Chemical Constituents from the Colombian Medicinal Plant *Maytenus laevis*

Hiroyuki Nakagawa,[†] Yoshihisa Takaishi,*[,†] Yoshinori Fujimoto,[‡] Carmenza Duque,[§] Cristina Garzon,[⊥] Mitsunobu Sato,[#] Masato Okamoto,[#] Tetsuya Oshikawa,[#] and Sharif Uddin Ahmed[#]

Faculty of Pharmaceutical Sciences, University of Tokushima, Sho-machi, Tokushima 770-8505, Japan, Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan, Depatmento de Quimica, and Institito de Ciencias Naturales, Universidad Nacional de Colombia, AA 14490, Bogota, Colombia, and Schoool of Dentistry, University of Tokushima, Kuramoto-cho, Tokushima 770-8504, Japan

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The methanol extract of the bark of the Colombian medicinal plant *Maytenus laevis* gave six new compounds and 28 known compounds. The structures of the new and known compounds were elucidated on the basis of spectroscopic evidence. Several of these compounds were screened for cytokine-inducing activity on human PBMCs to investigate antitumor effects, and canophyllol (**12**) demonstrated the most effective induction of the cytokines.

Species belonging to the genus *Maytenus* (Celastraceae) have been used as a traditional medicine in the Amazonian region against cancer, rheumatism, and inflammation.[1−3] The bark of *Maytenus laevis*, known by a vernacular name meaning shaking waist, "chuchuhuasha" or "chuchuasi", is used as an alcoholic infusion, generally in "aguardiente", for the treatment of rheumatism and as a tonic and an aphrodisiac.[4−6] Antitumor, antiinflammatory, and radioprotective activities have been reported from *M. laevis* by Gonzalez et al.[2] In the present study, we report the isolation and structural elucidation of six new and 28 known compounds from the methanol extract of the bark of *M. laevis*. The cytokine-inducing activity of some of the isolated compounds is also reported.

Repeated column chromatography of the ethyl acetate-soluble fraction from the methanol extract of the bark of *M. laevis* yielded six new compounds, including one sesquiterpene pyridine alkaloid, one flavonoid, two triterpenoids, one lignan, one iridoid, and 28 known compounds.

Compound **1** was assigned the molecular formula $C_{41}H_{47}O_{17}N$ on the basis of HRFABMS. The IR spectrum revealed hydroxy and ester carbonyl bands (3457 and 1744 cm^{-1}). The ^1H NMR spectrum showed four acetyl groups (δ_H 2.33, 2.22, 2.12, and 1.42), one benzoyl group [δ_H 7.96 (2H, d, J = 7.4 Hz), 7.57 (1H, t, J = 7.4 Hz), and 7.43 (2H, t, J = 7.7 Hz)], two tertiary methyl groups (δ_H 1.72, 1.64), two sets of methylene protons [δ_H 6.00, 3.71 (each 1H, d, J = 11.4 Hz), 5.48, 4.81 (each 1H, d, J = 13.6 Hz)], and seven methine protons [δ_H 7.07, 5.76, 5.54, 5.41, 4.84, 4.14, and 2.36]. It also showed one evonic acid moiety, 2,3-disubstituted pyridine (δ_H 8.77, 8.14, and 7.34), two secondary methyl groups [δ_H 1.44 and 1.20 (each 3H, d, J = 6.9 Hz)], and two methine protons [δ_H 4.72 and 2.59 (each 1H, q, J = 6.9 Hz)]. The ^{13}C NMR spectra of **1** indicated the presence of eight methyl carbons, two methylene carbons attached to an oxygen function, six methine carbons attached to an oxygen function, one methine carbon, four ester carbonyl carbons, four quaternary carbons, one benzoyl group [δ_C 165.0 (s), 133.8 (d), 129.9 (d) × 2, 129.3 (s), and 128.8 (d) × 2], and one evonic acid moiety [δ_C 174.4 (s), 168.2 (s), 165.0 (s), 150.9 (d), 138.8 (d), 126.0 (s), 121.7 (d), 45.2 (d), 36.6 (d), 12.1 (q), and 9.8 (q)]. Owing to the presence of ester carbons and a pyridine ring moiety, **1** was assumed to be a sesquiterpene pyridine alkaloid derived from dihydroagarofuran polyol esters found in the Celastraceae.[7−9] The NMR spectra of **1** suggested that it was an evonine-type sesquiterpene alkaloid,[7−13] possessing four acetyl groups and one benzoyl group. The $^1H-^1H$ COSY and the coupling pattern among the seven methine protons [δ_H 5.76 (H-1), 4.14 (H-2), 4.84 (H-3), 7.07 (H-5), 2.36 (H-6), 5.54 (H-7), and 5.41 (H-8)] revealed their connections in the dihydroagarofuran core. In the HMBC spectrum of **1**, the proton signal at δ_H 5.76 (H-1) was correlated with the carbon signals at δ_C 53.0 (C-9) and 60.5 (C-11); the proton signal at δ_H 1.64 (H-12) with the carbon signals at δ_C 78.3 (C-3), 70.7 (C-4), and 94.8 (C-10); the proton signal at δ_H 7.07 (H-5) with the carbon signal at δ_C 94.8 (C-10); the proton signal at δ_H 5.41 (H-8) with the carbon signal at δ_C 53.0 (C-9); the proton signal at δ_H 1.72 (H-14) with the carbon signals at δ_C 50.6 (C-6), 84.3 (C-13), and 70.5 (C-15). The proton signal at δ_H 4.84 (H-3) was correlated with the carbon signal at δ_C 174.4 (C-11′); the proton signal at δ_H 2.59 (H-8′) with the carbon signals at δ_C 36.6 (C-7′) and 174.4 (C-11′); the proton signal at δ_H 8.14 (H-4′) with the carbon signal at δ_C 168.2 (C-12′); the proton signal at δ_H 6.00 (H-15) with the carbon signal at δ_C 168.2 (C-12′) in the HMBC spectrum; these facts clearly indicated that the macrocyclic structure was formed by ester linkages between the dihydroagarofuran core and evonic acid at positions C-3 and 15.

