Four New Triterpenoids from *Maytenus ilicifolia*

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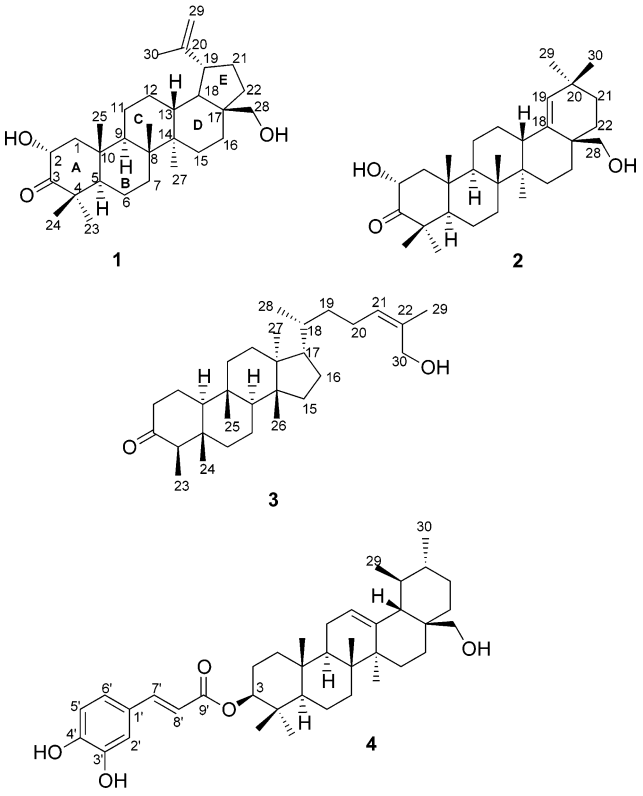
Four new triterpenoids with various skeletons, maytefolins A−C (**1**−**3**) and uvaol-3-caffeate (**4**), were isolated from the leaves of a Brazilian medicinal plant, *Maytenus ilicifolia*, together with five known triterpenoids. Of these triterpenoids only erythrodiol exhibited significant cytotoxicity against KB/S, KB/VJ300, and KU 19-20 cells.

In the search for biologically active compounds in Brazilian medicinal plants,[1] we studied the constituents of the leaves of *Maytenus ilicifolia* Mart. ex Reissek (Celastraceae). *M. ilicifolia*, popular name "Espinheira-santa" in Brazil, is a tree found in the south of South America. The leaves of this plant are widely used to treat gastrointestinal disorders, rheumatism, diureasis, and fever.[2] Previous studies of this plant have resulted in the isolation of several triterpenoids,[3] alkaloids,[4] and glucosides.[5] In this paper, we describe the isolation and structural elucidation of four new triterpenoids, maytefolins A−C (**1**−**3**) and uvaol-3-caffeate (**4**), as well as their cytotoxicity against KB/S, KB/VJ300, and KU19-20 cells.

The leaves of *M. ilicifolia* were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. EtOAc-soluble materials were chromatographed over an ODS column. The fractions obtained were separated using a silica gel column and reversed-phase HPLC to afford **1**−**4**, together with five known triterpnoids, betulin,[6] betulin-3-caffeate,[6] moradiol,[7] erythrodiol,[8] and erythrodiol-3-caffeate.[9]

The molecular formula, $C_{30}H_{48}O_3$, of **1** was established by HREIMS, and the IR spectrum implied the presence of hydroxyl groups, a carbonyl group, and an exomethylene group. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data aided by 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^1H NMR, ^{13}C NMR, and HMQC experiments of **1** indicated the presence of one ketone carbonyl, one sp^3 oxymethine, one oxymethylene, one exomethylene, one olefinic methyl, one sp^2 quaternary carbon, five sp^3 quaternary carbons, five sp^3 methines, nine methylenes, and five methyl carbons. Because two of seven unsaturations were thus accounted for, it was concluded that **1** contained five rings. HMBC correlations (Figure 1, Supporting Information) suggested that **1** had a lupane skeleton. Full ^1H NMR assignments are detailed in the Experimental Section, and ^{13}C assignments are given in Table 1. The NOESY cross-peaks of H-2/H_3-24, H-2/H_3-25, H-18/H_3-30, H-18/H-29a, and H-19/H_2-28 showed that a hydroxyl group at C-2 and a propenyl group at C-19 are α-oriented and a hydroxy methylene group at C-17 is *β*-oriented. Thus, maytefolin A was concluded to be 2α,28-dihydroxy-3-oxo-20-lupene (**1**).



