Lupane Triterpenoids from *Maytenus* Species

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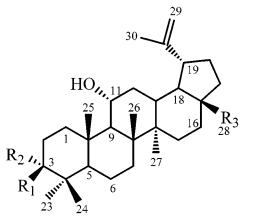
Five new lupane triterpenes (**1**−**5**), in addition to 24 known ones, were isolated from *Maytenus cuzcoina* and *M. chiapensis*. Their structures were elucidated on the basis of spectroscopic analysis, including homonuclear and heteronuclear correlation NMR (COSY, ROESY, HSQC, and HMBC) experiments. The compounds were assayed for antimicrobial and cytotoxic activities, with 3-*epi*-betulinic acid and 28,30-dihydroxy-3-oxolup-20(29)-ene showing moderate cytotoxicity.

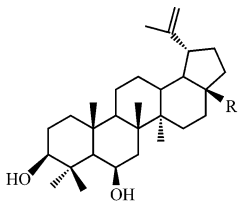
Species of the genus *Maytenus* (Celastraceae) are being investigated for bioactive compounds since they are widely used in traditional medicine and agriculture in North Africa, South and Central America, and Central and East Asia.[1] As a part of our studies of species of this genus, we have previously reported on dihydro-β-agarofuran sesquiterpenes as multidrug-resistance inhibitors[2,3] and antitumor promoters,[4] and sesquiterpene pyridine alkaloids with insecticidal activity[5] from *Maytenus cuzcoina* Loesener, a plant endemic to the Cusco region of Peru, and *Maytenus chiapensis* Lundell, a species that grows in El Salvador.

Nearly 200 different triterpene skeletons are known from natural sources or enzymatic reactions that are structurally consistent with being cyclization products of squalene, oxidosqualene, or bis-oxidosqualene.[6] Triterpenoids from the Celastraceae include those belonging to the lupane, oleane, friedelane, taraxerane, glutinane, ursane, dammarane, and baccharane series.[7]

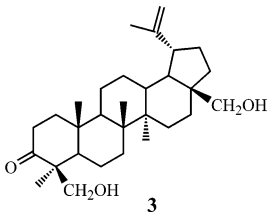
In a continuation of our work on *M. cuzcoina* and *M. chiapensis*, we report herein on the isolation and structural elucidation of five new lupane triterpenoids (**1**−**5**), whose structures were determined by application of 1D and 2D NMR techniques, including COSY, HSQC, HMBC, and ROESY experiments. In addition, 24 known lupane triterpenes were also isolated and were identified as 3β,6β-dihydroxylup-20(29)-ene (**6**),[8] nepeticin,[9] rigidenol,[10] 6β,28-dihydroxy-3-oxolup-20(29)-ene,[8] 6β-hydroxy-3-oxolup-20(29)-en-28-oic acid,[11] resinone,[12] glochidone,[13] lupeol,[13] betulin,[14] betulinic aldehyde,[15] 3-*epi*-betulin,[14] 3-*epi*-betulinic aldehyde,[15] 3-*epi*-betulinic acid,[15] lupenone,[13] betulone,[14] betulone aldehyde,[15] betulonic acid,[14] glochidiol,[13] 3-*epi*-glochidiol,[13] glochidonol,[13] messagenin,[16] 28,30-dihydroxy-3-oxolup-20(29)-ene,[17] lupan-3β-caffeate,[18] and betulin-3β-caffeate[18] in comparison of their spectral data with values reported in the literature.



1 R_1= H, R_2= OH, R_3= CH_2OH
4 R_1= R_2= O, R_3=CHO



2 R= CH_2OH
6 R= CH_3



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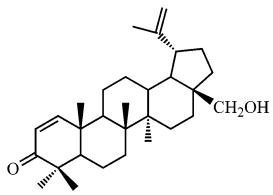

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Results and Discussion

Repeated chromatography of the CH_2Cl_2 extract of the leaves of *M. chiapensis* and the *n*-hexane−Et_2O (1:1) extract of the root bark of *M. cuzcoina* on silica gel and Sephadex LH-20 yielded five new lupane triterpenoids (**1**−**5**).