On comparing the ^{13}C NMR spectroscopic data of **1** with the literature, compound **1** was found to be similar to hyponine C,[14] except for the presence of five acetyl groups in hyponine C compared to four in **1**. From the HMBC spectrum of **1**, the locations of the ester groups were determined as follows: the proton signals at δ_H 7.07 (H-5) and 5.41 (H-8) showed long-range correlations with the carbonyl carbons of the acetyl groups at δ_C 170.0 and 169.2, respectively. The ^1H chemical shifts of H-1 (δ_H 5.76), H-2 (δ_H 4.14), and H-7 (δ_H 5.54) revealed acylation at C-1 and C-7. Thus, the four acetyl groups were assigned at positions C-1, -5, -7, and -8. The benzoyl group was assigned at position C-11 from an NOE correlation between the proton at δ_H 7.96 (*ortho*-Bz) and the proton at δ_H 4.81 (H-11). In

* To whom correspondence should be addressed. Tel: 81-886337275. Fax: 81-886339501. E-mail: takaishi@ph.tokushima-u.ac.jp.
[†] Faculty of Pharmaceutical Sciences, University of Tokushima.
[‡] Tokyo Institute of Technology.
[§] Depatmento de Quimica, Universidad Nacional de Colombia.
[⊥] Institito de Ciencias Naturales, Universidad Nacional de Colombia.
[#] Schoool of Dentistry, University of Tokushima.

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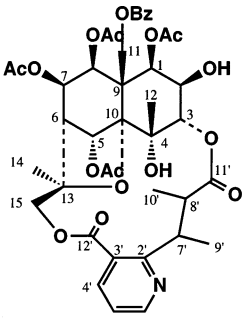

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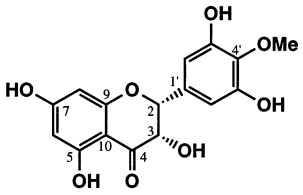

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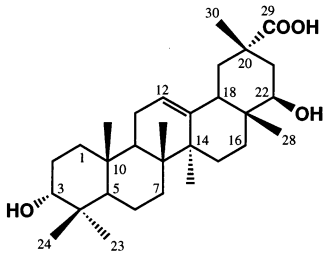

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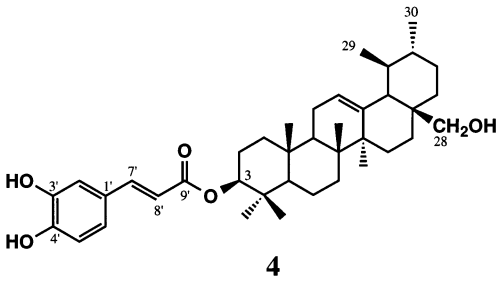

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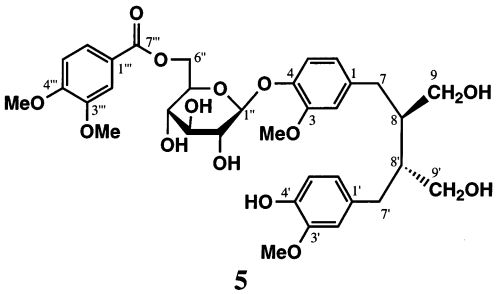

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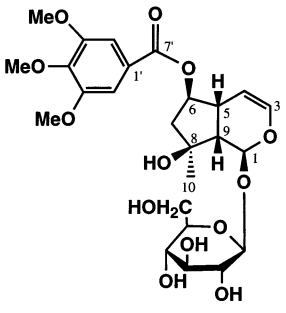

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the NOESY spectrum, the proton at δ_H 7.07 (H-5) was correlated with the protons at δ_H 5.48 (H-11), 2.36 (H-6), and 1.64 (H-12); the proton at δ_H 5.41 (H-8) with the protons at δ_H 5.76 (H-1) and 5.54 (H-7); the proton at δ_H 5.76 (H-1) with the protons at δ_H 5.41 (H-8) and 4.14 (H-2). The proton at δ_H 2.36 (H-6) was correlated with the proton at δ_H 3.71 (H-15). This evidence proved that the relative configuration of the ester groups was 1β, 2β, 5α, 7β, and 8β, and that of the two methyl groups was 12β, 14α. Therefore, the structure of **1** was formulated as 7-(acetyloxy)-O^{11}-benzoyl-$O^{2,11}$-deacetyl-7-deoxoevonine, as shown.

Compound **2** was assigned the molecular formula $C_{16}H_{14}O_8$ on the basis of HRFABMS. The ^{1}H NMR spectrum showed four aromatic protons [δ_H 6.56 (2H, brs), 5.97 and 5.92 (each 1H, brs)], two methine protons [δ_H 5.26 and 4.20 (each 1H, d, J = 2.1 Hz)], and one methoxyl group (δ_H 3.80). The ^{13}C NMR spectra of **2** indicated the presence of two methine carbons attached to an oxygen function (δ_C 85.4 and 75.8), 12 aromatic carbons δ_C 171.3 (s), 168.6 (s), 167.0 (s), 154.3 × 2 (s), 139.4 (s), 135.9 (s), 110.4 × 2 (d), 104.6 (s), 99.9 (d), and 99.0 (d)], one carbonyl carbon (δ_C 199.0), and one methoxyl group (δ_C 63.5). The above spectroscopic data suggested that **2** was a flavonoid and were very similar to those of 4′-*O*-methyl-2,3-dihydromyricetin (=pallasiin).[15] However, the ^{1}H NMR spectrum of **2** was considerably different from that of pallasiin in regard to the coupling constant of H-2 and H-3. The value of the coupling constant in **2** was $J_{2,3}$ = 2.1 Hz versus $J_{2,3}$ = 11.0 Hz in pallasiin. From this difference, **2** was suggested not to be a 2,3-*trans*-3-hydroxyflavanone as pallasiin, but to be a 2,3-*cis* isomer. The absolute configurations at C-2 and C-3 were deduced from the circular dichroism (CD) spectrum of **2**. The CD spectrum of **2** showed negative and positive Cotton effects at 292 and 339 nm, respectively. The CD curves and maxima of **2** showed the characteristic pattern of a 2*R*,3*S*-dihydroflavanol.[16−19] Therefore, **2** was determined as (2*R*,3*S*)-4′-*O*-methyl-2,3-dihydromyricetin.

