (**4**). One acetoxy group was easily concluded to be at the C-3 hydroxy group in both compounds, since the proton resonance of the hydroxy-bearing methine proton assignable to H-3 [δ 4.88 (br s) in **6**; δ 4.94 (br s) in **7**] was almost identical with that [δ 4.94 (br s)] found in **1**. In the ^{1}H-NMR spectra of **6** and **7**, lowfield one-proton triplets [δ 5.76 (1H, t, J=9 Hz) in **6**; δ 5.50 (1H, t, J=9 Hz) in **7**] could be assigned by ^{1}H–^{1}H correlation spectroscopy (COSY) examination to glucosyl H-3 and H-4, respectively, thus confirming the location of the remaining acetyl group in each compound. On the

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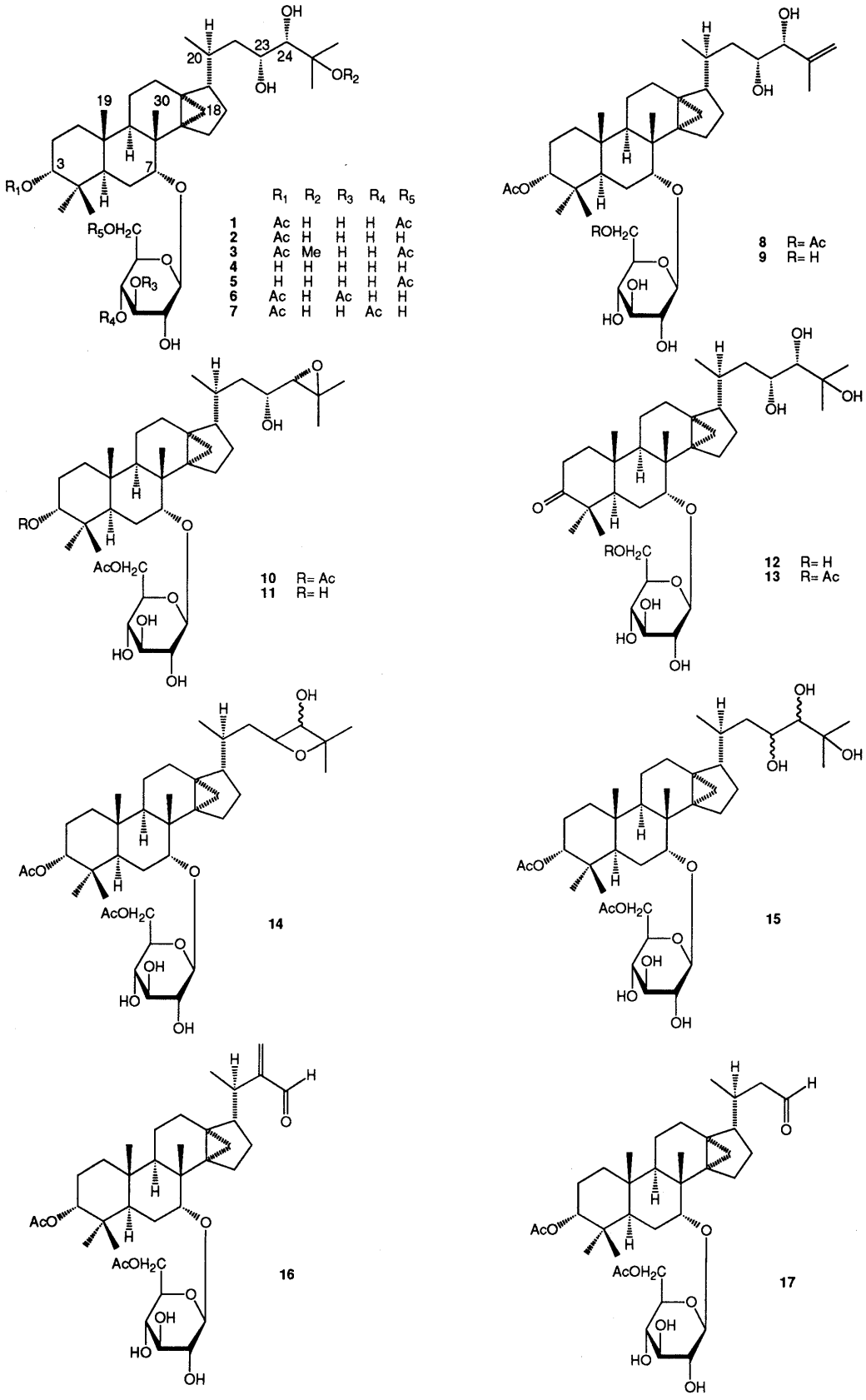

Fig. 1

basis of the above-mentioned evidence, the structures of **6** and **7** were established as 3α,7α,23(*R*),24(*S*),25-pentahydroxy-14,18-cycloapotirucallanyl 7-*O*-β-D-(3′-*O*-acetyl)glucopyranoside (**6**) and 3α,7α,23(*R*),24(*S*),25-pentahydroxy-14,18-cycloapotirucallanyl 7-*O*-β-D-(4′-*O*-acetyl)glucopyranoside (**7**), respectively.