Maytefolin B (**2**) showed a molecular ion peak [*m*/*z* 456.36] in an EIMS spectrum; however, HREIMS showed a dehydroxymethyl ion peak at *m*/*z* 425.3405, $(M - CH_2OH)^+$. Thus, the molecular formula of **2** was also deduced as $C_{30}H_{48}O_3$. The ^1H and ^{13}C NMR (Table 1) and HMQC data of **2** closely resembled those of **1** except for ring E. Ring E had one trisubstitued olefin (δ_C 134.8, δ_H 5.16 brs, δ_C 138.2), two singlet methyl carbons (δ_C 31.3, δ_H 0.96 s and δ_C 29.2, δ_H 0.97 s), and one sp^3 quaternary carbon (δ_C 32.3). HMBC experiments showed correlations of H_3-29 to C-19, C-20, C-30, and C-21, H_3-30 to C-20 and C-21, and H-19 to C-17 and C-13. The NOESY spectrum of **2** showed cross-peaks of H_3-29/H-19 and H-19/H-12. Thus, **2** was concluded to be 2α,28-dihydroxy-3-oxo-18-oleanene.

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Table 1. ^{13}C NMR Data (δ, ppm) of Compounds **1**−**4**

	1[a]	**2**[a]	**3**[a]	**4**[b]
1	49.1	50.1	22.8	33.6
2	69.4	69.4	41.6	24.1
3	216.7	216.6	213.1	80.9
4	47.8	47.8	58.3	38.9
5	57.9	58.0	42.4	56.0
6	19.0	19.0	40.8	17.9
7	34.0	34.3	20.3	39.2
8	41.1	41.0	49.8	40.9
9	50.1	50.8	37.9	48.4
10	38.0	38.0	59.1	37.6
11	21.1	21.3	36.7	23.9
12	25.0	26.0	30.0	122.5
13	37.3	38.5	46.2	140.1
14	42.9	43.3	48.1	42.8
15	27.0	27.3	34.0	24.5
16	29.1	31.2	28.1	23.8
17	47.7	39.5	50.4	38.9
18	48.7	138.2	35.7	55.1
19	47.8	134.8	36.7	40.2
20	150.2	32.3	24.5	40.4
21	29.7	33.2	129.2	28.5
22	33.9	31.6	133.9	36.3
23	24.6	24.6	6.8	26.8
24	24.1	21.4	14.7	17.3
25	16.7	17.2	18.5	17.2
26	16.2	16.3	19.3	16.1
27	14.7	14.6	15.8	21.7
28	60.6	65.6	18.5	69.6
29	109.9	31.3	21.3	17.3
30	19.1	29.2	61.7	19.0
1′				127.7
2′				122.4
3′				116.4
4′				148.7
5′				146.3
6′				115.2
7′				145.3
8′				116.2
9′				167.2

[a] In $CDCl_3$. [b] In CD_3COCD_3.

The molecular formula of maytefolin C (**3**) was determined as $C_{30}H_{50}O_2$ (HREIMS), and the IR spectrum indicated the presence of hydroxyl and ketone groups. The NMR data of **3** (Experimental Section and Table 1) were similar to those of astertarone A, which was previously isolated from the root of *Aster tartaricus*,[10] except for the absence of one methyl group and the presence of a hydroxymethyl group instead. These data suggested that **3** was a 30-hydroxyastertarone A. The stereochemistry of **3** was established by the NOESY experiment. Compound **3** showed significant NOE correlations between H-4 and H-10, H-23 and H-24, H-24 and H-25, H-25 and H-26, H-26 and H-17, and H-27 and H-18 (Figure 3, Supporting Information), which were consistent with those observed for astertarone A.[10] Thus, **3** was concluded to be 18*R*-D:A-friedoeuph-21-en-30-ol-3-one, and it possesses the same A, B, and C rings and C/D ring junction as D:A-friedooleanan-29-ol-3-one, which has also been isolated from *M. ilicifolia*.[11]

The molecular formula of **4** was determined as $C_{39}H_{56}O_5$ (HRFABMS). The IR spectrum indicated the presence of a hydroxyl (3240 cm^{-1}) and an α,β-unsaturated ester carbonyl (1684, 1604 cm^{-1}) group. The ^1H NMR, ^{13}C NMR, HMQC, $^1H-^1H$ COSY, and HMBC spectra of **4** indicated the presence of a caffeoyl unit, and the data for its triterpene moiety were identical with those for uvaol.[12] Furthermore, the caffeoyl unit connected to the O-3 position of the uvaol moiety as deduced by the HMBC correlation of H-3 (δ_H 4.59) to C-9′ (δ_C 167.2). Thus, **4** was uvaol-3-caffeate.