Compound **3** was assigned the molecular formula $C_{30}H_{48}O_4$ on the basis of HRFABMS. The ^{1}H NMR spectrum showed seven tertiary methyl groups (δ_H 1.80, 1.26, 1.22, 1.15, 1.07, 1.00, and 0.91), one olefinic proton [δ_H 5.41 (1H, brs)], and two methine protons [δ_H 4.00 (1H, brdd, J = 4.5, 2.7 Hz), 3.61 (1H, brs)] attached to an oxygen function. The ^{13}C NMR spectra of **3** indicated the presence of seven methyl carbons, nine methylene carbons, and two methine carbons (δ_C 75.3 and 75.2) attached to an oxygen function, three methine carbons, seven quaternary carbons, and one carboxylic acid (δ_C 181.4). This suggested that **3** was a triterpenoid derivative. Many types of triterpenoids have been isolated from Celastraceae plants;[20−22] compound **3** was assumed to be an oleanane-type triterpenoid from the carbon number (C_{30}) and the presence of seven tertiary methyl groups, one olefinic methine, and one carboxylic acid.

A detailed analysis of the ^{1}H−^{1}H COSY and HMBC spectral data as well as the ^{1}H and ^{13}C NMR data led to the conclusion that the structure of **3** was 3,22-dihydroxyolean-12-en-29-oic acid. The ^{13}C NMR data of **3** were similar to those of 3β,22β-dihydroxyolean-12-en-29-oic acid (**22**) except for C-3.[23] These data suggested that the A−E ring systems were the same as in **22**. The configuration of hydroxy groups on C-3 and C-22 were determined as follows. The signal at δ_H 3.61 (brs) suggested an α-configuration for the hydroxy group at C-3, because its coupling constant was small and the C-24 carbon signal of **3** suggested a downfield shift (δ_C 22.8) in comparison with that of **22** (δ_C 16.6). The 22-hydroxy group was deduced to be in a β-configuration from the NOESY spectrum; the proton at δ_H 1.26 (H-28) correlated with the protons at δ_H 2.08 (H-21β) and 2.46 (H-18); the proton at δ_H 4.00 (H-22) with the proton at δ_H 2.64 (H-21α); the proton at δ_H 1.80 (H-30) with the proton at δ_H 2.46 (H-18). Ring E is thus in a chair conformation that placed the C-20α carboxylic acid and the C-22β hydroxy group in an equatorial and axial

orientation, respectively. From the above evidence, **3** was shown to be 3α,22β-dihydroxyolean-12-en-29-oic acid. Four stereoisomeric forms of 3,22-dihydroxyolean-12-en-29-oic acid are possible with differing configurations at C-3 and C-22. We also isolated the remaining three isomers (**20**, **22**, and **24**). Although those isomers have been individually isolated from several plants,[23−25] it is interesting that this was the first time that all isomers were isolated from one plant.

Compound **4** was assigned the molecular formula $C_{39}H_{56}O_5$ on the basis of HRFABMS. The ^{1}H NMR spectrum showed seven methyl groups [δ_H 1.09 (3H, s), 0.98 (6H, s), 0.92 (3H, s), 0.91 (3H, d, J = 5.9 Hz), 0.89 (3H, s), and 0.79 (3H, d, J = 5.8 Hz)], three olefinic protons [δ_H 7.51, 6.21 (each 1H, d, J = 15.9 Hz), and 5.11 (1H, brs)], one set of methylene protons [δ_H 3.52 and 3.14 (each 1H, d, J = 11.0 Hz)], six methine protons [δ_H 4.59 (1H, t, J = 8.9 Hz), 1.55, 1.37, 1.36, 0.87, and 0.82 (each 1H, m)], and three aromatic protons [δ_H 7.03 (1H, d, J = 1.8 Hz), 6.93 (1H, dd, J = 8.2, 1.8 Hz), and 6.80 (1H, d, J = 8.2 Hz)]. The coupling pattern of the aromatic protons showed the presence of a 1,3,4-substituted ring. In addition, the coupling constant of the protons at δ_H 7.51 and 6.21 was 15.9 Hz, indicating the presence of two *trans*-olefinic protons. From these results, compound **4** was judged to be the caffeoyl ester of a triterpene.

The ^{13}C NMR spectral data of **4** were very similar to those of uvaol[26] except for the chemical shift of C-3 and the *O*-caffeoyl group. In the HMBC spectrum, the proton at δ_H 4.59 (H-3) correlated with the carbons at δ_C 17.0 (C-24) and 167.9 (C-9′), indicating that the *O*-caffeoyl group was located at C-3. Owing to the correlation of H-3 and H-5 present in the NOESY spectrum, the relative configuration of the *O*-caffeoyl group was indicated to be 3β. Therefore, the structure of **4** was determined as 28-hydroxy-12-ursene-3β-yl-caffeate (=uvaol-3β-yl-caffeate).