The same $(M-H)^-$ ion peak (*m/z* 737) and molecular formula ($C_{40}H_{66}O_{12}$) seen in **1** were obtained in the negative FAB-MS and HR FAB-MS of cumingianoside K (**15**). The ^{1}H-NMR spectrum of **15** resembled that of

Table 1. ^{1}H-NMR Data (δ, J in Hz) for Compounds **1**, **4**—**10**, **12**—**15** in Pyridine-d_5 + D_2O (400 MHz)

	1	**4**	**5**	**6**	**7**	**8**
H-3	4.94 (br s)	3.63 (br s)	3.62 (br s)	4.88 (br s)	4.94 (br s)	4.93 (br s)
H-5	2.34 (br d, 12)	2.50 (d, 12)	2.52 (d, 12)	2.32 (br d, 12)	2.39 (br d, 12)	2.39 (d, 12)
H-7	4.03 (br s)	4.13 (br s)	4.01 (br s)	4.10 (br s)	4.10 (br s)	4.01 (br s)
H-18	0.51 (d, 6)	0.59 (d, 5.5)	0.60 (d, 5.5)	0.56 (d, 6)	0.45 (d, 6)	0.53 (d, 6)
	0.65 (d, 6)	0.89 (d, 5.5)	0.83 (d, 5.5)	0.70 (d, 6)	0.59 (d, 6)	0.69 (d, 6)
H-19	0.91 (s)	0.93 (s)	0.95 (s)	0.88 (s)	0.89 (s)	0.91 (s)
H-21	1.11 (d, 7)	1.10 (d, 6)	1.11 (d, 6)	1.10 (d, 6)	1.10 (d, 6)	1.17 (d, 6.5)
H-23	4.51 (br t, 7)	4.50 (br t, 6)	4.50 (br t, 7)	4.52 (br t, 6.5)	4.51 (br t, 7)	4.07 (m)
H-24	3.57 (br s)	3.59 (br s)	3.58 (br s)	3.58 (br s)	3.60 (br s)	4.21 (d, 6)
H-26	1.59 (s)	1.60 (s)	1.59 (s)	1.59 (s)	1.59 (s)	4.98, 5.22 (each br s)
H-27	1.63 (s)	1.64 (s)	1.62 (s)	1.63 (s)	1.62 (s)	1.93 (s)
H-28	1.13 (s)	1.30 (s)	1.31 (s)	1.04 (s)	1.13 (s)	1.11 (s)
H-29	0.90 (s)	0.90 (s)	0.91 (s)	0.85 (s)	0.89 (s)	0.89 (s)
H-30	1.09 (s)	1.07 (s)	1.11 (s)	1.04 (s)	1.02 (s)	1.09 (s)
Glc-1	4.74 (d, 8)	4.82 (d, 8)	4.73 (d, 7)	4.82 (d, 8)	4.81 (d, 7.5)	4.72 (d, 8)
2	3.88 (dd, 8, 9)	3.96 (dd, 8, 9.5)	3.91 (dd, 7, 9)	3.93 (dd, 8, 9)	3.93 (dd, 7.5, 9)	3.87 (t, 8)
3	4.67 (t, 9)	4.22 (t, 9.5)	4.16 (t, 9)	5.76 (t, 9)	4.25 (t, 9)	4.14 (t, 8)
4	4.02 (t, 9)	4.08 (t, 9.5)	4.01 (t, 9)	4.14 (t, 9)	5.50 (t, 9)	3.96 (t, 8)
5	3.96 (ddd, 2, 5.5, 9)	3.94 (ddd, 3, 6, 9.5)	3.93 (m)	3.98 (m)	3.97 (ddd, 3, 6, 9)	3.92 (m)
6	4.76 (dd, 5.5, 11.5)	4.27 (dd, 6, 11.5)	4.67 (dd, 6, 12)	4.30 (dd, 5.5, 11.5)	4.08 (dd, 6, 12)	4.67 (dd, 5.5, 11.5)
	4.93 (dd, 2, 11.5)	4.52 (dd, 3, 11.5)	4.89 (dd, 2, 12)	4.49 (dd, 2.5, 11.5)	4.16 (dd, 3, 12)	4.89 (dd, 2.5, 11.5)
$-COCH_3$	1.94 (s)		2.06 (s)	1.89 (s)	1.89 (s)	1.89 (s)
	2.05 (s)			2.08 (s)	2.08 (s)	2.03 (s)

