Flavonol Glycosides and Steroidal Saponins from the Leaves of *Cestrum nocturnum* and Their Cytotoxicity

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Phytochemical analysis of the leaves of *Cestrum nocturnum* (Solanaceae) resulted in the isolation of two new flavonol glycosides (**1**, **2**) and seven steroidal saponins (**3**−**9**), including four new ones (**4**, **6**, **7**, and **9**). The structures of the new compounds were determined by spectroscopic analysis, including 2D NMR data, and the results of hydrolytic cleavage. Cytotoxic activities of the isolated compounds against human oral squamous cell carcinoma-(HSC-2) cells and normal human gingival fibroblasts are reported.

The genus *Cestrum* contains more than 300 species, and most of them are native to warm subtropical and tropical areas of America. *Cestrum nocturnum* L. (Solanaceae), the subject of this report, is an evergreen shrub indigenous to South America and the West Indies and is now cultivated in many parts of the world for ornamental purposes. Interestingly, the flowers of this plant are fragrant only at night, hence, its species name. The flower's volatile compounds were identified as phenylacetylaldehyde and linalool.[1] The leaves of *C. nocturnum* have pharmacological significance in Chinese folk medicine and have been used for the treatment of burns and swellings, being applied externally.[2] A few glycosides such as (25*R*)-spirost-5-ene-2α,3*β*-diol pentaglycosides (nocturnoside A),[3] (25*R*)-spirost-5-en-3*β*-ol tetraglycoside (nocturnoside B),[4] and phenolic glucosides (cesternosides A and B)[5] were isolated from the leaves of *C. nocturnum*. However, studies of the plant to date have been fragmentary, and there has been no systematic exploration of its secondary metabolites. Our detailed investigation of *C. nocturnum* leaves led to the isolation of two new flavonol glycosides (**1**, **2**) and seven steroidal saponins (**3**−**9**), including four new ones (**4**, **6**, **7**, and **9**). In this paper, we report the identification of these compounds and their cytotoxic activity against human oral squamous cell carcinoma (HSC-2) cells and normal human gingival fibroblasts (HGF).

Results and Discussion

Fresh leaves of *C. nocturnum* (1.2 kg) were extracted with MeOH at room temperature for 7 days. After removal of solvent, the crude extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column and divided into H_2O−MeOH (7:3), MeOH, and EtOAc eluate fractions. The MeOH fraction was subjected to Si gel and ODS Si gel column chromatography and gel filtration using Sephadex LH-20, to give two new flavonol glycosides (**1**, **2**) and seven steroidal saponins (**3**−**9**).

The positive-ion FABMS of **1** showed pseudomolecular ion peaks at *m*/*z* 999 $[M + Na]^+$ and 977 $[M + H]^+$, whereas the negative-ion FABMS gave an $[M - H]^-$ ion at *m*/*z* 975. These data, elemental analysis, the ^{13}C NMR spectrum showing a total of 45 signals, and DEPT data indicated the molecular formula of **1** to be $C_{45}H_{52}O_{24}$. The IR spectrum showed absorption bands of hydroxyl, conjugated carbonyl, and aromatic groups. The ^1H NMR spectrum contained signals for *p*-disubstituted aromatic protons (*δ* 8.56 and 7.40), symmetrically tetrasubstituted aromatic protons (*δ* 6.80), *m*-coupled aromatic protons (*δ* 6.50 and 6.48), a pair of *trans*-alkene protons (*δ* 7.79 and 6.51), three methoxyl protons (*δ* 3.77 × 2 and 3.67), and three anomeric protons (*δ* 6.32, 5.61, and 5.16). Alkaline methanolysis of **1** with 3% NaOMe in MeOH yielded a deacyl derivative (**1a**) and methyl (*E*)-3,5-dimethoxy-4-hydroxycinnmate. The UV spectrum of **1a** suggested a flavone or flavonol derivative.[8] Acid hydrolysis of **1a** with 1 M HCl in dioxane−H_2O (1:1) yielded 4′,5-dihydroxy-7-methoxyflavonol[9] as the aglycon and D-glucose and L-rhamnose as the carbohydrate components. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded Si gel column using MeCN−H_2O (3:1) as solvent. To determine the sugar sequence of **1**, spectral analysis was carried out with the deacyl derivative (**1a**). The negative-ion FABMS of **1a** showed fragment ion peaks at *m*/*z* 623 and 607, the former was assignable to the loss of a rhamnosyl group from the parent ion at *m*/*z* 769 $[M - H]^-$, and the latter corresponded to the loss of a glucosyl group. This was suggestive of a branched triglycoside moiety. $^1H-^1H$ COSY experiments allowed the sequential assignments of the signals of each monosaccharide. HMQC was applied to associate the protons with the corresponding carbon resonances. Comparison of the carbon chemical shifts thus assigned, with those of reference methyl glycosides,[10] taking into account the known effects of *O*-glycosylation, indicated that **1a** contained a terminal *β*-D-glucopyranosyl unit; a 2,6-disubstituted *β*-D-glucopyranosyl unit; and a terminal α-L-rhamnopyranosyl unit. The *β*-orientations of the anomeric centers of the two glucosyl moieties were supported by the relatively large *J* values of their anomeric protons. The ^{13}C NMR shifts of C-3 and C-5 indicated the α-anomeric orientation of the rhamnopyranosyl residue.[10] In the HMBC spectrum, correlation peaks were observed from *δ* 6.47 (anomer proton of substituted glucosyl) to *δ* 134.5 (C-3 of aglycon), *δ* 5.61