Compound **1** was assigned the molecular formula $C_{30}H_{50}O_3$ by HREIMS. The IR spectrum suggested that it contained hydroxyl groups (3404 cm^{-1}) and a terminal double bond (2923, 1595, and 885 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed five methyl groups [δ_H 0.85, 0.94, 1.01, 1.02, 1.05 (each 3H, s)], an isopropenyl group [δ_H 1.68 (3H, s), 4.60 (1H, br s), and 4.71 (1H, br s)], and two oxymethine protons in proximity to a hydroxyl group [δ_H 3.36 (1H, s) and 3.91 (1H, dt, J = 5.3, 10.7 Hz)]; signals for a hydroxymethyl group (δ_H 3.33, 3.74, 2H, d_{AB}, J = 10.8 Hz) and a typical lupene H_β-19 proton signal at δ_H 2.39 (1H, dt, J = 4.9, 10.7 Hz) were also observed. These data provided evidence that **1** belongs to the lupane family. Its ^{13}C NMR spectrum (Table 2) revealed 30 carbon signals, which were assigned by DEPT experiments as six methyls, nine methylenes, five methines, five quaternary carbons, two alcoholic methines, one hydroxymethine, and two olefinic carbons. The two sp^2 carbons observed at δ_C 149.8 (s) and 110.2 (t) in the ^{13}C NMR spectrum confirmed the $\Delta^{20,29}$-functionality of a lupene skeleton. Assignments of the ^{1}H and ^{13}C signals performed by extended 2D NMR methods indicated **1** is an analogue of 3-*epi*-betulin[14] bearing one secondary hydroxyl group. The position of this hydroxyl group at C-11 was determined through an HMBC experiment in which the oxymethine proton signal (δ_H 3.91) showed ^2J correlations with C-9 (δ_C 55.6) and C-12 (δ_C 37.6) and ^3J correlations with C-13 (δ_C 36.3) and C-10 (δ_C 39.1). The stereochemistry of the hydroxyl group at C-3 was assigned as α on the basis of the small J values (δ 3.36, br s, $W_{1/2}$ = 6.9 Hz), which indicated an equatorial position for H-3 on the β-face and an axial orientation for the

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Table 1. ^{1}H NMR (400 MHz) Data (δ, $CDCl_3$, J are given in parentheses) of **1**−**6**

H	1	2	3	4	5	6
1	1.47 2.28	0.90 1.67	1.52 1.90[a]	2.66	7.09 d (10.1)	0.91 1.67
2	1.48 1.98	1.61[a]	2.40[a] 2.60	2.42	5.79 d (10.1)	1.59
3	3.36 br s	3.12 t (7.3)				3.14 t (7.3)
5	1.24	0.68 br s	1.60	1.48[a]	1.55	0.68 br s
6	1.42	4.51 br s	1.43, 1.50	1.52	1.39	4.53 br s
7	1.47	1.61[a]	1.45	1.55	1.56	1.61
9	1.45[a]	1.30[a]	1.40[a]	1.48[a]	1.65[a]	1.30
11	3.92 dt (5.3, 10.7)	1.30[a], 1.45	1.40[a]	3.90 dt (5.3, 10.7)	1.48	
12	1.10, 1.91	1.03[a], 1.64	1.67	2.10	1.65[a]	
13	1.75	1.74	1.70[a]	2.25	1.72	1.76
15	1.10, 1.62	1.65	1.70[a]	0.75	1.80	
16	0.99, 1.85	1.03[a], 1.85	1.05, 1.87	2.08	1.52	
18	1.59	1.58	1.65	1.75	1.68	1.38
19	2.39 dt (4.9, 10.8)	2.39 dt (5.6, 10.4)	2.40[a] m	2.89 dt (4.9, 10.8)	2.40 dt (5.7, 10.7)	2.39 m
21	1.95	1.40	1.95	2.01	1.98	
22	1.45[a]	1.90	1.90[a]	1.80	1.91	
23	0.94 s	1.04 s	1.26 s	1.08 s	1.13 s	1.06 s
24	0.85 s	1.14 s	3.43, 3.97 d_{AB} (11.2)	1.04 s	1.08 s	1.15 s
25	1.05 s	1.18 s	0.86 s	1.06 s	1.06 s	1.20 s
26	1.02 s	1.34 s	1.02 s	0.95[a] s	1.04 s	1.36 s
27	1.01 s	0.94 s	0.99 s	0.95[a] s	0.99 s	0.92 s
28	3.33, 3.74 d_{AB} (10.8)	3.32, 3.79 d_{AB} (10.7)	3.33, 3.78 d_{AB} (10.6)	9.61 s	3.35, 3.80 d_{AB} (10.6)	0.80 s
29	4.60 br s 4.71 br s	4.57 br s 4.68 br s	4.58 br s 4.68 br s	4.65 br s 4.78 br s	4.60 br s 4.69 br s	4.59 br s 4.70 br s
30	1.68 s	1.67 s	1.68 s	1.73 s	1.69 s	1.69 s