Compound **5** was assigned the molecular formula $C_{35}H_{44}O_{14}$ on the basis of HRFABMS. The ^{1}H and ^{13}C NMR spectra showed four methoxyl groups, four sets of methylenes, two methine protons, nine aromatic protons, and one sugar moiety. The ^{1}H and ^{13}C NMR spectra, analyzed by 2D NMR, suggested the presence of one secoisolariciresinol,[27] one β-glucose, one 1,3,4-trisubstituted benzoyl group, and two methoxyl groups. The benzoyl group was defined as veratroyl on the basis of the HMBC spectrum. Moreover, the correlation between the anomeric proton (δ_H 4.83, H-1″) and H-5 (δ_H 6.89) in the NOESY spectrum and the downfield shifts of C-1″ (δ_C 102.8) and C-6″ (δ_C 65.2) of the β-glucose moiety, when compared with those (δ_C 96.8 and 61.8) of β-D-glucose,[28] suggested that the glucose moiety and the veratroyl group were located at C-4 and C-6″, respectively. A comparison of the NMR data of **5** with (−)-secoisolariciresinol 4-*O*-β-D-(6-*O*-vanilloyl)glucopyranoside[29] showed almost the same chemical shift values, except for one methoxy group. Thus, **5**, $[\alpha]_D$ −31.2°, was identified as (−)-secoisolariciresinol 4-*O*-β-D-(6-*O*-veratroyl)glucopyranoside.

Compound **6** was assigned the molecular formula $C_{25}H_{34}O_{13}$ on the basis of HRFABMS. The ^{1}H NMR spectrum showed three methoxyl groups [δ_H 3.87 (6H, s), 3.82 (3H, s)], one set of methylene protons [δ_H 2.28 (1H, dd, J = 14.3, 6.2 Hz), 2.06 (1H, dd, J = 14.3, 3.4 Hz)], two olefinic protons [δ_H 6.24 (1H, dd, J = 6.3, 2.1 Hz), 4.99 (1H, dd, J = 6.3, 2.7 Hz)], two methine protons [δ_H 5.51 (1H, d, J = 2.5 Hz), 5.06 (1H, m)] attached to oxygen functions, two methine protons [δ_H 3.01 (1H, m), 2.62 (1H, brd, J = 9.0 Hz)], two aromatic methine protons [δ_H 7.36 (2H, brs)], and one sugar moiety [δ_H 4.66 (1H, d, J = 7.9 Hz), 3.88 (1H, m), 3.65 (1H, dd, J = 11.8, 5.8 Hz), 3.36 (1H, t, J = 8.7 Hz), 3.34 (1H, m), 3.26 (1H, m), and 3.18 (1H, t, J = 8.7 Hz)]. The ^{13}C NMR spectra of **6** indicated the presence of one carboxylic carbon (δ_C 167.4), three methoxyl carbons (δ_C 61.2, 56.8 × 2), one methylene carbon, two olefinic carbons (δ_C 141.3, 104.4), two methine carbons (δ_C 93.5, 81.2) attached to an oxygen function, two methine carbons, six aromatic carbons [δ_C 154.4 × 2, 143.7, 126.9 (each s), and 108.2 × 2 (d)], and one sugar moiety [δ_C 99.4, 78.3, 78.0, 74.8, 71.7 (each d), and 62.9 (t)]. A detailed analysis of the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra suggested that **6** was an ajugol-type iridoid glycoside esterified with a 3,4,5-trimethoxybenzoyl group. The ^{1}H and ^{13}C NMR data of **6** were very similar to those of ajugol[30] except for the 3,4,5-trimethoxybenzoyl moiety. In the ^{13}C NMR spectrum, the carbon signal at δ_C 81.2 (C-6) shifted downfield (Δ +3.0 ppm) and the carbon signals at δ_C 39.6 (C-5) and 47.7 (C-7) shifted upfield (Δ −1.7 and −2.3 ppm, respectively) in comparison with those of ajugol. These facts indicated that the 3,4,5-trimethoxybenzoyl group was attached at C-6. Thus, the structure of **6** was determined as 6-*O*-(3′,4′,5′-trimethoxybenzoyl)ajugol.

Known compounds were also isolated; ebenifoline E-II (**7**),[31] mayteine (**8**),[9] *p*-hydroxybenzoic acid (**9**),[32] ebenifoline E-III (**10**),[33] lambertic acid (**11**),[34] canophyllol (**12**),[25] 3-oxoolean-12-en-oic acid (**13**),[35] β-sitosterol (**14**),[36] wilforlide B (**15**),[37] abruslactone A (**16**),[25] 3-epiabruslactone A (**17**),[38] salaspermic acid (**18**),[39] 22β-hydroxy-3-oxoolean-12-en-oic acid (**19**),[23] maytenfolic acid (**20**),[25] mearnsetin (**21**),[15] 3β,22β-dihydroxyolean-12-en-29-oic acid (**22**),[23] triptocallic acid A (**23**),[40] triptocallic acid D (**24**),[24] (−)-4′-*O*-methylepigallocatechin (**25**),[41] 3′,4′-di-*O*-methyl-(−)-epicatechin (**26**),[42] epikatonic acid (**27**),[43] 6-*O*-3′,4′-dimethoxybenzoyl ajugol (**28**),[44] isoverbascoside (**29**),[45] *ent*-isolariciresinol (**30**),[46] 6-*O*-*p*-hydroxybenzoyl ajugol (**31**),[44] 6-*O*-4″-hydroxy-3″-methoxybenzoyl ajugol (**32**),[47] ourateaproanthocyanidin A (**33**),[48] and ajugol (**34**)[30] were identified from spectral data comparison with the literature, respectively.

Furthermore, several of the isolated compounds were assayed for cytokine-inducing activity on human peripheral blood mononuclear cells (PBMCs) to investigate antitumor effects. OK-432 used as a positive control induced all of the cytokines examined except for interleukin (IL)-4 (Table 1). It has been reported that OK-432 elicits antitumor effects by stimulating immunocompetent cells, such as macrophages, T cells, and natural killer cells and induces multiple cytokines including IL-1, IL-2, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ.[49−53] One or 10 μg/mL of the compounds was added into the PBMC culture. Forty-eight hours later, cytokines in the supernatants were measured. Data are shown in Table 1. All of the cytokines examined were not detected in a supernatant derived from untreated PBMC culture. IL-6, one of the helper T lymphocytes 2 (Th2)-type cytokines, was induced by the treatment with most of the compounds tested, especially compounds **8**, **12**, and **15** (>1000 pg/mL). IL-12 and TNF-α, Th1-type cytokines, were secreted by the PBMCs stimulated with several compounds, while compound **12** was most effective in the induction of the cytokines. None of the compounds induced IFN-γ, IL-4, or IL-10 in the current in vitro system (data not shown).