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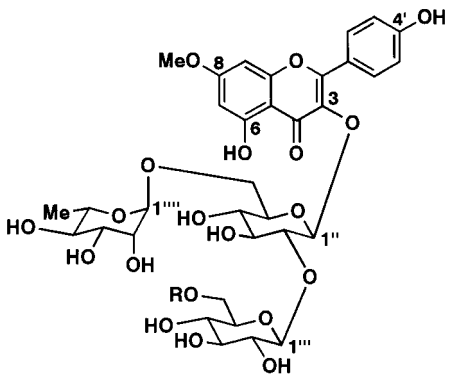
(anomer proton of terminal glucosyl) to δ 83.8 (C-2 of substituted glucosyl), and δ 5.18 (anomer proton of rhamnosyl) to δ 67.5 (C-6 of substituted glucosyl). Thus, the glucosyl-(1→2)-[rhamnosyl-(1→6)]-glucosyl structure and its linkage to C-3 of the aglycon were revealed. Finally, our attention was focused on the ester linkage position of (*E*)-3,5-dimethoxy-4-hydroxycinnamic acid. When the ^{1}H and ^{13}C NMR spectra of **1** were compared with those of **1a**, downfield shifts due to *O*-acylation were detected at the H_2-6 (δ 5.05) and C-6 (δ 64.5) of the terminal glucosyl residue. Furthermore, an HMBC correlation was observed from δ 5.05 to the carbonyl carbon resonance of the acyl group at δ 167.5. All of these data for **1** were consistent with the structure 4′,5-dihydroxy-7-methoxyflavonol 3-*O*-[6-*O*-(*E*)-3,5-dimethoxy-4-hydroxycinnamoyl-β-D-glucopyranosyl]-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **2** was shown to have the molecular formula $C_{33}H_{40}O_{19}$ by positive-ion and negative-ion FABMS (*m*/*z* 763 $[M + Na]^+$ and 739 $[M - H]^-$), ^{13}C NMR with DEPT data, and elemental analysis. The ^{1}H NMR spectrum of **2** showed signals for *p*-disubstituted aromatic protons (δ 8.55 and 7.36), *m*-coupled aromatic protons (6.57 and 6.53), and methoxyl protons (δ 3.69). These signals were attributable to the aglycon moiety and were very similar to those of **1a**. In addition, three anomeric protons were noted (δ 6.42, 5.66, and 5.21). Acid hydrolysis of **2** with 1 M HCl gave 4′,5-dihydroxy-7-methoxyflavonol, and D-glucose, D-xylose, and L-rhamnose as the carbohydrate moieties. The fragment ion peaks observed in the negative-ion FABMS at *m*/*z* 607 and 593 were assigned to $[M - \text{xylosyl} - H]^-$ and $[M - H - \text{rhamnosyl}]^-$, respectively, suggesting that **2** had a branched triglycoside unit. The ^{13}C NMR shifts of the sugar part, which were assigned by interpretation of the ^{1}H−^{1}H COSY spectrum followed by HMQC data, indicated that it was composed of a terminal β-D-xylopyranosyl, a 2,6-disubstituted β-D-glucopyranosyl, and a terminal α-L-rhamnopyranosyl unit. These findings implied that **2** differed from **1a** only by the monosaccharide attached to C-2 of the inner glucosyl group; that is, the terminal glucose was replaced by β-D-xylopyranose in **2**. This was confirmed by an HMBC correlation between the signals of the anomeric proton of the xylose moiety at δ 5.66 and C-2 of the inner glucose at δ 82.4. Thus, **2** was identified as 4′,5-dihydroxy-7-methoxyflavonol 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **4** was assigned the molecular formula $C_{50}H_{80}O_{24}$ using positive-ion and negative-ion FABMS (*m*/*z* 1087 $[M + Na]^+$ and 1063 $[M - H]^-$), ^{13}C NMR with DEPT data, and elemental analysis. The ^{1}H NMR spectrum of **4** showed two three-proton singlet signals (δ 0.97 and 0.95) and two three-proton doublets (δ 1.23 and 0.69), which were recognized as typical steroid methyls. Signals for four anomeric protons (δ 5.58, 5.26, 5.22, and 4.92) were also observed. Acid hydrolysis of **4** with 1 M HCl liberated D-glucose, D-galactose, and D-xylose. The ^{13}C NMR shifts of **4** were essentially the same as those of **3**.[6] However, the molecular formula of **4** had one more oxygen than did **3**, and the presence of a hydroxyl group at C-17 was suggested by the appearance of a quaternary carbon signal at δ 90.1, along with downfield shifts of the signals due to C-13 (+4.7 ppm), C-16 (+8.9 ppm), and C-20 (+2.8 ppm), when the ^{13}C NMR spectrum of **4** was compared with that of **3**. This was confirmed by two- or three-bond coupled ^{1}H/^{13}C correlations from the carbon signal at δ 90.1 to H-15α [δ 2.20 (1H, m)], H-16 [δ 4.46 (1H, t-like, J = 6.9 Hz)], Me-18 [δ 0.95 (3H, *s*)], H-20, and Me-21. An NOE correlation between Me-18 and H-20 indicated α-orientation of the C-17 hydroxyl group. The structure of **4** was thus formulated to be (25*R*)-2α,17α-dihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.



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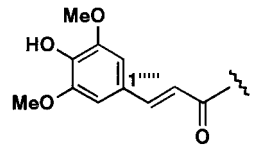

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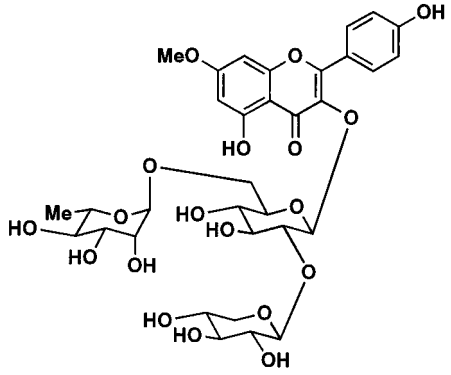

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Compound **6** ($C_{56}H_{90}O_{29}$) was isolated as an amorphous powder. The ^{1}H NMR spectrum of **6** showed signals for five anomeric protons (δ 5.58, 5.20, 5.17, 5.12, and 4.92), together with signals for four steroid methyls (δ 1.27, 1.13, 0.98, and 0.72). Acid hydrolysis of **6** with 1 M HCl gave an aglycon (**10**), D-glucose, D-galactose, and D-xylose. The NMR data of **10** suggested that it was a (25*R*)-spirost-5-en derivative with one more hydroxyl function in addition to the C-2α and C-3β hydroxyl groups. The ^{13}C NMR spectrum of **10** showed three hydroxymethine carbon signals (δ 76.9, 72.7, and 70.1) and δ 76.9 and 72.7 resonances readily assigned to C-3 and C-2, respectively, by comparison with reported ^{13}C NMR data.[11] The signal at δ 70.1 was correlated to a proton signal at δ 4.37 (1H, dd, J = 5.2, 3.9 Hz) in the HMQC spectrum, which in turn showed long-range correlations with C-13 (δ 40.7) and C-17 (δ 62.0) in the HMBC spectrum. Furthermore, downfield shifts of C-14 (+4.0 ppm), C-16 (+2.5 ppm), and C-18 (+2.6 ppm) were observed when the ^{13}C NMR spectrum of **10** was compared with that of (25*R*)-spirost-5-ene-2α,3β-diol.[11] Thus, a C-15 hydroxyl group in **10** was evident. In the NOESY spectrum of **10**, NOE correlations were observed from H-15 to H-14 [δ 1.10 (1H, dd, J = 10.8, 3.9 Hz)], H-16 [δ 4.49 (1H, dd, J = 8.5, 5.2 Hz)], and both H-7α [δ 2.43 (1H, m)] and H-7β

[δ 1.73 (1H, t-like, J = 10.8 Hz)]. This confirmed the β-configuration of the C-15 hydroxyl group. Thus, **10** was characterized as (25*R*)-spirost-5-ene-2α,3β,15β-triol, which is a new steroidal sapogenin.