	9	**10**	**12**	**13**	**14**	**15**
H-3	4.92 (br s)	4.94 (br s)			4.93 (br s)	4.92 (br s)
H-5	2.38 (br d, 12)	2.42 (d, 12)	2.44 (br d, 10)	2.43 (m)	2.40 (br d, 13)	2.38 (d, 12)
H-7	4.12 (br s)	4.04 (br s)	4.07 (br s)	3.92 (br t)	4.00 (br s)	4.01 (br s)
H-18	0.52 (d, 6)	0.56 (d, 5.5)	0.75 (d, 6)	0.75 (d, 5.5)	0.50 (d, 6)	0.58 (d, 6)
	0.70 (d, 6)	0.67 (d, 5.5)	0.95 (d, 6)	0.93 (d, 5.5)	0.62 (d, 6)	0.70 (d, 6)
H-19	0.90 (s)	0.93 (s)	0.90 (s)	0.90 (s)	0.90 (s)	0.89 (s)
H-21	1.15 (d, 6.5)	1.13 (d, 5.5)	1.12 (d, 6)	1.14 (d, 6.5)	1.09 (d, 6.5)	1.25 (d, 7)
H-23	4.07 (m)	3.91 (br t, 8)	4.53 (br t, 6)	4.53 (br t, 7)	4.98 (br t, 6.5)	4.36 (ddd, 3, 8, 8)
H-24	4.23 (d, 5.5)	2.97 (d, 8)	3.60 (s)	3.60 (s)	4.68 (d, 6.5)	3.76 (d, 8)
H-26	5.00, 5.25 (each br s)	1.33 (s)	1.61 (s)	1.61 (s)	1.51 (s)	1.69 (s)
H-27	1.94 (s)	1.36 (s)	1.64 (s)	1.64 (s)	1.61 (s)	1.71 (s)
H-28	1.11 (s)	1.13 (s)	1.38 (s)	1.37 (s)	1.12 (s)	1.11 (s)
H-29	0.89 (s)	0.90 (s)	1.10 (s)	1.11 (s)	0.89 (s)	0.89 (s)
H-30	1.05 (s)	1.11 (s)	1.04 (s)	1.08 (s)	1.07 (s)	1.06 (s)
Glc-1	4.80 (d, 8)	4.75 (d, 8)	4.79 (d, 7)	4.69 (d, 8)	4.73 (d, 8)	4.73 (d, 8)
2	3.94 (dd, 8, 9)	3.90 (dd, 8, 9)	3.98 (dd, 7, 9)	3.92 (t, 8)	3.87 (dd, 8. 9)	3.87 (dd, 8, 9)
3	4.19 (t, 9)	4.17 (t, 9)	4.21 (t, 9)	4.19 (m)	4.16 (t, 9)	4.16 (t, 9)
4	4.07 (t, 9)	4.00 (t, 9)	4.12 (t, 9)	3.98 (m)	4.00 (t, 9)	3.96 (t, 9)
5	3.94 (m)	3.97 (m)	3.96 (m)	3.98 (m)	3.95 (ddd, 2, 6, 9)	3.95 (ddd, 1.5, 5, 9)
6	4.28 (dd, 5.5, 11)	4.71 (dd, 5.5, 11.5)	4.30 (dd, 6, 11)	4.66 (dd, 5, 11)	4.69 (dd, 6, 11)	4.67 (dd, 5, 12)
	4.52 (dd, 2.5, 11)	4.92 (dd, 2, 11.5)	4.53 (dd, 3, 11)	4.88 (br d, 11)	4.92 (dd, 2, 11)	4.91 (dd, 1.5, 12)
$-COCH_3$	1.97 (s)	1.93 (s)		2.11 (s)	1.96 (s)	1.94 (s)
		2.05 (s)			2.05 (s)	2.04 (s)

cumingianoside A (**1**); differences were found only in the coupling patterns and the chemical shifts of the hydroxy-bearing methine signals at δ 3.76 (1H, d, J=8 Hz) and δ 4.36 (1H, ddd, J=3, 8, 8 Hz) assignable to H-24 and H-23, respectively, as compared with those [δ 3.57 (1H, br s, H-24), 4.51 (1H, br t, J=7 Hz, H-23)] found in **1**. This observation indicated that the hydroxy groups at C-23 and/or C-24 possess different configuration(s) from those in **1**. The carbon resonances of compound **15**, except for the chemical shifts for the side chain moiety at C-17 (see Table 2), were in good accord with those of **1**. This observation again suggested that **1** and cumingianoside K possess almost identical structures except for the configuration(s) at C-23 and/or C-24. The small quantity of available sample has prevented a definitive determination of the stereochemistries of C-23 and -24; formula **15** indicates this ambiguity.

Cumingianoside L (**12**) gave a ^{1}H-NMR spectrum similar to that of **4**, except for the absence of an oxygen-bearing methine signal assignable to H-3. The negative FAB-MS of **12** exhibited an $(M-H)^-$ ion peak at m/z 651, which was 2 mass units less than that (m/z 653) of **4**. The appearance of a carbonyl carbon resonance (δ 217.3), along with the absence of the hydroxy-carrying C-3 resonance, suggested the presence of a carbonyl group at the C-3 position instead of a hydroxy group. This was further supported by the observation of ^{1}H–^{13}C long-range correlations between the 4-$(CH_3)_2$ and the

Table 2. ^{13}C-NMR Data (δ) for Compounds **1**, **4**—**10**, **12**—**15** in Pyridine-d_5 + D_2O (100 MHz)