[a] Overlapping signals.

Table 2. ^{13}C NMR (100 MHz) Data[a] (δ, $CDCl_3$) of **1**−**6**

C	1	2	3	4	5	6
1	35.5 t	40.7 t	39.3 t	42.4 t	125.2 d	40.7 t
2	25.6 t	27.5 t	34.3 t	34.3 t	159.2 d	29.7 t
3	75.9 d	79.1 d	221.4 s	218.6 s	205.0 s	79.1 d
4	37.8 s	39.6 s	50.7 s	47.6 s	44.6 s	39.6 s
5	48.9 d	55.6 d	55.3 d	54.9 d	53.5 d	55.6 d
6	18.0 t	68.9 d	19.2 t	19.5 t	19.0 t	69.0 d
7	35.1 t	42.0 t	33.6 t	34.2 t	33.7 t	42.1 t
8	42.6 s	40.0 s	40.7 s	42.1 s	41.8 s	39.9 s
9	55.6 d	51.0 d	49.5 d	54.7 d	44.5 d	51.9 d
10	39.1 s	36.7 s	36.6 s	38.2 s	39.5 s	36.7 s
11	70.5 d	21.0 t	21.7 t	70.1 d	21.2 t	21.1 t
12	37.6 t	25.3 t	25.2 t	37.6 t	25.2 t	25.3 t
13	36.3 d	36.4 d	37.5 d	37.6 d	37.5 d	37.2 d
14	42.9 s	42.9 s	42.8 s	42.3 s	43.0 s	43.0 s
15	27.0 t	27.1 t	27.0 t	28.8 t	27.0 t	27.5 t
16	33.8 t	33.6 t	34.0 t	28.9 t	29.1 t	35.5 t
17	47.8 s	47.7 s	47.8 s	59.3 s	47.7 s	43.1 s
18	48.2 d	48.8 d	48.6 d	59.3 s	48.6 d	48.4 d
19	47.6 d	47.7 d	47.8 d	47.2 d	47.8 d	48.0 d
20	149.8 s	150.4 s	150.3 s	149.1 s	150.3 s	150.9 s
21	29.1 t	29.7 t	29.7 t	29.8 t	29.7 t	29.9 t
22	29.3 t	29.1 t	29.1 t	32.9 t	33.9 t	40.0 t
23	22.3 q	27.6 q	22.1 q	20.7 q	21.4 q	27.6 q
24	28.7 q	16.9 q	65.3 t	27.4 q	27.8 q	16.8 q
25	16.3 q	17.7 q	17.0 q	16.8 q	19.2 q	17.7 q
26	17.2 q	16.9 q	15.6 q	16.7 q	16.5 q	16.9 q
27	14.1 q	15.1 q	14.7 q	14.1 q	14.6 q	14.9 q
28	60.6 t	60.4 t	60.5 t	206.0 d	60.6 t	18.0 q
29	110.2 t	109.7 t	109.8 t	110.7 t	109.9 t	109.4 t
30	19.1 q	19.1 q	19.1 q	19.0 q	19.0 q	19.3 q

[a] Data are based on DEPT, HSQC, and HMBC experiments.

hydroxyl group on the α-face, and confirmed by the correlations of H-3 with Me-23 and Me-24 (δ_H 0.94 and 0.85) and the absence of interactions between H-3 and H-5 in a NOESY experiment. Similarly, the relative stereochemistry of the hydroxyl group at C-11 was assigned as α on the basis of the NOESY spectrum, which showed a correlation between the oxymethine proton at δ_H 3.92 and Me-26. Compound **1** was therefore assigned the structure 11α-hydroxy-*epi*-betulin.