The structure of pentaglycoside moiety of **6** was considered to be the same as that of the known compound **5**.[3] However, it seemed that there were several mistakes in assignment of the ^{13}C NMR signals in the literature.[3] We established unambiguous assignments of the anomeric proton signals and all of the carbon signals of the pentaglycoside moiety by using HSQC−TOCSY techniques and varying the mixing times from 20 to 100 ms at 20-ms intervals. At 20 ms of mixing time, relayed cross-peaks from each anomeric proton were observed at the C-1 and C-2 carbons. At 100 ms, cross-peaks extended to the terminal carbon of each monosaccharide (C-5 for xylose and C-6 for glucose) except for the galactose originating signals. On the H-1 track of the galactose, relayed-peaks terminated at C-4, but two remaining signals at δ 75.6 (CH) and 60.6 (CH_2) were reasonably assigned to C-5 and C-6, respectively. Based on the above analysis, the ^{13}C NMR assignment of the pentaglycoside moiety was completed as shown in Table 1. The sugar sequence and its linkage position to C-3 of the aglycon of **6** were ascertained to be the same as those of **5** by the observation of the HMBC correlation from each anomeric proton across the glycosidic bond to the carbon of the other substituted monosaccharide or the aglycon. The structure of **6** was determined to be (25*R*)-2α,15β-dihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **7** ($C_{56}H_{90}O_{29}$) was a new combination of a known steroidal sapogenin and sugar. The prominent ^{1}H NMR signals due to the aglycon moiety at δ 1.22, 0.97, 0.94, 0.69, and 5.30 were superimposable on those of **4**, whereas the signals due to the anomeric protons (δ 5.57, 5.20, 5.17, 5.11, and 4.90) were consistent with those of **5** and **6**. The ^{13}C NMR data and the result of acid hydrolysis, as well as the above ^{1}H NMR data, indicated the structure of **7** to be (25*R*)-2α,17α-dihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

The ^{1}H NMR spectrum of compound **9** ($C_{51}H_{82}O_{21}$) contained four anomeric proton signals (δ 6.41, 6.29, 5.83, and 5.00), and four steroid methyl proton signals (δ 1.14, 1.09, 0.82, and 0.70). Acid hydrolysis of **9** with 1 M HCl yielded (25*R*)-spirost-5-ene-2α,3β-diol,[11] D-glucose, and L-rhamnose. The ^{13}C NMR signals arising from the tetraglycoside moiety composed of three α-L-rhamnosyl and one β-D-glucosyl units were in good agreement with those of **8**.[7] Thus, **9** was identified as (25*R*)-2α-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside.

The isolated compounds were evaluated for their cytotoxic activity against HSC-2 cells and HGF (Table 2). The steroidal saponins, especially **5**−**9**, exhibited considerable cytotoxicity against HSC-2 cells, with LD_{50} values ranging from 2.0 μg/mL to 13 μg/mL, whereas the flavonol glycosides (**1**, **2**) were not cytotoxic. It is noteworthy that **5**−**7** showed 5−10-fold higher cytotoxic activities against HSC-2 cells than against normal HGF. In contrast, **8** and **9** were cytotoxic against both HSC-2 cells and HGF. Thus, the structure of the sugar portion appears to play an important role in the tumor-specific cytotoxicity of these steroidal saponins.

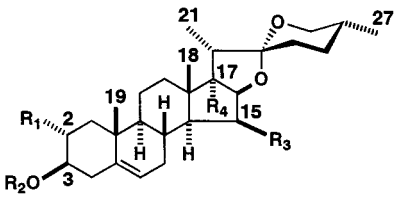
Table 1. ^{13}C NMR Data for Compounds **4**, **6**, **7**, and **9**[a]

C	**4**	**6**	**7**	**9**
1	45.7	45.7	45.8	46.0
2	70.0	70.1	70.1	70.2
3	84.6	84.6	84.6	85.2
4	37.6	37.6	37.6	37.2
5	140.1	140.3	140.1	140.0
6	121.9	122.0	122.0	122.0
7	32.3	31.6	32.3	32.2
8	32.0	27.5	32.0	31.2
9	50.1	50.5	50.1	50.2
10	37.9	38.2	37.9	38.0
11	21.0	21.5	21.0	21.2
12	32.1	42.3	32.1	39.8
13	45.1	40.6	45.1	40.5
14	52.9	60.5	52.9	56.5
15	31.7	70.1	31.8	32.3
16	90.0	83.5	90.0	81.1
17	90.1	61.9	90.1	62.9
18	17.1	19.0	17.1	16.3
19	20.5	20.3	20.5	20.4
20	44.8	42.9	44.8	42.0
21	9.8	14.7	9.8	15.0
22	109.8	110.1	109.8	109.3
23	31.7	31.4	31.8	31.9
24	28.8	29.2	28.8	29.3
25	30.4	30.6	30.4	30.6
26	66.7	67.2	66.7	66.9
27	17.3	17.4	17.3	17.3
1′	103.3	103.3	103.3	101.2
2′	72.7	72.7	72.7	77.8
3′	75.5	75.4	75.4	77.7
4′	79.3	79.1	79.0	77.8
5′	75.7	75.6	75.7	77.2
6′	60.5	60.6	60.6	61.0
1″	104.7	103.9	104.0	102.1
2″	81.3	80.6	80.6	72.5
3″	87.0	87.0	87.0	72.8
4″	70.4	70.4	70.4	74.1
5″	77.6	77.5	77.5	69.6
6″	62.9	62.9	62.9	18.6
1‴	104.8	104.4	104.4	102.3
2‴	76.1	74.7	74.7	72.9
3‴	78.1	87.8	87.8	73.3
4‴	71.3	69.7	69.7	80.4
5‴	78.4	77.8	77.8	68.4
6‴	62.7	62.4	62.3	18.9
1⁗	104.9	105.4	105.4	103.3
2⁗	75.1	75.6	75.6	72.7
3⁗	78.7	78.0	78.0	72.9
4⁗	70.8	71.6	71.5	74.0
5⁗	67.3	78.4	78.4	70.4
6⁗		62.5	62.5	18.5
1′′′′′		104.9	104.9	
2′′′′′		75.2	75.2	
3′′′′′		78.5	78.5	
4′′′′′		70.7	70.7	
5′′′′′		67.2	67.2	

[a] Spectra were measured in C_5D_5N.