Experimental Section

General Experimental Procedures. NMR spectra (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both using TMS as internal standard) were measured on a Bruker ARX-400 instrument and a Bruker AVANCE-400 instrument. MS were

Table 1. Cytokine Induction by the Compounds on Human PBMCs[a]

	secreted cytokines					
	IL-6 (pg/mL)		IL-12 (pg/mL)		TNF-α (pg/mL)	
compd	1 μg/mL	10 μg/mL	1 μg/mL	10 μg/mL	1 μg/mL	10 μg/mL
1	136.1	110.1	N.D.	N.D.	N.D.	N.D.
2	279.4	470.4	N.D.	N.D.	N.D.	N.D.
3	32.2	16.2	N.D.	N.D.	N.D.	N.D.
4	28.5	607.1	N.D.	N.D.	N.D.	27.9
7	463.0	260.8	17.3	N.D.	26.7	N.D.
8	1245.2	1576.2	14.3	14.7	22.0	24.6
10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11	59.8	1454.4	N.D.	N.D.	N.D.	N.D.
12	2579.3	2579.2	47.3	104.3	132.5	322.9
15	1477.6	451.1	N.D.	N.D.	N.D.	N.D.
16	22.5	777.2	N.D.	28.3	N.D.	22.7
17	N.D.	308.4	N.D.	N.D.	N.D.	N.D.
18	857.3	109.6	21.6	N.D.	54.5	N.D.
20	380.0	76.1	N.D.	N.D.	N.D.	N.D.
21	783.6	203.4	14.3	N.D.	31.3	N.D.
22	57.5	297.6	N.D.	N.D.	N.D.	N.D.
23	705.7	10.6	N.D.	N.D.	N.D.	N.D.
24	793.2	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	662.9	N.D.	N.D.	N.D.	N.D.
26	473.4	198.6	N.D.	N.D.	N.D.	N.D.
27	16.2	22.9	N.D.	N.D.	N.D.	N.D.
28	849.5	38.1	27.2	N.D.	12.6	N.D.
29	101.5	175.5	N.D.	N.D.	N.D.	N.D.
32	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
33	28.1	174.4	N.D.	N.D.	N.D.	N.D.
OK-432	1824.5	2555.2	462.7	542.3	315.8	278.4

[a] Human PBMCs (1×10^6 /mL) were stimulated with the compounds (1 or 10 μg/mL) for 48 h at 37 °C, then cytokines in the supernatants of these cultures were analyzed by ELISA. Data represent mean value of triplicate samples. N.D. = not detected (<7.8 pg/mL).

obtained on a JEOL JMSD-300 instrument. CC: silica gel 60 (Merck), Toyopearl HW-40 (Tosoh), Sephadex LH-20 (Pharmacia), and Diaion HP-20 (Nippon Rensui); HPLC: GPC (gel-permeation chromatography: Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC (Si_1: YMC-Park SIL-06 SH-043-5-06, 250 × 20 mm, hexane−EtOAc system; Si_2: Hibar RT 250-25, LiChrosorb Si 60, $CHCl_3$−MeOH system); ODS: Hibar RT 250-25, LiChrosorb RP 18. IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer), and the CD spectrum was measured on a JASCO CD-J600 spectropolarimeter. Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material. The bark of *Maytenus laevis* was collected from Leticia in Colombia in August 2000 and identified by one of the authors (C.G.). A voucher specimen (OOJC007) is deposited in the Institute de Ciencias Naturales, Universidad Nacional de Colombia, Colombia.