	1	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**15**
1	34.5	34.0	34.0	34.4	34.4	34.1	34.5	34.5	34.3	34.4	34.5	34.5
2	23.4	26.6	26.4	23.4	23.4	23.4	23.4	23.4	39.7	39.7	23.4	23.4
3	78.2	75.9	75.6	78.1	78.2	78.1	78.2	78.2	217.3	218.3	78.0	78.2
4	37.0	38.0	37.7	36.9	37.0	37.7	37.8	37.0	47.3	47.4	37.0	37.0
5	41.5	40.4	40.3	41.4	41.2	41.5	41.3	41.5	45.6	45.7	41.4	41.5
6	20.4	21.1	21.5	20.5	20.2	20.7	20.3	20.7	22.5	23.3	20.7	20.7
7	78.1	78.3	79.1	77.9	77.4	78.2	77.4	78.2	78.6	80.0	78.3	78.2
8	35.5	35.7	35.5	35.5	35.5	35.5	35.6	35.5	35.7	35.7	35.6	35.5
9	45.4	45.2	45.2	45.4	45.2	45.3	45.3	45.3	44.4	44.6	45.3	45.3
10	37.7	37.9	37.8	37.7	37.7	37.0	37.1	37.7	36.9	37.0	37.7	37.7
11	17.3	17.5	17.4	17.4	17.2	17.5	17.5	17.5	17.8	18.2	17.4	17.5
12	28.2	28.3	28.3	28.1	28.0	28.1	28.1	28.3	28.2	28.5	28.1	28.3
13	27.3	27.4	27.2	27.4	27.3	27.2	27.3	27.4	27.4	27.4	27.3	27.4
14	39.7	39.6	39.4	39.5	39.4	39.5	39.6	39.5	39.0	39.0	39.4	39.5
15	25.6	25.8	25.5	25.6	25.6	25.7	25.8	26.1	25.6	25.6	25.6	26.1
16	26.1	26.4	26.1	26.4	26.3	26.0	26.4	26.5	26.4	26.3	26.1	26.5
17	53.5	53.7	53.6	53.4	53.5	53.0	53.0	52.8	53.5	53.7	53.4	52.8
18	17.5	17.6	17.6	17.3	17.4	17.2	17.1	17.3	17.9	18.2	17.2	17.3
19	16.3	16.6	16.6	16.2	16.2	16.3	16.2	16.2	16.2	16.6	16.2	16.2
20	33.1	33.3	33.1	33.1	33.2	34.1	34.1	34.3	33.1	33.2	33.7	34.3
21	19.8	19.8	19.7	19.7	19.7	20.4	20.4	20.7	19.9	20.1	19.9	20.7
22	39.4	39.8	39.6	39.7	39.7	38.3	38.4	41.4	39.5	39.8	35.6	41.4
23	69.7	69.7	69.6	69.6	69.6	72.4	72.4	73.9	69.6	69.8	81.0	73.9
24	77.2	77.2	77.0	77.2	77.2	79.2	79.2	80.0	77.2	77.4	73.3	80.0
25	73.7	73.9	73.7	73.8	73.7	147.5	147.5	74.5	73.8	74.1	86.6	74.5
26	27.7	27.8	27.7	27.7	27.7	112.4	112.5	24.6	27.8	27.9	24.1	24.6
27	27.2	27.1	27.1	27.1	27.1	18.9	19.0	29.0	27.1	27.2	28.6	29.0
28	27.7	29.1	29.0	27.8	27.7	27.7	27.7	27.8	26.9	27.2	27.7	27.8
29	22.3	23.0	22.9	22.2	22.2	22.3	22.2	22.3	21.4	21.6	22.2	22.3
30	20.4	20.5	20.4	20.3	20.2	20.3	20.3	20.3	20.3	20.6	20.3	20.3
Glc-1	100.2	100.5	101.1	99.9	99.6	100.3	99.8	100.3	101.3	102.2	100.2	100.3
2	75.0	75.5	75.1	73.1	75.3	74.9	75.3	75.0	75.3	75.1	75.0	75.0
3	78.3	78.4	78.2	79.9	75.7	78.3	78.5	78.3	78.6	78.3	78.2	78.3
4	71.6	72.5	71.5	70.3	73.5	71.6	72.4	71.6	72.4	71.8	71.6	71.6
5	74.7	78.0	74.5	77.8	75.8	74.6	78.0	74.6	78.0	74.7	74.6	74.6
6	64.7	63.5	64.8	62.8	62.8	64.7	63.4	64.7	63.4	65.1	64.7	64.7
$-COCH_3$	20.9		20.9	21.1	21.1	20.9	21.2	20.9		21.2	20.9	20.9
	21.1			21.3	21.2	21.1		21.2			21.1	21.2
$-COCH_3$	170.9		171.4	171.1	170.9	170.9	171.0	171.0		171.5	171.0	171.0
	170.8			170.9	170.9	170.8		170.9			170.8	170.9

carbonyl carbon resonance at δ 217.3. The carbon resonances due to C1, C2, C4, and C5 were shifted downfield [C1 (Δ 0.3 ppm), C2 (Δ 13.1 ppm), C4 (Δ 9.3 ppm), C5 (Δ 5.2 ppm)] as compared with those found in **4**, whereas the other carbon resonances were in good agreement with those found in **4**. Thus, compounds **12** and **4** have almost identical structures with the exception of the carbonyl group at C-3 position in the former. On the basis of these spectral data, the structure of **12** was concluded to be 7α,23(*R*),24(*S*),25-pentahydroxy-14,18-cycloapotirucallanyl-3-one 7-*O*-β-D-(3′-*O*-acetyl)glucopyranoside (**12**).