Table 2. Cytotoxic Activity of Compounds **1**−**9** against HSC-2 Cells and HGF

compounds	LD_{50} (μg/mL)	
	HSC-2	HGF
1	>400	>400
2	>400	>400
3	12	37
4	13	58
5	2.7	31
6	4.4	22
7	5.5	34
8	2.0	2.8
9	5.5	9.1



	R_1	R_2	R_3	R_4
3	OH	S-1	H	H
4	OH	S-1	H	OH
5	OH	S-2	H	H
6	OH	S-2	OH	H
7	OH	S-2	H	OH
8	H	S-3	H	H
9	OH	S-3	H	H

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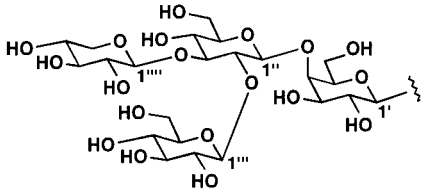

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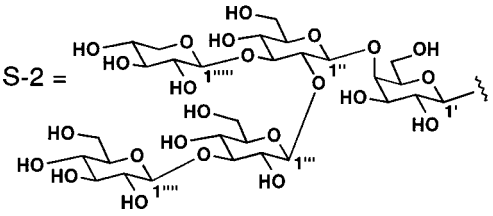

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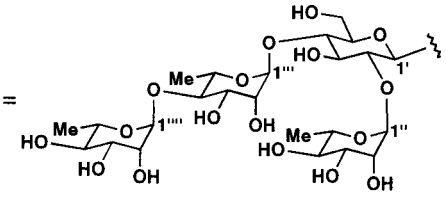

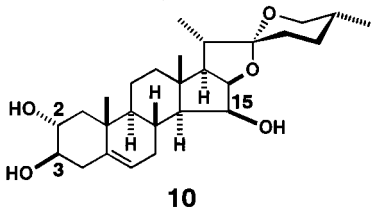

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Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, and UV spectra on a JASCO V-520 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H NMR, Karlsruhe, Germany) spectrometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer, using a dithiothreitol−dithioerythritol (3:1) matrix. Elemental analysis was carried out using an Elemental Vario EL elemental analyzer (Hanau, Germany). Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan), Si gel (Fuji-Silysia Chemical, Aichi, Japan), ODS Si gel (Nacalai Tesque, Kyoto, Japan), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP_{18} F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system composed of a Tosoh CCPM pump (Tokyo, Japan), a Tosoh CCP PX-8010 controller, a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and Rheodyne injection port with a 20-μL sample loop. A Kaseisorb LC NH_2-60-5 column (4.6 mm i.d. × 250 mm, 5 μm, Tokyo-Kasei, Tokyo, Japan) was employed for HPLC analysis. The following reagents were obtained from the indicated companies: Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY); fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS); penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), and α-minimum essential medium (α-MEM) (Sigma, St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Plant Material. The fresh leaves of *C. nocturnum* cultivated in the Tokyo Metropolitan Medicinal Plant Garden (Tokyo, Japan) were collected in October 1998, and identified by Y. S. A voucher specimen of the plant is on file in our laboratory (voucher no. CN-98-007., Laboratory of Medicinal Plant Science).

Extraction and Isolation. The plant material (fresh wt, 1.2 kg) was extracted with MeOH at room temperature for 7 days. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (77 g) was passed through a Diaion HP-20 column eluting successively with H_2O−MeOH (7:3), MeOH, and EtOAc. Column chromatography (CC) of the MeOH eluate portion (44.5 g) on Si gel and elution with a stepwise gradient mixture of $CHCl_3$−MeOH (19:1; 9:1; 4:1; 2:1), and finally with MeOH alone, gave 11 fractions (I−XI). Fraction V was subjected to CC on Si gel eluting with $CHCl_3$−MeOH−H_2O (40:10:1; 30:10:1; 25:10:1), ODS Si gel with MeOH−H_2O (4:1), and MeCN−H_2O (1:1; 2:3) and on Sephadex LH-20 with MeOH to give **8** (29.1 mg) and **9** (8.8 mg). Fraction VI was further separated by subjecting it to a Si gel column eluting with $CHCl_3$−MeOH−H_2O (40:10:1; 25:10:1) and a Sephadex LH-20 column with MeOH into fraction VIa and VIb. Fraction VIa was chromatographed on Si gel eluting with MeOH−H_2O (4:1) to yield **3** (80.1 mg) and **4** (83.5 mg). Fraction VIb was refined using Si gel CC eluting with $CHCl_3$−MeOH−H_2O (30:10:1) and ODS Si gel CC with MeOH−H_2O (3:2; 1:1) and MeCN−H_2O (7:13) to furnish **1** (74.2 mg) and **2** (52.3 mg). Fraction VII was subjected to CC on Si gel eluting with $CHCl_3$−MeOH−H_2O (20:10:1) and ODS Si gel with MeOH−H_2O (4:1) to yield **5** (1.34 g) as a pure compound and a mixture of **6** and **7**. The mixture was separated by repeated CC on ODS Si gel eluting with MeCN−H_2O (2:3) to furnish **6** (95.7 mg) and **7** (146 mg).