Extraction and Isolation. The dried bark (1.9 kg) of *M. laevis* was extracted with MeOH. The MeOH extracts were concentrated in vacuo to give a residue (248 g), which was suspended in H_2O and partitioned sequentially with EtOAc and *n*-BuOH. The EtOAc layer was concentrated to give a residue (113 g), which was subjected to silica gel column chromatography (1.2 kg, 11 × 100 cm). The column was eluted with solvents of increasing polarity (*n*-hexane−EtOAc, EtOAc, EtOAc−MeOH, MeOH) to give 12 major fractions (1−12). Fraction 2 (3.9 g) was chromatographed on a Toyopearl HW-40 column ($CHCl_3$−MeOH, 2:1) to give four fractions (2.1−2.4). Fraction 2.3 was separated by ODS (MeOH−H_2O, 9:1) to give 18 fractions (2.3.1−2.3.18). Combined fractions 2.3.1 and 2.3.2 crystallized from MeOH to give **14** (33 mg). Fraction 2.3.15 was separated by preparative TLC ($CHCl_3$−MeOH, 95:5) to give **12** (11 mg) and **13** (3.5 mg). Fraction 2.4 was subjected to Si HPLC ($CHCl_3$−MeOH, 98:2) to give **11** (33 mg). Fraction 3 (1.5 g) was chromatographed on a Toyopearl HW-40 column and silica gel column ($CHCl_3$−MeOH) to give five fractions (3.1−3.5). Fraction 3.3 was separated by GPC ($CHCl_3$), GPC (MeOH), Si HPLC ($CHCl_3$−MeOH), and preparative TLC ($CHCl_3$−MeOH) to give **27** (5.9 mg). Fraction 3.4 was subjected to Si HPLC ($CHCl_3$−MeOH) and Si HPLC (*n*-hexanes−EtOAc) to give **4** (7.4 mg). Fraction 4 (4.5 g) was chromatographed on a Toyopearl HW-40 column to give 10 fractions (4.1−4.10). Fraction 4.2 was separated by Si HPLC (*n*-hexane−EtOAc) to give **7** (18 mg), **8** (163 mg), and seven other fractions (4.2.1−4.2.7). Fraction 4.2.6 was separated by preparative TLC ($CHCl_3$−MeOH) to give **1** (4.5 mg) and **10** (13 mg). Fraction 4.3 (yield 0.6 g of 2.4 g) was subjected to ODS (MeOH−H_2O) to give **15** (8.8 mg), **16** (7.0 mg), **17** (8.0 mg), **18** (6.5 mg), and 17 other fractions (4.3.1−4.3.16). Fraction 4.3.10 was further separated by Si HPLC ($CHCl_3$−MeOH) to give **19** (3.5 mg), **20** (18 mg), and nine other fractions (4.3.10.1−4.3.10.9). Fractions 4.3.10.5 and 4.3.10.6 were separated by GPC (MeOH) to give **22** (8.0 mg) and **23** (4.9 mg), respectively. Fraction 4.3.13 was subjected to Si HPLC ($CHCl_3$−MeOH) and GPC (MeOH) to give **24** (7.6 mg). Fraction 4.3.14 was separated by GPC (MeOH) to give **3** (6.7 mg). Combined fractions 4.6 and 4.7 were separated by Si HPLC (hexane−EtOAc) to give **9** (28 mg). Fraction 4.10 was subjected to Si HPLC (*n*-hexane−EtOAc) and preparative TLC ($CHCl_3$−MeOH) to give **2** (14 mg) and **21** (6.1 mg). Fraction 5 (1.5 g) was subjected to Toyopearl HW-40, GPC (MeOH), and ODS (MeOH−H_2O) to give **25** (36 mg) and **26** (5.6 mg). Fraction 7 (yield 3.0 g of 9.2 g) was chromatographed on a Sephadex LH-20 (MeOH) column to give 10 fractions (7.1−7.10). Fraction 7.3 was separated by ODS (MeOH−H_2O) to give **30** (2.7 mg). Fraction 7.7 was subjected to ODS (MeOH−H_2O) and GPC (MeOH) to give **33** (11 mg). Fraction 10 (yield 10 g of 19 g) was chromatographed on a Diaion HP-20 [H_2O−MeOH (1:0, 9:1, 8:2, 7:3, 1:1, MeOH)] column to give 13 fractions (10.1−10.13). Fraction 10.6 was subjected to GPC (MeOH), ODS (MeOH−H_2O), and preparative TLC ($CHCl_3$−MeOH−H_2O) to give **34** (0.9 mg). Combined fractions 10.8 and 10.9 were separated by LH-20 (MeOH) and GPC (MeOH) to give **29** (7.9 mg), **31** (4.2 mg), and **32** (8.3 mg). Fraction 10.10 was subjected to LH-20 (MeOH), GPC (MeOH), and ODS (MeOH−H_2O) to give **5** (2.9 mg), **6** (3.8 mg), and **28** (19 mg).

Treatment of Human PBMCs and Cytokine-Inducing Assay. PBMCs were isolated from heparinized venous blood by Ficoll-Hypaque gradient density centrifugation according to standard procedures.[54] PBMCs (1×10^6/mL) were cultured in RPMI 1640 medium containing 10% fetal calf serum in the presence or absence of the compounds (1 or 10 μg/mL) for 48 h at 37 °C, then cytokines in the supernatants of these cultures were analyzed by commercial ELISA kits. OK-432 (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), a *Streptococcus pyogenes*-derived immunopotentiator that is commonly used for immunotherapy in malignancies, was used as a positive control. ELISA kits for human IFN-γ, TNF-α, IL-4, IL-6, IL-10, and IL-12 were purchased from BioSource International, Inc. (Camarillo, CA).

Compound 1: white amorphous powder; $[\alpha]_D$ +4.3° (*c* 0.2, $CHCl_3$−MeOH); IR (KBr) ν_{max} 3457, 2929, 1744, 1371, 1249, 1118, 1057 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.77 (1H, brs, H-6′), 8.14 (1H, brd, *J* = 6.6 Hz, H-4′), 7.96 (2H, d, *J* = 7.4 Hz, *ortho*-Bz), 7.57 (1H, t, *J* = 7.4 Hz, *para*-Bz), 7.43 (2H, d, *J* = 7.7 Hz, *meta*-Bz), 7.34 (1H, brs, H-5′), 7.07 (1H, brs, H-5), 6.00 (1H, d, *J* = 11.4 Hz, H-15a), 5.76 (1H, d, *J* = 3.8 Hz, H-1), 5.54 (1H, t, *J* = 5.5 Hz, H-7), 5.48 (1H, d, *J* = 13.6 Hz, H-11a), 5.41 (1H, d, *J* = 5.8 Hz, H-8), 4.84 (1H, d, *J* = 2.2 Hz, H-3), 4.81 (1H, d, *J* = 13.6 Hz, H-11b), 4.72 (1H, q, *J* = 6.9 Hz, H-7′), 4.14 (1H, brs, H-2), 3.71 (1H, d, *J* = 11.4 Hz, H-15b), 2.59 (1H, q, *J* = 6.9 Hz, H-8′), 2.36 (1H, d, *J* = 3.8 Hz, H-6), 2.33 (3H, s, 1-Ac), 2.22 (3H, s, 5-Ac), 2.12 (3H, s, 7-Ac), 1.72 (3H, s, H-14), 1.64 (3H, s, H-12), 1.44 (1H, d, *J* = 6.9 Hz, H-9′), 1.42 (3H, s, 8-Ac), 1.20 (1H, d, *J* = 6.9 Hz, H-10′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 75.5 (C-1), 70.3 (C-2), 78.3 (C-3), 70.7 (C-4), 74.1 (C-5), 50.6 (C-6), 69.1 (C-7), 71.8 (C-8), 53.0 (C-9), 94.8 (C-10), 60.5 (C-11), 23.2 (C-12), 84.3 (C-13), 18.6 (C-14), 70.5 (C-15), 165.0 (C-2′), 126.0 (C-3′), 138.8 (C-4′), 121.7 (C-5′), 150.9 (C-6′), 36.6 (C-7′), 45.2 (C-8′), 12.1 (C-9′), 9.8 (C-10′), 174.4 (C-11′), 168.2 (C-12′), 170.3, 21.6 (1-Ac), 170.0, 21.8 (5-Ac), 170.2, 21.1 (7-Ac), 169.2, 20.1 (8-Ac), 165.0, 129.3, 129.9 × 2, 128.8

× 2, 133.8 (11-Bz); HRFABMS *m/z* 826.2911 [M + H]$^+$ (calcd for $C_{41}H_{48}O_{17}N$, 826.2922).