The ^{1}H-NMR spectrum of cumingianoside M (**13**) was quite similar to that of cumingianoside L (**12**). It also showed a three-proton singlet at δ 2.11, suggestive of an acetoxy group. The $(M-H)^-$ ion peak at *m/z* 693 in the negative FAB-MS of **13**, which was 42 mass units more than that (*m/z* 651) of **12**, was consistent with this finding. Treatment of **13** with 2% NaOMe–MeOH furnished a hydrolysate, which was identified as cumingianoside L (**12**). The ^{1}H- and ^{13}C-NMR chemical shifts and the coupling patterns of the glucosyl signals in **13** were in good accord with those seen in cumingianoside A (**1**), indicating that the acetyl group was bound to the C-6 position in the glucose moiety. Based on these observations, the structure of cumingianoside M was represented by formula **13**.

The negative FAB-MS of cumingianoside N (**9**) exhibited an $(M-H)^-$ ion peak at *m/z* 677. The presence of an exomethylene group in **9** was revealed by two one-proton olefinic signals [δ 5.00, 5.25 (each br s)] in the ^{1}H-NMR spectrum as well as ^{13}C resonances [δ 112.5 (t), 147.5 (s)] that were similar to those of cumingianoside D (**8**). Compound **9** contained an acetyl group, as shown by a three-proton singlet at δ 1.97 in the ^{1}H-NMR spectrum. In the ^{1}H-NMR spectrum of **9**, the glucosyl C-6 methylene proton signals were shifted upfield [δ 4.28 (1H, dd, J = 5.5, 11 Hz) and 4.52 (1H, dd, J = 2.5, 11 Hz)] as compared with those [δ 4.67 (1H, dd, J = 5.5, 11.5 Hz), 4.89 (1H, dd, J = 2.5, 11.5 Hz)] of **8**, while the chemical shifts for the signals due to the aglycone moiety were in good accord with those of **8**. Thus, the location of the acetyl group was concluded to be at the C-3 position, and the structure of cumingianoside N (**9**) was established to be 3-*O*-acetyl-3α,7α,23(*R*),24(*S*)-tetrahydroxy-14,18-cycloapotirucall-25-enyl 7-*O*-β-D-glucopyranoside (**9**).

Table 3. Cytotoxicity (EC_{50} in μM) of Compounds **4**, **5**, **9**, **12**, **13** and **15** against Human Cancer Cell Lines *in Vitro*

	4	**5**	**15**	**12**	**13**	**9**
Panel/cell line						
Leukemia						
CCRF-CEM	>10	>10	>10	>10	4.13	>10
HL-60 (TB)	>10	>10	9.45	>10	2.25	>10
K-562	>10	>10	9.12	>10	5.52	>10
MOLT-4	>10	>10	>10	>10	3.71	>10
RPMI-8226	>10	>10	5.18	>10	3.57	>10
SR	NT	NT	NT	4.34	>10	NT
Non-small cell lung cancer						
A549/ATCC	>10	>10	>10	>10	>10	>10
EKVX	>10	>10	>10	>10	>10	>10
HOP-62	>10	>10	>10	>10	>10	>10
HOP-92	>10	>10	9.45	>10	3.18	>10
NCI-H226	>10	>10	>10	>10	>10	>10
NCI-H23	>10	>10	>10	>10	>10	>10
NCI-H322M	>10	>10	>10	>10	>10	>10
NCI-H460	>10	>10	>10	>10	>10	>10
NCI-H522	>10	>10	>10	>10	>10	>10
Colon cancer						
COLO 205	>10	>10	>10	>10	5.84	>10
HCC-2998	>10	>10	>10	>10	>10	>10
HCT-116	>10	>10	>10	>10	7.09	>10
HCT-15	>10	>10	>10	>10	>10	>10
HT29	>10	>10	>10	>10	4.22	>10
KM12	>10	>10	>10	>10	6.42	>10
SW-620	>10	>10	>10	>10	>10	>10
CNS cancer						
SF-268	>10	>10	>10	>10	>10	>10
SF-295	>10	>10	>10	>10	>10	>10
SF-539	>10	>10	>10	>10	>10	>10
SNB-19	>10	>10	>10	>10	>10	>10
SNB-75	>10	>10	>10	>10	5.34	>10
U251	>10	>10	>10	>10	8.13	>10
Melanoma						
LOX IMVI	>10	>10	>10	>10	3.71	>10
MALME-3M	>10	>10	>10	>10	>10	>10
M14	>10	>10	>10	>10	>10	>10
SK-MEL-2	>10	>10	5.64	>10	4.93	>10
SK-MEL-28	>10	>10	>10	>10	3.25	>10
SK-MEL-5	>10	>10	>10	>10	7.65	>10
UACC-257	>10	>10	>10	>10	>10	>10
UACC-62	>10	>10	>10	>10	>10	>10
Ovarian cancer						
IGROV1	>10	>10	>10	>10	>10	>10
OVCAR-3	>10	>10	>10	>10	>10	>10
OVCAR-4	>10	>10	>10	>10	>10	>10
OVCAR-5	>10	>10	>10	>10	>10	>10
OVCAR-8	>10	>10	>10	>10	>10	>10
SK-OV-3	>10	>10	>10	>10	>10	>10
Renal cancer						
786-0	>10	>10	>10	>10	>10	>10
A498	>10	>10	>10	>10	>10	>10
ACHN	>10	>10	>10	>10	>10	>10
CAKI-1	>10	>10	>10	>10	>10	>10
RXF-393	>10	>10	>10	>10	>10	>10
SN12C	>10	>10	>10	>10	>10	>10
TK-10	>10	>10	>10	>10	>10	>10
UO-31	>10	>10	>10	>10	>10	>10
Prostate cancer						
PC-3	>10	>10	>10	>10	9.81	>10
DU-145	>10	>10	>10	>10	>10	>10
Breast cancer						
MCF7	>10	>10	6.61	>10	4.78	>10
MCF7/ADR-RES	>10	>10	>10	>10	>10	>10
MDA-MB-231/ATCC	>10	>10	>10	>10	6.44	>10
HS 578T	>10	>10	>10	>10	>10	>10
MDA-MB-435	>10	>10	>10	>10	9.00	>10
MDA-N	>10	>10	>10	>10	>10	>10
BT-549	>10	>10	>10	>10	>10	>10
T-47D	>10	>10	>10	>10	5.68	>10