Compound 1: pale-yellow amorphous powder; $[\alpha]^{24}_D$ −29.2° (*c* 0.26, $CHCl_3$−MeOH, 1:1); UV (MeOH) λ_{max} 329 nm (log ϵ 4.43), 268 nm (log ϵ 4.31); UV (MeOH + 1 M NaOH) λ_{max} 391 nm; IR (film) ν_{max} 3387 (OH), 2934 (CH), 1697 (C=O), 1655 (C=O), 1598, 1515, 1498, and 1454 (aromatic rings), 1345, 1283, 1261, 1214, 1168, 1115, 1080, 984, 914, 887 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.50 (1H, d, J = 2.2 Hz, H-6), 6.48 (1H, d, J = 2.2 Hz, H-8), 3.67 (3H, s, OMe), 8.56 (2H, d, J = 8.8 Hz, H-2′ and H-6′), 7.40 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.32 (1H, d, J = 7.6 Hz, H-1″), 4.45 (1H, dd, J = 8.4, 7.6 Hz, H-2″), 4.32 (1H, dd, J = 8.4, 8.4 Hz, H-3″), 3.94 (1H, H-4″), 3.95 (1H, H-5″), 4.37 (1H, br d, J = 10.8 Hz, H-6″a), 3.87 (1H, dd, J = 10.8, 5.3 Hz, H-6″b), 5.61 (1H, d, J = 7.4 Hz, H-1‴), 4.25 (1H, dd, J = 8.1, 7.4 Hz, H-2‴), 4.29 (1H, dd, J = 8.1, 8.1 Hz, H-3‴), 4.18 (1H, dd, J = 8.1, 8.1 Hz, H-4‴), 4.22 (1H, H-5‴), 5.05 (2H, d, J = 3.3 Hz, H_2-6‴), 5.16 (1H, d, J = 1.2 Hz, H-1⁗), 5.23 (1H, dd, J = 3.5, 1.2 Hz, H-2⁗), 4.26 (1H, dd, J = 9.2, 3.5 Hz, H-3⁗), 4.08 (1H, dd, J = 9.2, 9.2 Hz, H-4⁗), 4.05 (1H, dq, J = 9.2, 5.4 Hz, H-5⁗), 1.44 (3H, d, J = 5.4 Hz, Me-6⁗), 6.80 (2H, s, H-2⁗⁗ and H-6⁗⁗), 7.79 (1H, d, J = 15.8 Hz, H-7⁗⁗), 6.51 (1H, d, J = 15.8 Hz, H-8⁗⁗), 3.77 (3H × 2, s, OMe × 2); ^{13}C NMR (C_5D_5N) δ 157.8 (C-2), 134.7 (C-3), 178.8 (C-4), 162.4 (C-5), 98.4 (C-6), 165.5 (C-7), 92.3 (C-8), 106.3 (C-5a), 157.3 (C-8a), 55.7 (OMe), 122.2 (C-1′), 132.2 (C-2′), 116.3 (C-3′), 161.7 (C-4′), 116.3 (C-5′), 132.2 (C-6′), 100.2 (C-1″), 84.0

(C-2″), 78.2 (C-3″), 71.3 (C-4″), 77.2 (C-5″), 67.6 (C-6″), 106.2 (C-1‴), 76.1 (C-2‴), 78.2 (C-3‴), 71.4 (C-4‴), 75.8 (C-5‴), 64.5 (C-6‴), 102.2 (C-1⁗), 72.1 (C-2⁗), 72.5 (C-3⁗), 73.9 (C-4⁗), 69.5 (C-5⁗), 18.4 (C-6⁗), 125.0 (C-1⁗′), 106.5 (C-2⁗′ and C-6⁗′), 149.0 (C-3⁗′ and C-5⁗′), 140.4 (C-4⁗′), 145.9 (C-7⁗′), 115.2 (C-8⁗′), 167.5 (C-9⁗′), 56.3 (OMe); FABMS (positive mode) *m/z* 999 $[M + Na]^+$, 977 $[M + H]^+$; FABMS (negative mode) *m/z* 975 $[M - H]^-$, 829 $[M - H -$ rhamnosyl$]^-$, 769 $[M - H -$ (acyl group)$]^-$, 607 $[M - H -$ glucosyl $-$ (acyl group)$]^-$; *anal.* C 52.37%, H 5.85%, calcd for $C_{45}H_{52}O_{24}\cdot 3H_2O$, C 52.43%, H 5.67%.

Alkaline Methanolysis of 1. Compound **1** (36.5 mg) was treated with 3% NaOMe in MeOH (4 mL) at room temperature for 2 h. The reaction mixture was neutralized by passing through an Amberlite IR-120B (Organo, Tokyo, Japan) column and purified by Si gel CC, eluting initially with $CHCl_3$ and then with $CHCl_3$−MeOH−H_2O (20:10:1) to give **1a** (23.8 mg) and methyl 3,5-dimethoxy-4-hydroxycinnamate (7.2 mg).[12]