Compound 2: yellow amorphous powder; $[\alpha]_D$ −59.5° (*c* 0.7, MeOH); IR (KBr) ν_{max} 3492, 2938, 2359, 1639, 1594, 1462, 1369, 1166 cm^{-1}; CD (*c* 0.0000898, MeOH) [θ] (nm) −3.4 × 10^{-4} (292) (negative maximum), 0 (317), +1.6 × 10^{-4} (339) (positive maximum); ^{1}H NMR (CD_3OD, 400 MHz) δ 6.56 (2H, brs, H-2′, 6′), 5.97 (1H, brs, H-8), 5.92 (1H, brs, H-6), 5.26 (1H, d, J = 2.1 Hz, H-2), 4.20 (1H, d, J = 2.1 Hz, H-3), 3.80 (3H, brs, 4′-OMe); ^{13}C NMR (CD_3OD, 100 MHz) δ 85.4 (C-2), 75.8 (C-3), 199.0 (C-4), 168.6 (C-5), 99.9 (C-6), 171.3 (C-7), 99.0 (C-8), 167.0 (C-9), 104.6 (C-10), 135.9 (C-1′), 110.4 × 2 (C-2′, 6′), 154.3 (C-3′, 5′), 139.4 (C-4′), 63.5 (4′-OMe); HRFABMS *m/z* 333.0580 [M − H]$^-$ (calcd for $C_{16}H_{13}O_8$, 333.0610).

Compound 3: white amorphous powder; $[\alpha]_D$ +44.4° (*c* 0.7, $CHCl_3$−MeOH); IR (KBr) ν_{max} 3438, 2945, 1705 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 5.41 (1H, brs, H-12), 4.00 (1H, brdd, J = 4.5, 2.7 Hz, H-22), 3.61 (1H, brs, H-3), 2.74 (1H, t, J = 13.8 Hz, H-19a), 2.64 (1H, dd, J = 13.7, 2.8 Hz, H-21a), 2.46 (1H, brd, J = 13.8 Hz, H-18), 2.08 (1H, m, H-21b), 2.03 (1H, m, H-2a), 1.98 (2H, m, H-11), 1.97 (1H, m, H-16a), 1.87 (1H, m, H-9), 1.84 (1H, m, H-15a), 1.80 (3H, s, H-30), 1.78 (2H, m, H-1a, 2b), 1.68 (1H, m, H-19b), 1.67 (1H, m, H-5), 1.55 (1H, *m*, H-7a), 1.52 (2H, m, H-6), 1.44 (1H, m, H-16b), 1.42 (1H, m, H-1b), 1.33 (1H, m, H-7b), 1.26 (3H, s, H-28), 1.22 (3H, s, H-24), 1.15 (3H, s, H-27), 1.07 (3H, s, H-26), 1.01 (1H, m, H-15b), 1.00 (3H, s, H-25), 0.91 (3H, s, H-23); ^{13}C NMR (C_5D_5N, 100 MHz) δ 33.8 (C-1), 26.4 (C-2), 75.2 (C-3), 37.9 (C- 4), 49.2 (C-5), 18.6 (C-6), 33.2 (C-7), 40.2 (C-8), 47.8 (C-9), 37.4 (C-10), 23.8 (C-11), 123.3 (C-12), 144.2 (C-13), 42.5 (C-14), 26.3 (C-15), 28.9 (C-16), 38.0 (C-17), 44.7 (C-18), 41.5 (C-19), 42.5 (C-20), 37.7 (C-21), 75.3 (C-22), 29.2 (C-23), 22.8 (C-24), 15.7 (C-25), 17.3 (C-26), 25.5 (C-27), 20.9 (C-28), 181.4 (C-29), 24.9 (C-30); HRFABMS *m/z* 471.3519 [M − H]$^-$ (calcd for $C_{30}H_{47}O_4$, 471.3474).

Compound 4: yellow amorphous powder; $[\alpha]_D$ +21.5° (*c* 0.7, MeOH); IR (KBr) ν_{max} 3432, 2925, 1681, 1605, 1274, 1182 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51 (1H, d, J = 15.9 Hz, H-7′), 7.03 (1H, d, J = 1.8 Hz, H-2′), 6.93 (1H, dd, J = 8.2, 1.8 Hz, H-6′), 6.80 (1H, d, J = 8.2 Hz, H-5′), 6.21 (1H, d, J = 15.9 Hz, H-8′), 5.11 (1H, brs, H-12), 4.59 (1H, t, J = 8.9 Hz, H-3), 3.52, 3.14 (each 1H, d, J = 11.0 Hz, H-28), 2.26 (2H, m, H-11), 1.92 (2H, m, H-2), 1.88 (1H, m, H-1a), 1.55 (1H, m, H-9), 1.53 (1H, m, H-7a), 1.52 (1H, m, H-6a), 1.51 (1H, m, H-22a), 1.44 (2H, m, H-21), 1.39 (1H, m, H-6b), 1.38 (1H, m, H-22b), 1.37 (1H, m, H-18), 1.36 (1H, m, H-19), 1.35 (1H, m, H-7b), 1.15 (1H, brs, H-15a), 1.09 (3H, s, H-27), 1.08 (1H, m, H-1b), 1.00 (1H, m, H-15b), 0.98 (6H, s, H-25, 26), 0.92 (3H, s, H-24), 0.91 (3H, d, J = 5.8 Hz, H-30), 0.89 (3H, s, H-23), 0.87 (1H, m, H-20), 0.85 (2H, m, H-16), 0.82 (1H, m, H-5), 0.79 (3H, d, J = 5.9 Hz, H-29); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 38.6 (C-1), 23.8 (C-2), 81.1 (C-3), 38.1 (C-4), 55.4 (C-5), 18.3 (C-6), 32.9 (C-7), 40.2 (C-8), 47.7 (C-9), 36.9 (C-10), 23.5 (C-11), 125.1 (C-12), 138.9 (C-13), 42.2 (C-14), 26.1 (C-15), 23.7 (C-16), 38.1 (C-17), 54.2 (C-18), 39.5 × 2 (C-19, 20), 30.7 (C-21), 35.3 (C-22), 28.2 (C-23), 17.0 (C-24), 15.8 (C-25), 16.8 (C-26), 23.4 (C-27), 69.9 (C-28), 17.5 (C-29), 21.4 (C-30), 127.2 (C-1′), 114.0 (C-2′), 144.8 (C-3′), 147.2 (C-4′), 115.3 (C-5′), 122.1 (C-6′), 150.0 (C-7′), 115.7 (C-8′), 167.9 (C-9′); HRFABMS *m/z* 627.4072 [M + Na]$^+$ (calcd for $C_{39}H_{56}O_5Na$, 627.4025).