NT: not tested.

The negative FAB-MS of cumingianoside O (**14**) showed an $(M-H)^-$ ion peak at *m/z* 719. Compound **14** possesses two acetyl groups as revealed by ^{1}H-NMR signals at δ 1.96 and 2.05 (each 3H, s). The presence of an epoxy group in **14** was suggested by ^{13}C-NMR examinations combined with the elemental analysis data ($C_{40}H_{64}O_{11}$). Comparison of the ^{13}C resonances of **14** with those found in cumingianoside E (**10**), which possesses an epoxy group at C24–C25, indicated that the epoxy group was present in the C17 side chain. On acetylation with acetic acid and pyridine, **14** yielded a hexaacetate (**14a**), which exhibited the $(M+Na)^+$ ion peak at *m/z* 911. The ^{1}H-NMR spectrum of **14a** showed methine signals at δ 4.98 (br t, $J=6.5$ Hz) and 5.34 (d, $J=6.5$ Hz), assignable to H-23 and H-24, respectively, based on ^{1}H–^{1}H COSY examination, and the latter was shifted downfield compared with both methine signals [δ 4.98 (br t, $J=6.5$ Hz, H-23), 4.68 (d, $J=6.5$ Hz, H-24)] found in **14**. This observation indicated that the epoxy group in **14** was present at C23–C25. The locations of the acetyl groups were confirmed to be at the C-3 and the glucosyl C-6 hydroxy groups, since in the ^{13}C-NMR spectrum of **14**, the carbon resonances due to C-3 and glucosyl C-6 coincided with those of **10**. On the basis of these observations, the structure of **14** was concluded to be 3-*O*-acetyl-3α,7α,24-trihydroxy-23,25-epoxy-14,18-cycloapotirucallanyl 7-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (**14**). However, owing to the small amount of sample, the absolute configurations of C-23 and -24 remain to be determined.

The cytotoxic activities of cumingianosides G (**4**), H (**5**), K (**15**), L (**12**), M (**13**), and N (**9**) against 59 human cancer cell lines *in vitro* are summarized in Table 3. Among these compounds, cumingianoside M (**13**) showed significant (<4 μM) cytotoxicity, especially against leukemia (MOLT-4: 3.71 μM; RPMI8226: 3.57 μM; HL60TB: 2.25 μM) and melanoma (LOXIMV1: 3.71 μM; SK-MEL-28: 3.25 μM) cell lines. Cumingianosides K (**15**) and L (**12**) showed cytotoxicity only at relatively high concentrations (cumingianoside K (**15**); leukemia cell lines: RPMI8226: 5.18 μM; HL-60TB: 9.45 μM; K-562: 9.12 μM; melanoma cells: SK-MEL-2: 5.64 μM; cumingianoside L (**12**); leukemia cells: SR: 4.34 μM), while the other compounds (**4**, **5**, **9**) showed no cytotoxicity (>10 μM). Except for **5**, the non-cytotoxic cumingianosides do not possess an acetyl group at the glucosyl C-6 position; this observation is consistent with previous results.[2b] Changing the C-3 acetoxy groups in **1** to a carbonyl group, as seen in cumingianoside M (**13**), drastically decreased the cytotoxicity (for example, compare the corresponding EC_{50} values in MOLT-4 cells: **1** <0.00625 μM, **13** 3.71 μM), indicating that the acetoxy group at C-3 may also play an important role in the cytotoxicity. This is consistent with the observation that cumingianoside H (**5**) had no cytotoxicity, although it does possess an acetyl group at the glucosyl C-6 position. The weak cytotoxicity found with cumingianoside K (**15**), which differs in structure from cumingianoside A (**1**) only in the configurations of the hydroxy groups at C-23 and/or C-24, suggested that the stereochemistry of C-23 and C-24 might be important for potent cytotoxicity.