Compound 1a: pale-yellow amorphous powder; $[\alpha]^{24}_D$ +5.9° (*c* 0.17, $CHCl_3$−MeOH, 1:1); UV (MeOH) λ_{max} 348 nm (log ϵ 4.22), 268 nm (log ϵ 4.29); UV (MeOH + 1 M NaOH) λ_{max} 385 nm; IR (film) ν_{max} 3376 (OH), 2929 (CH), 1656 (C=O), 1596, 1497, and 1445 (aromatic rings), 1416, 1346, 1280, 1212, 1168, 1136, 1070, 916, 886 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.58 (1H, d, *J* = 2.2 Hz, H-6), 6.56 (1H, d, *J* = 2.2 Hz, H-8), 3.69 (3H, s, OMe), 8.54 (2H, d, *J* = 8.8 Hz, H-2′ and H-6′), 7.41 (2H, d, *J* = 8.8 Hz, H-3′ and H-5′), 6.47 (1H, d, *J* = 7.6 Hz, H-1″), 4.44 (1H, dd, *J* = 8.8, 7.6 Hz, H-2″), 4.35 (1H, dd, *J* = 8.8, 8.8 Hz, H-3″), 3.94 (1H, dd, *J* = 8.8, 8.8 Hz, H-4″), 3.99 (1H, ddd, *J* = 8.8, 6.2, 2.4 Hz, H-5″), 4.38 (1H, dd, *J* = 11.2, 2.4 Hz, H-6″a), 3.88 (1H, dd, *J* = 11.2, 6.2 Hz, H-6″b), 5.61 (1H, d, *J* = 7.8 Hz, H-1‴), 4.20 (1H, dd, *J* = 9.0, 7.8 Hz, H-2‴), 4.28 (1H, dd, *J* = 9.0, 9.0 Hz, H-3‴), 4.32 (1H, dd, *J* = 9.0, 9.0 Hz, H-4‴), 4.00 (1H, ddd, *J* = 9.0, 4.3, 2.4 Hz, H-5‴), 4.52 (1H, dd, *J* = 11.7, 2.4 Hz, H-6‴a), 4.39 (1H, dd, *J* = 11.7, 4.3 Hz, H-6‴b), 5.18 (1H, d, *J* = 1.3 Hz, H-1⁗), 4.24 (1H, dd, *J* = 3.3, 1.3 Hz, H-2⁗), 4.29 (1H, dd, *J* = 9.2, 3.3 Hz, H-3⁗), 4.09 (1H, dd, *J* = 9.2, 9.2 Hz, H-4⁗), 4.06 (1H, dq, *J* = 9.2, 5.6 Hz, H-5⁗), 1.44 (3H, d, *J* = 5.6 Hz, Me-6⁗); ^{13}C NMR (C_5D_5N) δ 157.8 (C-2), 134.5 (C-3), 178.9 (C-4), 162.5 (C-5), 98.5 (C-6), 165.6 (C-7), 92.4 (C-8), 106.3 (C-5a), 157.3 (C-8a), 55.8 (OMe), 122.1 (C-1′), 132.2 (C-2′), 116.3 (C-3′), 161.7 (C-4′), 116.3 (C-5′), 132.2 (C-6′), 99.9 (C-1″), 83.8 (C-2″), 78.4 (C-3″), 71.2 (C-4″), 77.4 (C-5″), 67.5 (C-6″), 106.1 (C-1‴), 76.1 (C-2‴), 78.4 (C-3‴), 71.4 (C-4‴), 78.8 (C-5‴), 62.6 (C-6‴), 102.1 (C-1⁗), 72.1 (C-2⁗), 72.5 (C-3⁗), 73.9 (C-4⁗), 69.5 (C-5⁗), 18.4 (C-6⁗); FABMS (positive mode) *m/z* 793 $[M + Na]^+$; FABMS (negative mode) *m/z* 769 $[M - H]^-$, 623 $[M - H -$ rhamnosyl$]^-$, 607 $[M - H -$ glucosyl$]^-$.

Acid Hydrolysis of 1a. A solution of **1a** (6.9 mg) in 1 M HCl (dioxane−H_2O, 1:1, 4 mL) was heated at 95 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on Si gel using a discontinuous gradient of $CHCl_3$−MeOH (12:1 to 1:1) to give 4′,5-dihydroxy-7-methoxyflavonol (2.8 mg) and a sugar fraction (3.3 mg). The sugar fraction was dissolved in H_2O and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), which was then analyzed by HPLC under the following conditions: solvent, MeCN−H_2O (3:1); flow rate, 0.8 mL/min; detection, optical rotation. Identification of D-glucose and L-rhamnose present in the sugar fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R (min) 8.86 (L-rhamnose, negative optical rotation), 15.69 (D-glucose, positive optical rotation).

Compound 2: pale-yellow amorphous powder; $[\alpha]^{24}_D$ −47.6° (*c* 0.21, $CHCl_3$−MeOH, 1:1); UV (MeOH) λ_{max} 348 nm (log ϵ 4.19), 268 nm (log ϵ 4.25); UV (MeOH + 1 M NaOH) λ_{max} 388 nm; IR (film) ν_{max} 3377 (OH), 2930 (CH), 1656 (C=O), 1597, 1498, and 1445 (aromatic rings), 1347, 1280, 1213, 1168, 1138, 1065, 917, 887 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.53 (1H, d, *J* = 2.2 Hz, H-6), 6.57 (1H, d, *J* = 2.2 Hz, H-8), 3.69 (3H, s, OMe), 8.55 (2H, d, *J* = 8.8 Hz, H-2′ and H-6′), 7.36 (2H, d, *J* = 8.8 Hz, H-3′ and H-5′), 6.42 (1H, d, *J* = 7.7 Hz, H-1″), 4.44 (1H, dd, *J* = 8.7, 7.7 Hz, H-2″), 4.35 (1H, dd, *J* = 8.7, 8.7 Hz, H-3″), 3.97 (1H, dd, *J* = 8.7, 8.7 Hz, H-4″), 4.02 (1H, br dd, *J* = 8.7, 6.1 Hz, H-5″), 4.38 (1H, br d, *J* = 11.2 Hz, H-6″a), 3.88 (1H, dd, *J* = 11.2, 6.1 Hz, H-6″b), 5.66 (1H, d, *J* = 6.2 Hz, H-1‴), 4.22 (1H, H-2‴), 4.21 (1H, H-3‴), 4.20 (1H, H-4‴), 4.49 (1H, dd, *J* = 11.4, 3.9 Hz, H-5‴a), 3.78 (1H, dd, *J* = 11.4, 8.6 Hz, H-5‴b), 5.21 (1H, br s, H-1⁗), 4.30 (1H, H-2⁗), 4.31 (1H, H-3⁗), 4.09 (1H, H-4⁗), 4.08 (1H, H-5⁗), 1.45 (3H, d, *J* = 5.5 Hz, Me-6⁗); ^{13}C NMR (C_5D_5N) δ 157.7 (C-2), 134.7 (C-3), 178.8 (C-4), 162.5 (C-5), 98.4 (C-6), 165.7 (C-7), 92.4 (C-8), 106.3 (C-5a), 157.3 (C-8a), 55.8 (OMe), 122.1 (C-1′), 132.1 (C-2′), 116.3 (C-3′), 161.7 (C-4′), 116.3 (C-5′), 132.1 (C-6′), 100.3 (C-1″), 82.4 (C-2″), 78.6 (C-3″), 71.3 (C-4″), 77.3 (C-5″), 67.9 (C-6″), 105.9 (C-1‴), 75.1 (C-2‴), 77.3 (C-3‴), 71.3 (C-4‴), 66.8 (C-5‴), 102.3 (C-1⁗), 72.1 (C-2⁗), 72.5 (C-3⁗), 73.9 (C-4⁗), 69.6 (C-5⁗), 18.4 (C-6⁗); FABMS (positive mode) *m/z* 763 $[M + Na]^+$; FABMS (negative mode) *m/z* 739 $[M - H]^-$, 607 $[M - H -$ xylosyl$]^-$, 583 $[M - H -$ rhamnosyl$]^-$; *anal.* C 51.84%, H 6.10%, calcd for $C_{33}H_{40}O_{19}\cdot 3/2H_2O$, C 51.62%, H 5.65%.