Compound 5: yellow amorphous powder; $[\alpha]_D$ −31.2° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3499, 1710, 1641, 1601, 1515, 1273, 1132, 1073, 1029 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.67 (1H, dd, J = 8.4, 1.8 Hz, H-6‴), 7.53 (1H, d, J = 1.8 Hz, H-2‴), 7.00 (1H, d, J = 8.4 Hz, H-5‴), 6.89 (1H, d, J = 8.2 Hz, H-5), 6.64 (1H, d, J = 8.4 Hz, H-5′), 6.63 (1H, d, J = 1.8 Hz, H-2), 6.52 (1H, brs, H-2′), 6.50 (1H, m, H-6′), 6.31 (1H, dd, J = 8.2, 1.7 Hz, H-6), 4.83 (1H, m, H-1″), 4.65 (1H, dd, J = 11.7, 2.1 Hz, H-6″a), 4.44 (1H, dd, J = 11.7, 7.3 Hz, H-6″b), 3.89 (3H, s, 4‴-OMe), 3.81 (3H, s, 3‴-OMe), 3.76 (1H, m, H-5″), 3.73 (3H, s, 3-OMe), 3.68 (3H, s, 3′-OMe), 3.55 (4H, brd, J = 4.9 Hz, H-9, 9′), 3.52 (1H, m, H-2″), 3.51 (1H, m, H-3″), 3.44 (1H, m, H-4″), 2.61 (2H, m, H-7a, 7′a), 2.52 (2H, m, H-7b, 7′b), 1.83 (2H, m, H-8, 8′); ^{13}C NMR (CD_3OD, 100 MHz) δ 137.3 (C-1), 114.4 (C-2), 150.4 (C-3), 146.0 (C-4), 117.6 (C-5), 122.6 (C-6), 36.2 (C-7), 44.1 (C-8), 62.1 (C-9), 133.8 (C-1′), 113.3 (C-2′), 144.8 (C-3′), 145.6 (C-4′), 115.8 (C-5′), 122.7 (C-6′), 36.2 (C-7′), 44.1 (C-8′), 62.1 (C-9′), 102.8 (C-1″), 74.9 (C-2″), 77.8 (C-3″), 72.1 (C-4″), 75.6 (C-5″), 65.2 (C-6″), 123.8 (C-1‴), 113.7 (C-2‴), 150.2 (C-3‴), 155.0 (C-4‴), 112.0 (C-5‴), 125.1 (C-6‴), 167.6 (C-7‴), 56.6 × 3 (3, 3‴, 4‴-OMe), 56.3 (3′-OMe); HRFABMS *m/z* 711.2655 [M + Na]$^+$ (calcd for $C_{35}H_{44}O_{14}Na$, 711.2629).

Compound 6: yellow amorphous powder; $[\alpha]_D$ −78.6° (*c* 0.3, MeOH); IR (KBr) ν_{max} 3387, 1710, 1659, 1591, 1505, 1461, 1417, 1334, 1228, 1128, 764 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.36 (2H, brs, H-2′, 6′), 6.24 (1H, dd, J = 6.3, 2.1 Hz, H-3), 5.51 (1H, d, J = 2.5 Hz, H-1), 5.06 (1H, m, H-6), 4.99 (1H, dd, J = 6.3, 2.7 Hz, H-4), 4.66 (1H, d, J = 7.9 Hz, Glc-1), 3.88 (1H, m, Glc-6a), 3.87 (6H, s, H-3′, 5′), 3.82 (3H, s, H-4′), 3.65 (1H, dd, J = 11.8, 5.8 Hz, Glc-6b), 3.36 (1H, t, J = 8.7 Hz, Glc-3), 3.34 (1H, m, Glc-5), 3.26 (1H, m, Glc-4), 3.18 (1H, t, J = 8.7 Hz, Glc-2), 3.01 (1H, m, H-5), 2.62 (1H, brd, J = 9.0 Hz, H-9), 2.28 (1H, dd, J = 14.3, 6.2 Hz, H-7a), 2.06 (1H, dd, J = 14.3, 3.4 Hz, H-7b), 1.41 (3H, s, H-10); ^{13}C NMR (CD_3OD, 100 MHz) δ 93.5 (C-1), 141.3 (C-3), 104.4 (C-4), 39.6 (C-5), 81.2 (C-6), 47.7 (C-7), 79.2 (C-8), 51.8 (C-9), 26.2 (C-10), 126.9 (C-1′), 108.2 × 2 (C-2′, 6′), 154.4 × 2 (C-3′, 5′), 143.7 (C-5′), 56.8 × 2 (3′, 5′-OMe), 61.2 (3′-OMe), 167.4 (C-7′), 99.4 (Glc-1), 74.8 (Glc-2), 78.0 (Glc-3), 71.7 (Glc-4), 78.3 (Glc-5), 62.9 (Glc-6); HRFABMS *m/z* 565.1870 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{13}Na$, 565.1897).

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