The structure of compound **2**, with the same molecular weight as **1**, was elucidated by ^{1}H and ^{13}C NMR studies (Tables 1 and 2), 2D correlations (^{1}H−^{1}H and ^{1}H−^{13}C), and a ROESY experiment. Compound **2** exhibits a structure similar to **1**, the differences being in the stereochemistry of the hydroxyl group at C-3 and the presence of a secondary hydroxyl group (δ_H 4.51 br s) at C-6 instead of C-11 as in **1**. Those differences were well established by an HMBC experiment in which H-6 (δ_H 4.51) showed ^3J correlations with C-8 (δ_C 40.0) and C-10 (δ_C 36.7), while H-5 (δ_H 0.68) was correlated with C-6 (δ_C 68.9). The relative stereochemistry of the hydroxyl group at C-6 was resolved as β by analysis of a ROESY experiment, where H-6 showed a NOE effect with Me-23 (δ_H 1.04) and H-5 (δ_H 0.68). This evidence allowed us to establish the structure of **2** as 6β-hydroxybetulin.

Compound **3** was assigned the molecular formula $C_{30}H_{48}O_3$ by HREIMS. The IR spectrum showed absorption bands for hydroxyl (3425 cm^{-1}) and carbonyl (1705 cm^{-1}) groups and a terminal double bond (3011, 1650, and 910 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed four methyl groups [δ_H 0.86, 0.99, 1.02, 1.26 (each 3H, s)], an isopropenyl group [δ_H 1.68 (3H, s), 4.58 (1H, br s), and 4.68 (1H, br s)], two hydroxymethyl groups [δ_H 3.33, 3.78 (d, J = 10.6 Hz, each 1H) and 3.43, 3.97 (2H, d_{AB}, J = 11.2 Hz)], and a typical lupene H$_\beta$-19 proton signal at δ_H 2.40 (1H, m) (Table 1). These data were confirmed by the ^{13}C NMR spectrum, which revealed signals for the corresponding two hydroxymethyl carbons (δ_C 60.5 and 65.3), one ketone carbonyl (δ_C 221.4), and two olefinic carbons (δ_C 109.8 and 150.3) (Table 2), characteristic of $\Delta^{20,29}$-functionality of a lupene skeleton. The two hydroxymethyl groups and the

ketone carbonyl were located on the triterpene skeleton by an HMBC experiment. Thus, correlation between Me-23 at δ_H 1.26 and a quaternary carbon at δ 50.7 (C-4), one ketonic carbon at δ_C 221.4, and a methylene carbon attached to an oxygen at δ_C 65.3 located one hydroxymethyl group at C-24 and the carbonyl group at C-3, while correlation of the protons at δ_H 3.33, 3.78 with C-16 (δ_C 34.0) and C-22 (δ_C 29.1) placed the other hydroxymethyl group at C-28. The stereochemistry of **3** was confirmed by a ROESY experiment, showing NOE effects of the hydroxymethyl groups at C-24 and C-28 to Me-25 (δ_H 0.86) and H-19 (δ_H 2.40), respectively, indicating their relative position as β. All of these data and comparison with those found in the literature for betulone[14] established the structure of **3** as 24-hydroxybetulone.