Acid Hydrolysis of 2. Compound **2** (13.6 mg) was subjected to acid hydrolysis as described for **1a** to give 4′,5-dihydroxy-7-methoxyflavonol (3.4 mg) and a sugar fraction (4.5 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1a** showed the presence of D-glucose, D-xylose, and L-rhamnose; t_R (min) 8.85 (L-rhamnose, negative optical rotation); 10.71 (D-xylose, positive optical rotation); 15.71 (D-glucose, positive optical rotation).

Compound 4: amorphous powder; $[\alpha]^{24}_D$ −70.8° (*c* 0.13, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3387 (OH), 2951, 2928, and 2876 (CH), 1457, 1434, 1376, 1243, 1210, 1158, 1073, 1055, 980, 919, 893, 863 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.58 (1H, d, *J* = 7.8 Hz, H-1‴), 5.29 (1H, br d, *J* = 4.8 Hz, H-6), 5.26 (1H, d, *J* = 7.8 Hz, H-1⁗), 5.22 (1H, d, *J* = 7.9 Hz, H-1″), 4.92 (1H, d, *J* = 7.7 Hz, H-1′), 4.46 (1H, t-like, *J* = 6.9 Hz, H-16), 4.06 (1H, m, H-2), 3.81 (1H, m, H-3), 3.51 (2H, br s, H_2-26), 2.27 (1H, q, *J* = 7.1 Hz, H-20), 2.20 (1H, m, H-15α), 1.48 (1H, m, H-15*β*), 1.23 (3H, d, *J* = 7.1 Hz, Me-21), 0.97 (3H, s, Me-19), 0.95 (3H, s, Me-18), 0.69 (3H, d, *J* = 5.5 Hz, Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) *m/z* 1087 $[M + Na]^+$; FABMS (negative mode) *m/z* 1063 $[M - H]^-$, 931 $[M - H -$ xylosyl$]^-$, 901 $[M - H -$ glucosyl$]^-$, 769 $[M - H -$ xylosyl $-$ glucosyl$]^-$, 607 $[M - H -$ xylosyl $-$ glucosyl × 2$]^-$; *anal.* C 52.55%, H 7.92%, calcd for $C_{50}H_{80}O_{24}\cdot 4H_2O$, C 52.81%, H 7.80%.

Acid Hydrolysis of 4. Compound **4** (27.9 mg) was subjected to acid hydrolysis as described for **1a** to give unidentified artifactual sapogenols (11.3 mg) and a sugar fraction (10.2 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1a** showed the presence of D-glucose, D-galactose, and D-xylose; t_R (min) 10.73 (D-xylose, positive optical rotation); 15.74 (D-glucose, positive optical rotation); 17.22 (D-galactose, positive optical rotation).

Compound 6: amorphous powder; $[\alpha]^{24}_D$ −60.0° (*c* 0.13, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3376 (OH), 2926 and 2873 (CH), 1456, 1435, 1418, 1374, 1259, 1243, 1210, 1154, 1075, 1040, 981, 919, 897, 867 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.58 (1H, d, *J* = 7.6 Hz, H-1‴), 5.36 (1H, br d, *J* = 4.7 Hz, H-6), 5.20 (1H, d, *J* = 7.9 Hz, H-1″), 5.17 (1H, d, *J* = 7.8 Hz, H-1⁗′), 5.12 (1H, d, *J* = 7.8 Hz, H-1⁗), 4.92 (1H, d, *J* = 7.7 Hz, H-1′), 4.48 (1H, m, H-16), 4.35 (1H, dd, *J* = 5.1, 3.9 Hz, H-15), 4.06 (1H, m, H-2), 3.84 (1H, m, H-3), 3.62 (1H, dd, *J* = 11.0, 3.2 Hz, H-26eq), 3.51 (1H, dd, *J* = 11.0, 11.0 Hz, H-26ax), 1.27 (3H, s, Me-18), 1.13 (3H, d, *J* = 6.6 Hz, Me-21), 0.98 (3H, s, Me-19), 0.72 (3H, d, *J* = 6.4 Hz, Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) *m/z* 1249 $[M + Na]^+$; FABMS (negative mode) *m/z* 1225 $[M - H]^-$, 1093 $[M - H -$ xylosyl$]^-$, 1063 $[M - H -$ glucosyl$]^-$, 931 $[M - H -$ xylosyl $-$ glucosyl$]^-$, 901 $[M - H -$ glucosyl × 2$]^-$, 769 $[M - H -$ xylosyl $-$ glucosyl × 2$]^-$, 607 $[M - H -$ xylosyl $-$ glucosyl × 3$]^-$; *anal.* C 52.06%, H 7.82%, calcd for $C_{56}H_{90}O_{29}\cdot 7/2H_2O$, C 52.13%, H 7.58%.

Acid Hydrolysis of 6. Compound **6** (36.6 mg) was subjected to acid hydrolysis as described for **1a** to give **10** (4.6 mg) and a sugar fraction (12.8 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1a** showed the presence of D-glucose, D-galactose, and D-xylose; t_R (min)

10.71 (D-xylose, positive optical rotation), 15.70 (D-glucose, positive optical rotation), 17.17 (D-galactose, positive optical rotation).

Compound 10: amorphous powder; $[\alpha]^{24}_D$ −84.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3387 (OH), 2950, 2927, and 2872 (CH), 1457, 1376, 1296, 1242, 1210, 1184, 1151, 1139, 1103, 1064, 1005, 981, 960, 920, 898, 866 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.48 (1H, br d, J = 5.1 Hz, H-6), 4.49 (1H, dd, J = 8.5, 5.2 Hz, H-16), 4.37 (1H, dd, J = 5.2, 3.9 Hz, H-15), 4.16 (1H, ddd, J = 11.8, 8.9, 4.4 Hz, H-2), 3.85 (1H, ddd, J = 11.3, 8.9, 5.5 Hz, H-3), 3.63 (1H, dd, J = 10.9, 3.8 Hz, H-26eq), 3.52 (1H, dd, J = 10.9, 10.9 Hz, H-26ax), 2.43 (1H, m, H-7α), 2.08 (1H, dd, J = 8.5, 8.5 Hz, H-17), 1.73 (1H, t-like, J = 10.8 Hz, H-7β), 1.31 (3H, s, Me-18), 1.15 (3H, s, Me-19), 1.14 (3H, d, J = 6.5 Hz, Me-21), 1.10 (1H, dd, J = 10.8, 3.9 Hz, H-14), 0.72 (3H, d, J = 6.5 Hz, Me-27); ^{13}C NMR (C_5D_5N) δ 46.7 (C-1), 72.7 (C-2), 76.9 (C-3), 41.0 (C-4), 141.5 (C-5), 121.4 (C-6), 31.7 (C-7), 27.6 (C-8), 50.8 (C-9), 38.8 (C-10), 21.6 (C-11), 42.4 (C-12), 40.7 (C-13), 60.7 (C-14), 70.1 (C-15), 83.6 (C-16), 62.0 (C-17), 19.0 (C-18), 20.6 (C-19), 42.9 (C-20), 14.7 (C-21), 110.1 (C-22), 31.5 (C-23), 29.2 (C-24), 30.6 (C-25), 67.2 (C-26), 17.4 (C-27); FABMS (positive mode) *m*/*z* 447 $[M + H]^+$.