Maytefolin A (**1**), uvaol-3-caffeate (**4**), betulin, its caffeate, erythrodiol, and its caffeate were assayed for their cytotoxicity and differentiation inducibility on human tumor cells including drug-resistant cells. Only erythrodiol showed significant activity with an IC_{50} of 7.0 μg/mL on KB/S cells, whereas all the other compounds had IC_{50} values higher than 10 μg/mL. None of these compounds showed any reversal effect on vincristine resistance in KB/VJ300 cells, and they induced no morphological changes on HL-60 cells at concentrations of 0.05−25 μg/mL. Therefore, it is considered that these compounds possess no differentiation activity.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were obtained on a Bruker AMX500 spectrometer using tetramethylsilane as the internal standard. HREIMS and HRFABMS spectra were obtained on JEOL HX-100 and JEOL-HX-110 spectrometers, respectively. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV and IR spectra were obtained on Shimadzu UV-260 and JASCO FT/IR-5300 spectrometers, respectively.

Plant Material. The leaf of *Maytenus ilicifolia* was purchased in Sao Paulo, Brazil. The plant was identified by Dr. G. Hashimoto (Centro de Pesquisas de Historia), and a voucher specimen with the identification number B 014 has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Isolation. The MeOH extract (116.4 g) of the leaf of *M. ilicifolia* (838 g) was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (47.9 g) was subjected to reversed-phase column chromatography (MCI Gel ODS 1MY, Mitsubishi Chemical Corp., step gradient with H_2O-CH_3CN) to give six fractions. The fifth fraction (1.10 g) eluted with H_2O-CH_3CN (20:80) and the sixth fraction (2.18 g) eluted with H_2O-CH_3CN (0:100) were further chromatographed over a silica gel column (n-C_6H_{12}−EtOAc) and then purified by reversed-phase HPLC (Capcellpak C18 UG 80, Shiseido, H_2O-CH_3CN) to afford maytefolins A (**1**, 7.4 mg, 0.00088%), B (**2**, 1.7 mg, 0.000202%), and C (**3**, 1.7 mg, 0.000202%), uvaol-3-caffeate (**4**, 10.4 mg, 0.00124%), betulin (42.5 mg, 0.00507%), betulin-3-caffeate (1.6 mg, 0.000191%), erythrodiol-3-caffeate (8.9 mg, 0.00106%), moradiol (4.4 mg, 0.000457%), and erythrodiol (1.5 mg, 0.000156%).

Maytefolin A (1): colorless amorphous solid; $[\alpha]^{23}_D$ +17.5° (c 0.092, $CHCl_3$); IR (KBr) ν_{max} 3433, 1704, 1641 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (1H, dd, J = 13.2, 7.0 Hz, H-1a), 1.4 (1H, m, H-1b), 4.53 (1H, dd, J = 12.6, 7.0 Hz, H-2), 1.4 (1H, m, H-9), 1.6 (1H, m, H-18), 2.4 (1H, m, H-19), 2.0 (1H, m, H-16a), 1.4 (1H, m, H-16b), 1.15 (3H, s, H-23), 1.09 (3H, s, H-24), 1.17 (3H, s, H-25), 1.08 (3H, s, H-26), 0.95 (3H, s, H-27), 3.34 (1H, d, J = 12.6 Hz, H-28a), 3.79 (1H, d, J = 12.6 Hz, H-28b), 4.59 (1H, d, J = 1.9 Hz, H-29a), 4.68 (1H, d, J = 1.9 Hz, H-29b), 1.67 (3H, s, H-30); ^{13}C NMR (Table 1); HREIMS m/z 456.3616 $[M]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3603).