Compound **4** was assigned the molecular formula $C_{30}H_{46}O_3$ by HREIMS. The most significant signals of its ^{1}H NMR spectrum were those corresponding to an aldehyde proton at δ_H 9.61 (1H, s), an isopropenyl group [δ_H 1.73 (3H, s, Me-30), and 4.65 (1H, br s) and 4.78 (1H, br s)], an oxymethine proton at δ_H 3.90 (1H, dt, J = 5.3, 10.7 Hz, H-11), a methylene group vicinal to a ketone (δ_H 2.42, m), and a typical lupene H_β-19 proton signal at δ_H 2.89 (1H, dt, J = 4.9, 10.8 Hz) (Table 1), which were confirmed by its ^{13}C NMR spectrum (Table 2). These data, 2D NMR experiments, and those given in the literature for rigidenol[10] revealed that **4** is rigidenol-28-aldehyde. The aldehyde group was located at C-28 by an HMBC experiment in which the aldehyde proton signal at δ_H 9.61 showed ^2J and ^3J correlations with C-17 (δ_C 59.3) and C-18 (δ_C 59.3), respectively.

The structure of compound **5** was determined as 28-hydroxyglochidone by ^{1}H and ^{13}C NMR studies (Tables 1 and 2), 2D ^{1}H−^{1}H and ^{1}H−^{13}C correlations, a ROESY experiment, and comparison with data in the literature for glochidone.[13] The position of the hydroxyl group on C-28 was established by the correlations between the signal at δ_H 3.35, 3.80 (d_{AB}, J = 10.6 Hz) and two methylene carbons at δ_C 29.1 (C-16) and δ_C 33.9 (C-22) observed in an HMBC experiment. The detailed ^{1}H and ^{13}C NMR assignments of the one known lupane triterpene, 3β,6β-dihydroxylup-20-(29)-ene (**6**),[8] which have not been previously reported, were achieved by 1D and 2D techniques, including DEPT, HMBC, HSQC, COSY, and ROESY experiments (see Experimental Section).

All the isolated compounds, except for **3**, **4**, **6**, betulinic aldehyde, 3-*epi*-betulinic aldehyde, and betulonic acid, were assayed for antimicrobial and cytotoxic activities. Only 6β-hydroxy-3-oxolup-20(29)-en-28-oic acid showed slight activity against spore-forming bacteria, such as *B. subtilis* (MIC 35 μg/mL), *B. cereus* (MIC 25 μg/mL), and *B. pumilus* (MIC 20 μg/mL). The other compounds were inactive (up to 40 μg/mL) against all microorganisms assayed.

Cytotoxic activity was evaluated against HeLa, Hep-2, and Vero cell lines. Compounds 3-*epi*-betulinic acid and 28,-30-dihydroxy-3-oxolup-20(29)-ene exhibited moderate activity against HeLa (IC_{50} 2.1 and 4.0 μg/mL, respectively) and Hep-2 (IC_{50} 3.1 and 7.1 μg/mL, respectively) cell lines, while nepeticin and glochidonol showed slight potency (IC_{50} < 10 μg/mL). The other compounds assayed were inactive (IC_{50} > 10 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR (film) spectra were recorded on a Bruker IFS 55 spectrophotometer, and UV spectra were collected in absolute EtOH on a JASCO V-560 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer. EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Purification was performed using silica gel (particle size 40−63 μM, Merck and HPTLC-Platten-Sil 20 UV_{254}, Panreac) and Sephadex LH-20 (Pharmacia).

Plant Material. *Maytenus cuzcoina* Loesener was collected at Huayllabamba-Urquillos, Province of Urabamba, Cusco (Peru), in December 1993, and a voucher ("cuz" 02765 A.T. 1004 MO) is deposited in the herbarium of Vargas, Department of Botany, in the National University of San Antonio Abad de Cusco. *Maytenus chiapensis* Lundell was collected at the Parque Nacional El Imposible, El Salvador, in August 1999, and was identified by Prof. Edi Montalvo; a voucher specimen (ISB-88) is on file in the Jardin Botánico La Laguna, El Salvador.