Experimental

The instruments and materials used in this work were as follows: Yazawa micro melting point BY-1 (melting point, uncorrected), JASCO DIP-4 digital polarimeter (specific rotations), JEOL JNM GX-400 spectrometer (100 MHz for ^{13}C-NMR spectra and 400 MHz for ^{1}H-NMR spectra), JEOL JMS HX-110 mass spectrometer (mass spectra), Kieselgel 60 (63—210 μm, E. Merck), Silica gel 60 spherical (70—230 μm, Nakalai Tesque), MCI gel CHP 20P (75—150 μm), Cosmosil 140C18 Prep (Nakalai Tesque), Fuji-gel Prep-Paksil (40—63 μm, packed column, 400 × 50 mm i.d., Fuji Gel), precoated Kieselgel 60 F_{254} plates (E. Merck), precoated HPTLC RP-18 F_{254} plates (E. Merck). HPLC was performed on YMC ODS (5 μm, 250 × 20 mm i.d., YMC Co., Ltd.). ^{1}H- and ^{13}C-NMR spectra were measured in pyridine-d_5 containing D_2O. Chemical shifts are expressed on the δ scale with tetramethylsilane as an internal standard. The signal assignment was based on comparison with data reported for compounds having similar structures, and confirmed with the aid of NMR spectral techniques (^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, nuclear Overhauser effect (NOE) spectroscopy (NOESY), NOE difference and long-range ^{1}H–^{13}C COSY spectra).

Extraction and Isolation The air-dried leaves of *Dysoxylum cumingianum* (8.7 kg), collected in Taiwan, were extracted with MeOH at room temperature. The MeOH solution was concentrated under reduced pressure to give the extract (1980 g), a part of which (549.7 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 and 90% aqueous MeOH to give fraction A (127 g) and fraction B (294.4 g). The aqueous layer was subsequently extracted with *n*-BuOH, yielding fractions C (4 g) and D (85.9 g). Fraction B (294.4 g) was subjected to chromatography over silica gel with $CHCl_3$ containing increasing amounts of MeOH to give 6 fractions: frs. 1 (11.7 g), 2 (41.9 g), 3 (14.5 g), 4 (38.5 g), 5 (33.8 g), and 6 (23.6 g). Repeated chromatography of fr. 2 on Cosmosil 140C18 Prep [MeOH–H_2O (4:1→1:0)], and MCI gel CHP20P [MeOH–H_2O (4:1→1:0)], followed by semi-preparative scale HPLC on a YMC ODS column (70% CH_3CN) gave **3** (1.1 g) and **11** (230 mg). Fraction 3 was further chromatographed on Cosmosil 140C18 Prep [MeOH–H_2O (3:2→1:0)] and then on MCI gel CHP20P [MeOH–H_2O (3:2→1:0)] to afford cumingianoside Q (**19**) (1.6 g) and a mixture of **1** and **7**. The mixture was separated by HPLC on a YMC ODS column (60% CH_3CN), yielding **1** (15 g) and **7** (10 mg). Fraction 4 was chromatographed on Cosmosil 140C18 Prep [MeOH–H_2O (3:2→1:0)] to furnish two fractions (frs. 4-1 and 4-2). Chromatography of fr. 4-1 on MCI gel CHP20P [MeOH–H_2O (3:2→1:0)] gave **13** (100 mg) and **9** (20 mg). Cosmosil 140C18 Prep chromatography [MeOH–H_2O (7:3→1:0) of fr. 5 yielded two fractions (frs. 5-1 and 5-2). Repeated chromatography of fr. 5-1 on MCI gel CHP20P [MeOH–H_2O (3:2→1:0)] and silica gel [$CHCl_3$–MeOH (50:1→5:1)] afforded **2** (1.6 g), while MCI gel CHP20P chromatography [MeOH–H_2O (4:1→1:0)] of fr. 5-2 afforded **5** (1.1 g). Fraction 6 was chromatographed on Cosmosil 140C18 Prep [MeOH–H_2O (7:1→1:0)] to give three fractions (frs. 6-1, 6-2, 6-3). Fraction 6-1 was chromatographed repeatedly on MCI gel CHP20P [MeOH–H_2O (4:1→1:0)] and silica gel [$CHCl_3$–MeOH (10:1→5:1)] to give **12** (40 mg). Fraction 6-2 was chromatographed on Fuji gel Prep-Paksil [$CHCl_3$–MeOH (25:1→3:1)] to give **4** (110 mg) and a mixture of **6** and **15**. MCI gel CHP20P [MeOH–H_2O (4:1→1:0)] chromatography of the mixture, followed by HPLC on a YMC ODS column (70% CH_3CN) furnished pure samples [**6** (11.5 mg), **15** (4 mg)]. Fraction 6-3 was chromatographed on MCI gel CHP 20P [MeOH–H_2O (4:1→1:0)] to give two fractions (frs. 6-3-1, 6-3-2). Fraction 6-3-1 was chromatographed on silica gel [$CHCl_3$–MeOH (50:1→10:1)], giving **8** (80 mg) and a mixture of **10** and **14**, which was subsequently separated by HPLC on a YMC ODS column (60% CH_3CN) to afford **10** (10 mg) and **14** (5 mg). Fraction 6-3-2 was chromatographed on silica gel [$CHCl_3$–MeOH (50:1→5:1)] and then subjected to HPLC on a YMC ODS column (65% CH_3CN) to give cumingianoside P (**18**) (100 mg).

General Procedures of Hydrolysis with 2% NaOMe–MeOH The sample (50—200 mg) in 2% NaOMe–MeOH (5—10 ml) was left at room temperature overnight. Then, the reaction mixture was neutralized with IR-120B resin, filtered, and concentrated to dryness. The residue was purified by silica gel chromatography [$CHCl_3$–MeOH (50:1→5:1)] to give a product, which was characterized by physical and spectral comparison with an authentic sample.