Maytefolin B (2): colorless amorphous solid; $[\alpha]^{23}_D$ −45.0° (c 0.08, $CHCl_3$); IR (KBr) ν_{max} 3445, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10 (1H, m, H-1a), 2.55 (1H, dd, J = 12.7, 6.8 Hz, H-1b), 4.56 (1H, m, H-2), 1.1 (1H, m, H-5), 1.4 (1H, m, H-7a), 1.5 (1H, m, H-7b), 1.1 (1H, m, H-15a), 1.74 (1H, brd J = 12.0, H-15b), 1.3 (1H, m, H-16a), 1.6 (1H, m, H-16b), 5.16 (1H, brs, H-19), 1.5 (2H, m, H-21), 1.8 (2H, m, H-22), 1.15 (3H, s, H-23), 1.10 (3H, s, H-24), 1.22 (3H, s, H-25), 1.11(3H, s, H-26), 0.75 (3H, s, H-27), 3.47 (1H, dd, J = 10.4, 7.7 Hz, H-28a), 3.63 (1H, dd, J = 10.4, 3.0 Hz, H-28b), 0.96 (1H, s, H-29), 0.97 (3H, s, H-30); ^{13}C NMR (Table 1); EIMS m/z 456.35 $[M]^+$; HREIMS m/z 425.3405 $[M - CH_2OH]^+$ (calcd for $C_{29}H_{45}O_2$, 425.3419).

Maytefolin C (3): colorless amorphous solid; $[\alpha]^{23}_D$ +15.0° (c 0.08, $CHCl_3$); IR (KBr) ν_{max} 3447, 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.9 (1H, m, H-1a), 2.32 (1H, dd, J = 13.6, 7.5 Hz, H-2a), 2.40 (1H, dd, J = 13.6, 4.7 Hz, H-2b), 2.27 (1H, q, J = 7.1 Hz, H-4), 1.3 (1H, m, H-6a), 1.7 (1H, m, H-6b), 1.3 (1H, m, H-16a), 1.9 (1H, m, H-16b), 1.45 (1H, m, H-17), 1.45 (1H, m,

H-18), 1.4 (1H, m, H-19), 1.9 (1H, m, H-20a), 2.15 (1H, m, H-20b), 5.30 (1H, brt, $J = 7.3$ Hz, H-21), 0.88 (3H, d, $J = 7.1$, $J = 5.6$ Hz, H-23), 0.72 (3H, s, H-24), 0.86 (3H, s, H-25), 0.78 (3H, s, H-26), 0.87 (3H, s, H-27), 0.91 (3H, d, H-28), 1.80 (3H, s, H-29), 4.14 (2H, d, $J = 5.2$ Hz, H-30); ^{13}C NMR (Table 1); HRSIMS m/z 442.3791 $[M]^+$ (calcd for $C_{20}H_{23}O_5$, 442.3811).

Uvaol-3-caffeate (4): colorless amorphous solid; $[\alpha]^{23}_D$ +54.5° (c 0.12, $CHCl_3$); IR (KBr) ν_{max} 3240 1684, 1604 cm^{-1}; ^{1}H NMR (CD_3COCD_3) δ 1.7 (1H, m, H-1a), 1.4 (1H, m, H-1b), 1.7 (1H, m, H-2a), 1.7 (2H, m, H-2b), 4.59 (1H, m, H-3), 1.0 (1H, m, H-5), 1.6 (1H, m, H-6a), 1.50 (1H, m, H-6b), 1.2 (1H, m, H-7a), 1.7 (1H, m, H-7b), 1.7 (1H, m, H-9), 2.0 (1H, m, H-11a), 2.1 (1H, m, H-11b), 5.15 (1H, s, H-12), 1.0 (1H, m, H-15a), 1.9 (1H, m, H-15b), 1.2 (2H, m, H-16), 1.4 (1H, m, H-18), 1.4 (1H, m, H-18), 1.4 (1H, m, H-19), 0.9 (1H, m, H-20), 1.3 (1H, m, H-21a), 1.4 (1H, m, H-21b), 1.4 (1H, m, H-22), 1.6 (1H, m, H-22b), 0.92 (3H, s, H-23), 0.97 (3H, s, H-24), 1.04 (3H, s, H-25), 1.04 (3H, s, H-26), 3.07 (1H, d, $J = 12.6$ Hz, H-28a), 3.55 (1H, d, $J = 12.6$ Hz, H-28b), 0.85 (3H, d, $J = 5.1$ Hz, H-29), 0.93 (3H, d, $J = 6.3$ Hz, H-30), 7.16 (1H, brs, H-2′), 6.87 (1H, d, $J = 6.7$ Hz, H-5′), 7.05 (1H, brs, H-6′), 7.53 (1H, d, $J = 16.0$ Hz, H-7′), 6.30 (1H, d, $J = 16.0$ Hz, H-8′); ^{13}C NMR (Table 1); HRFABMS m/z 627.4040 $[M + Na]^+$ (calcd for $C_{39}H_{56}O_5Na$, 627.4026).

Supporting Information Available: HMBC and NOESY correlations for compounds **1**−**3** and experimental details of cytotoxicity tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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