Extraction and Isolation. The root bark of *M. cuzcoina* (900.0 g) was extracted with *n*-hexane−Et_2O in a Soxhlet apparatus. The extract (25.5 g) was chromatographed on Sephadex LH-20 (*n*-hexane−$CHCl_3$−MeOH, 2:1:1) to afford 64 fractions. Thus, successive chromatographies on Sephadex LH-20 (*n*-hexane−$CHCl_3$−MeOH, 2:1:1), silica gel (CH_2Cl_2−Et_2O of increasing polarity), and preparative HPTLC developed with *n*-hexane−Et_2O (4:6) gave rise to compounds **1** (10.0 mg, R_f 0.41), **4** (2.0 mg, R_f 0.6), and **5** (3.5 mg, R_f 0.8), not previously described, in addition to the 17 known compounds: nepeticin (220.0 mg), rigidenol (200.0 mg), glochidone (75.0 mg), lupeol (10.0 mg), betulin (16.0 mg), betulinic aldehyde (5.0 mg), 3-*epi*-betulin (30.0 mg), 3-*epi*-betulinic aldehyde (5.0 mg), 3-*epi*-betulinic acid (28.0 mg), lupenone (7.0 mg), betulone (10.0 mg), betulone aldehyde (4.0 mg), betulonic acid (3.0 mg), glochidiol (40.0 mg), 3-*epi*-glochidiol (350.0 mg), glochidonol (125.0 mg), and lupan-3β-caffeate (7.0 mg).

The leaves of *M. chiapensis* (2.1 kg) were extracted with EtOH in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure provided 400.2 g of crude extract, which was partitioned into a CH_2Cl_2−H_2O (1:1, v/v) solution. Removal of the CH_2Cl_2 from the organic-soluble extract under reduced pressure yielded 77.0 g of residue, which was chromatographed on a silica gel column using mixtures of *n*-hexane−EtOAc of increasing polarity as eluant to afford 54 fractions. Fractions 15−35 (8.0 g) were subjected to column chromatography over Sephadex LH-20 (*n*-hexane−$CHCl_3$−MeOH, 2:1:1) and silica gel (CH_2Cl_2−acetone of increasing polarity). Preparative HPTLC developed with CH_2Cl_2−Et_2O (9:1) was used to purify the new compounds **2** (59.0 mg, R_f 0.30) and **3** (3.0 mg, R_f 0.33), in addition to the known compounds 3β,6β-dihydroxylup-20(29)-ene (**6**, 4.0 mg), 6β,28-dihydroxy-3-oxolup-20(29)-ene (25.0 mg), 6β-hydroxy-3-oxolup-20(29)-en-28-oic acid (30.0 mg), resinone (7.0 mg), glochidone (24.0 mg), lupeol (10.0 mg), betulin (45.0 mg), lupenone (50.0 mg), betulone (60.0 mg), betulone aldehyde (4.0 mg), 3-*epi*-glochidiol (60.0 mg), glochidonol (15.0 mg), messagenin (15.0 mg), 28,30-dihydroxy-3-oxolup-20(29)-ene (9.0 mg), and betulin-3β-caffeate (45.0 mg).

11α-Hydroxy-*epi*-betulin (1): colorless amorphous solid; $[\alpha]^{20}_D$ +29.0° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3404, 2923, 2852, 2852, 1733, 1595, 1459, 1026, 885 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 458 (M^+, 10), 440 (44), 422 (21), 409 (18), 391 (9), 255 (23), 219 (29), 199 (89), 189 (80), 175 (61), 159 (15), 135 (13), 119 (56), 109 (66), 95 (100), 81 (86), 69 (96); HREIMS *m/z* 458.3741 (calcd for $C_{30}H_{50}O_3$, 458.3760).

6β-Hydroxybetulin (2): colorless amorphous solid; $[\alpha]^{20}_D$ −0.8° (*c* 2.12, $CHCl_3$); IR (film) ν_{max} 3433, 3011, 2936, 2869, 1642, 1458, 1375, 1028, 974 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 458 (M^+, 43), 440 (10), 427 (40), 409 (20), 368 (15), 207 (48), 187 (79), 147 (32), 133 (42), 123 (94), 107 (52), 95 (72), 69 (97), 55 (100); HREIMS *m/z* 458.3723 (calcd for $C_{30}H_{50}O_3$, 458.3760).