Cumingianoside H (**5**): White amorphous powder, $[\alpha]_D^{25}$ −31.9° ($c=0.52$, $CHCl_3$), Positive FAB-MS *m/z*: 719 ($[M+Na]^+$), Negative

FAB-MS *m/z*: 695 ([M−H]$^-$), High-resolution FAB-MS Calcd for $C_{38}H_{64}NaO_{11}$: *m/z* 719.4346, Found *m/z*: 719.4345; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside I (**6**): White amorphous powder, $[\alpha]_D^{25}$ −33.5° (*c*=0.20, $CHCl_3$), 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*: 761.4451; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside J (**7**): White amorphous powder, $[\alpha]_D^{25}$ −38.8° (*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*: 761.4451; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside K (**15**): White amorphous powder, $[\alpha]_D^{25}$ −33.3° (*c*=0.58, $CHCl_3$), Positive FAB-MS *m/z*: 739 ([M+H]$^+$), 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*: 761.4430; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside L (**12**): Colorless needles (dil. MeOH), mp 145—148 °C, $[\alpha]_D^{25}$ −14.1° (*c*=0.52, $CHCl_3$), Positive FAB-MS *m/z*: 675 ([M+Na]$^+$), Negative FAB-MS *m/z*: 651 ([M−H]$^-$), High-resolution FAB-MS Calcd for $C_{36}H_{60}NaO_{10}$: *m/z* 675.4084, Found *m/z*: 675.4082; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside M (**13**): White amorphous powder, $[\alpha]_D^{25}$ −10.5° (*c*=0.7, $CHCl_3$), Positive FAB-MS *m/z*: 717 ([M+Na]$^+$), Negative FAB-MS *m/z*: 693 ([M−H]$^-$), High-resolution FAB-MS Calcd for $C_{38}H_{62}NaO_{11}$: *m/z* 717.4190, Found *m/z*: 717.4190; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside N (**9**): White amorphous powder, $[\alpha]_D^{25}$ −40.3° (*c*=0.59, $CHCl_3$), Positive FAB-MS *m/z*: 701 ([M+Na]$^+$), Negative FAB-MS *m/z*: 677 ([M−H]$^-$), High-resolution FAB-MS Calcd for $C_{38}H_{62}NaO_{10}$: *m/z* 701.4240, Found *m/z*: 701.4244; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside O (**14**): White amorphous powder, $[\alpha]_D^{25}$ −20.5° (*c*=0.10, $CHCl_3$), Positive FAB-MS *m/z*: 743 ([M+Na]$^+$), Negative FAB-MS *m/z*: 719 ([M−H]$^-$), High-resolution FAB-MS Calcd for $C_{40}H_{64}NaO_{11}$: *m/z* 743.4346, Found *m/z*: 743.4322; ^{1}H-NMR: Table 1, ^{13}C-NMR: Table 2.

Cumingianoside O Acetate (**14a**): **14** (1.7 mg) was treated with acetic anhydride (Ac_2O) (0.2 ml) and pyridine (C_5H_5N) (0.1 ml) at room temperature overnight. The reaction mixture was worked up as usual, and was chromatographed on silica gel [$CHCl_3$–MeOH (20:1)] to furnish **14a** (1.2 mg) as a white amorphous powder. Positive FAB-MS *m/z* 911 [M+Na]$^+$; ^{1}H-NMR (400 MHz, pyridine-d_5+D_2O) δ: 0.85, 0.86, 0.94, 1.06, 1.40, 1.55 (each 3H, s, CH_3×6), 1.09 (1H, d, *J*=6 Hz, H-21), 2.00—2.30 (each 3H, s, OAc×6), 4.86 (1H, br s, H-3), 4.03 (1H, br s, H-7), 4.15 (1H, ddd, *J*=2, 4.5, 9 Hz, glucosyl H-5), 4.50 (1H, dd, *J*=2, 12 Hz, glucosyl H-6), 4.55 (1H, dd, *J*=4.5, 12 Hz, glucosyl H-6′), 4.98 (br t, *J*=6.5 Hz, H-23), 5.00 (1H, d, *J*=7 Hz, glucosyl H-1), 5.34 (1H, d, *J*=6.5 Hz, H-24), 5.45 (1H, dd, *J*=7, 9 Hz, glucosyl H-2), 5.49 (1H, t, *J*=9 Hz, glucosyl H-4), 5.72 (1H, t, *J*=9 Hz, glucosyl H-3).

Biological Assay The *in vitro* cytotoxicity assay was carried out using the National Cancer Institute protocol. Details of the assay procedure have been reported.[6]

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3) Because the configuration of C-20 in the cumingianosides was not clear, the name of the 14,18-cycloapoeuphane-type skeleton used in ref. 2*a* and 2*b* was based on ref. 4. Since the configuration of C-20 has now been determined to be *S* by X-ray analysis,[2c] the skeleton type should be revised to 14,18-cycloapotirucallane.
4) Dev S., Nagasampagi B. A., "CRC Handbook of Terpenoids, Triterpenoids," Vol. I, CRC Press Inc., Boca Raton, Florida, 1989, pp. 32—33.
5) In ref. 2*b*, the ED_{50} values were shown incorrectly as μg/ml.
6) Boyd M. B., Paul K. D., *Drug. Development Res.*, **34**, 91—109 (1995).