Compound 7: amorphous powder; $[\alpha]^{24}_D$ −57.0° (*c* 0.20, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3388 (OH), 2952, 2930, 2901, and 2876 (CH), 1456, 1434, 1418, 1376, 1243, 1210, 1157, 1075, 1055, 1041, 980, 919, 893, 863 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.57 (1H, d, J = 7.6 Hz, H-1‴), 5.30 (1H, br d, J = 4.6 Hz, H-6), 5.20 (1H, d, J = 7.9 Hz, H-1″), 5.17 (1H, d, J = 7.8 Hz, H-1⁗), 5.11 (1H, d, J = 7.8 Hz, H-1⁗), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.45 (1H, t-like, J = 6.8 Hz, H-16), 4.07 (1H, m, H-2), 3.81 (1H, m, H-3), 3.52 (2H, br s, H_2-26), 2.26 (1H, q, J = 7.2 Hz, H-20), 1.22 (3H, d, J = 7.2 Hz, Me-21), 0.97 (3H, s, Me-19), 0.94 (3H, s, Me-18), 0.69 (3H, d, J = 5.4 Hz, Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 1249 $[M + Na]^+$; FABMS (negative mode) *m*/*z* 1225 $[M - H]^-$, 1093 [M − H − xylosyl]$^-$, 1063 [M − H − glucosyl]$^-$, 931 [M − H − xylosyl − glucosyl]$^-$, 901 [M − H − glucosyl × 2]$^-$, 769 [M − H − xylosyl − glucosyl × 2]$^-$, 607 [M − H − xylosyl − glucosyl × 3]$^-$; *anal.* C 50.98%, H 7.72%, calcd for $C_{56}H_{90}O_{29}\cdot 5H_2O$, C 51.06%, H 7.65%.

Acid Hydrolysis of 7. Compound **7** (21.6 mg) was subjected to acid hydrolysis as described for **1a** to give unidentified artifactual sapogenols (8.8 mg) and a sugar fraction (8.9 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1a** showed the presence of D-glucose, D-galactose, and D-xylose; t_R (min) 10.75 (D-xylose, positive optical rotation), 15.77 (D-glucose, positive optical rotation), 17.25 (D-galactose, positive optical rotation).

Compound 9: amorphous powder; $[\alpha]^{24}_D$ −93.3° (*c* 0.12, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3377 (OH), 2927, 2873, and 2857 (CH), 1454, 1377, 1278, 1259, 1242, 1128, 1052, 982, 916, 900, 866 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.41 (1H, br s, H-1″), 6.29 (1H, d, J = 1.0 Hz, H-1⁗), 5.83 (1H, br s, H-1‴), 5.35 (1H, br d, J = 4.7 Hz, H-6), 5.00 (1H, d, J = 7.3 Hz, H-1′), 4.54 (1H, m, H-16), 4.17 (1H, m, H-2), 3.83 (1H, m, H-3), 3.59 (1H, dd, J = 10.5, 2.7 Hz, H-26eq), 3.51 (1H, dd, J = 10.5, 10.5 Hz, H-26ax), 1.71 (3H, d, J = 6.2 Hz, Me-6″), 1.62 (3H, d, J = 6.0 Hz, Me-6⁗), 1.61 (3H, d, J = 6.2 Hz, Me-6‴), 1.14 (3H, d, J = 7.0 Hz, Me-21), 1.09 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.70 (3H, d, J = 5.6 Hz, Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 1053 $[M + Na]^+$; FABMS (negative mode) *m*/*z* 1029 $[M - H]^-$, 883 [M − H − rhamnosyl]$^-$, 737 [M − H − rhamnosyl × 2]$^-$; *anal.* C 57.67%, H 8.46%, calcd for $C_{51}H_{82}O_{21}\cdot 2H_2O$, C 57.40%, H 8.12%.

Acid Hydrolysis of 9. Compound **9** (4.1 mg) was subjected to acid hydrolysis as described for **1a** to give (25*R*)-spirost-5-ene-2α,3β-diol (1.1 mg) and a sugar fraction (1.5 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of that of **1a** showed the presence of L-rhamnose and D-glucose; t_R (min) 8.87 (L-rhamnose, negative optical rotation), 15.75 (D-glucose, positive optical rotation).

Cell Culture. HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM medium supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. HGF were isolated, as described previously.[13] Briefly, gingival tissues were obtained from healthy gingival biopsies from a 10-year-old girl, undergoing periodontal surgery. The tissue was cut into 1- to 2-mm^3 pieces, washed twice with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin, and placed into 25-cm^2 tissue-culture flasks. The explants were incubated in α-MEM supplemented with 30% FBS and antibiotics. When outgrowth of the cells was observed, the medium was replaced twice until the cells reached confluence. The cells were detached from the monolayer by trypsinization and recultured in 100-cm^2 tissue-culture flasks until confluent monolayers were again obtained. Cells between the fifth and seventh passages were used.

Assay for Cytotoxic Activity. Cells were trypsinized and inoculated at 6×10^3 per each 96-microwell plate (Falcon, Flat bottom, Treated polystyrene, Becton Dickinson, San Jose, CA), and incubated for 24 h. After washing once with PBS, they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM medium supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL DMSO, and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT matrix printer JL-10. The LD_{50} value, which reduces the viable cell number by 50%, was determined from the dose−response curve.

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