24-Hydroxybetulone (3): colorless amorphous solid; $[\alpha]^{20}_D$ +18.5° (*c* 0.68, $CHCl_3$); IR (film) ν_{max} 3425, 3011, 2943, 2870, 1705, 1650, 1461, 1377, 1035, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 456 (M^+, 4), 438 (23), 410 (2), 368 (3), 203 (8), 185 (7), 137 (17), 109

(12), 95 (21), 69 (100), 55 (37); HREIMS *m/z* 456.3531 (calcd for $C_{30}H_{48}O_3$, 456.3603).

Rigidenol-28-aldehyde (4): colorless amorphous solid; $[\alpha]^{20}_D$ +34.3° (*c* 0.21, $CHCl_3$); IR (film) ν_{max} 3426, 2925, 2854, 1702, 1640, 1459, 1382, 1074, 755 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 454 (M^+, 1), 436 (14), 420 (1), 407 (8), 269 (1), 217 (6), 203 (100), 189 (10), 149 (5), 121 (13), 105 (16), 93 (14), 81 (22); HREIMS *m/z* 454.3432 (calcd for $C_{30}H_{46}O_3$, 454.3447).

28-Hydroxyglochidone (5): colorless amorphous solid; $[\alpha]^{20}_D$ +33.0° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.39) nm; IR (film) ν_{max} 3428, 2924, 2853, 1728, 1667, 1459, 1377, 1283, 1261, 1035, 882, 822, 715 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 438 (M^+, 27), 420 (41), 408 (13), 322 (12), 279 (7), 255 (5), 243 (15), 227 (23), 215 (28), 205 (54), 187 (37), 175 (44), 150 (67), 137 (94), 121 (45), 91 (69), 69 (91), 57 (100); HREIMS *m/z* 438.3500 (calcd for $C_{30}H_{46}O_2$, 438.3499).

3*β*,6*β*-Dihydroxylup-20(29)-ene (6): colorless amorphous solid; $[\alpha]^{20}_D$ +3.2° (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 3438, 3010, 2946, 2844, 1640, 1460, 1380, 1040, 905, 755 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 442 (M^+, 5), 424 (4), 406 (83), 391 (49), 363 (15), 201 (21), 187 (100), 145 (30), 134 (38), 107 (29), 95 (32), 69 (22), 55 (24); HREIMS *m/z* 442.3782 (calcd for $C_{30}H_{50}O_2$, 442.3811).

Antimicrobial Activity. Activity was tested against Gram-positive (*Staphylococcus aureus* ATCC 6538, *S. epidermidis* CECT 232, *S. saprophyticus* CECT 235, *Enterococcus faecalis* CECT 795, *Bacillus* subtilis CECT 39, *B. cereus* CECT 496, *B. pumilus* CECT 29, *B. megaterium* CECT 44, *Mycobacterium smegmati* CECT 3032) and Gram-negative (*Escherichia coli* CECT 99, *Proteus mirabilis* CECT 170, *Salmonella* sp. CECT 456, and *Pseudomonas aeruginosa* AK 958) bacteria and the yeast *Candida albicans* UBC 1. The bacteria were maintained on nutrient agar (Oxoid or brain heart infusion agar, in the case of *E. faecalis* and *M. smegmati*) and the yeast on Sabouraud agar (Oxoid) at 37 °C. The minimal inhibitory concentration (MIC) of compounds previously dissolved in DMSO (dimethyl sulfoxide) was estimated by the broth microdilution method in 96-well microtiter plates.[19]

Cytotoxic Activity. HeLa (human carcinoma of cervix), Hep-2 (human carcinoma of larynx), and Vero (African green monkey kidney) cell lines were each grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Sigma), supplemented with 5% fetal calf serum (Gibco) and 1% of penicillin-streptomycin mixture (10.000 UI/mL). The cells were maintained at 37 °C in 5% CO_2 and 90% humidity. Cytotoxicity was assessed using the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay.[20] Exponentially growing HeLa, Hep-2, and Vero cells (2×10^4 cells/well) were incubated with different concentrations of the compounds for 48 h at 37 °C. After this time the optical density was measured using a microELISA reader (multiscan Plus II) at 550 nm after dissolving the MTT formazan with DMSO (100 mL). The percentage viability was plotted against the compound concentration, and the 50% cell viability (IC_{50}) was calculated from the curve.

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