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Synthesis and Anti-HIV Activity of Lupane and Olean-18-ene Derivatives. Absolute Configuration of 19,20-Epoxylupanes by VCD

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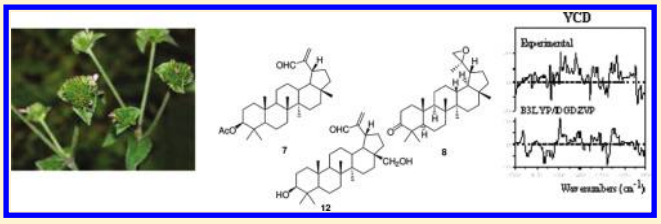
Supporting Information

ABSTRACT: Lupane triterpenoids **2** and **5**−**12** and oleanene derivatives **13** and **14** were prepared from lupeol (**1**), betulin (**3**), and germanicol (**4**). They were tested for anti-HIV activity, and some structure−activity relationships were outlined. The 20-(*S*) absolute configuration of epoxylupenone (**8**) was assessed by comparison of the observed and DFT-calculated vibrational circular dichroism spectra. The Compar-e*VOA* algorithm was employed to support the C-20 configuration assignment. The 20,29 double bond in lupenone (**2**) and 3-epilupeol (**15**) was stereoselectively epoxidized to produce 20-(*S*)-**8** and 20-(*S*)-**16**, respectively, an assignment in agreement with their X-ray diffraction structures.



Pentacyclic triterpenoids, secondary metabolites that arise from squalene cyclization,[1] are found in different plant organs, e.g., bark, cork, or wax covering leaves or peel. Distributed in several species, pentacyclic triterpenoids are found to be suitable starting materials for further pharmaceutical development. In the last 15 years, hundreds of publications have highlighted the broad spectrum of biological activities of lupane, oleanane, and ursane triterpenoids.[2] Lupane-type derivatives have attracted attention since they exhibit a broad range of biological and medicinal properties such as anticancer,[2,3] antitumoral,[4,5] anti-inflammatory,[6] anti-HIV,[7−11] and antimalarial[12] activities. The fundamental triterpenes lupeol (**1**)[13] and betulin (**3**) are accessible, abundant, and valuable bioactive natural products, while natural olean-18-ene derivatives have shown interesting anticarcinogenic activity.[14,15]

Driven by our interest in bioactive triterpenes,[7,16,17] in this paper we report the preparation of several derivatives from natural lupeol (**1**), betulin (**3**), and germanicol (**4**). These triterpenoids are the most abundant metabolites in the Argentinian species *Maytenus spinosa* (Griseb) Lourteig et ÓDonel.[18] Because of anti HIV-activity antecedents in related triterpenoids,[7] the obtained derivatives were assessed for their capacity to perturb X4- and R5-tropic HIV-1-envelope (Env)-mediated fusion membrane in a cell-to-cell fusion model.[19,20] Furthermore, the absolute configuration of epoxylupane derivatives was determined by vibrational circular dichroism (VCD) and confirmed by single-crystal X-ray studies.

RESULTS AND DISCUSSON

Scheme 1 shows the obtained lupeol derivatives. Thus, oxidation of **1** with Jones reagent in acetone afforded lupenone (**2**) in 61% yield, while esterification of **1** with Ac_2O/py yielded **5**. Allylic oxidation of **1**, **2**, and **5** with SeO_2 afforded the corresponding α,β-unsaturated aldehydes **6**, **11**, and **7**, respectively. The NMR spectrum of **11** showed the A ring α,β-unsaturated carbonyl moiety, which was confirmed by HMBC correlations between the doublets at δ 7.08 (J = 10.2 Hz, H-1) and 5.79 (J = 10.2 Hz, H-2) and the carbonyl carbon at δ 205.5 (C-3). Compounds **2** and **5** were converted into epoxy derivatives **8** and **9**, respectively, by treatment with MCPBA, while reaction of compound **2** with $NH_2OH{\cdot}HCl$ provided oxime **10** in 35% yield.

The derivatives obtained from betulin (**3**) and germanicol (**4**) are shown in Scheme 2. Treatment of **3** and **4** with Ac_2O/py yielded the corresponding acetates **12** and **13**, while epoxidation of the double bond of **13** afforded **14**. The absolute configuration of the epoxy derivative was established as 18*R*,19*S* based on the NOE effect observed between H-19 and Me-27.

In order to unequivocally assign the C-20 absolute configuration of the epoxidation products of the lupeol derivatives, their VCD data were studied. This analytical

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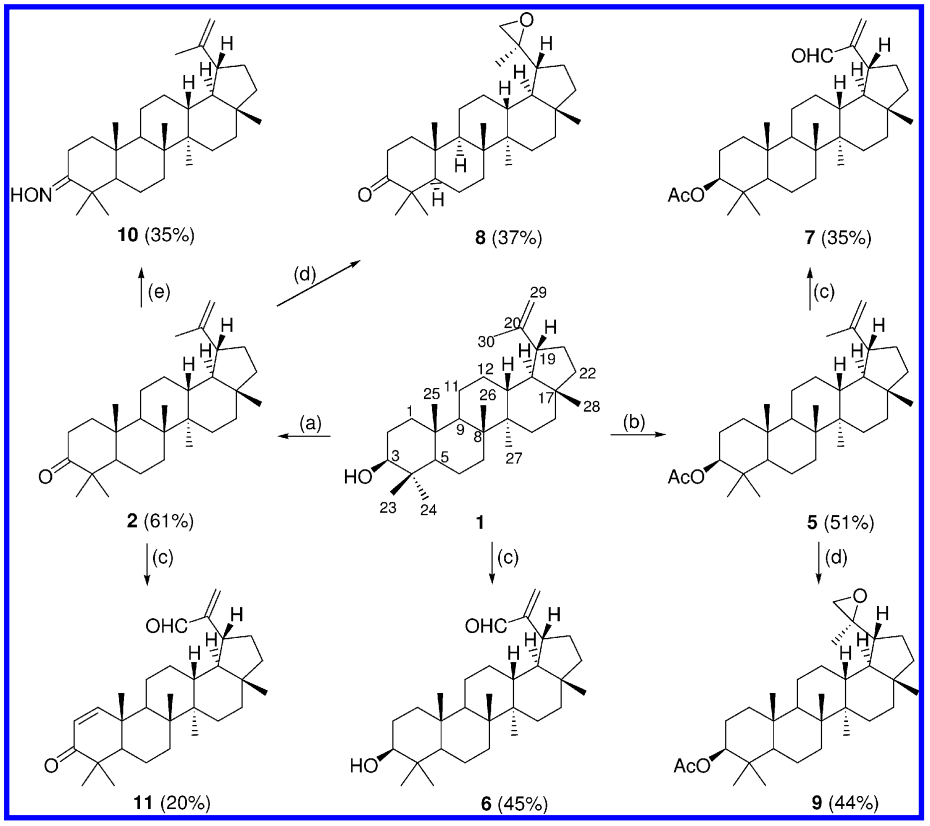
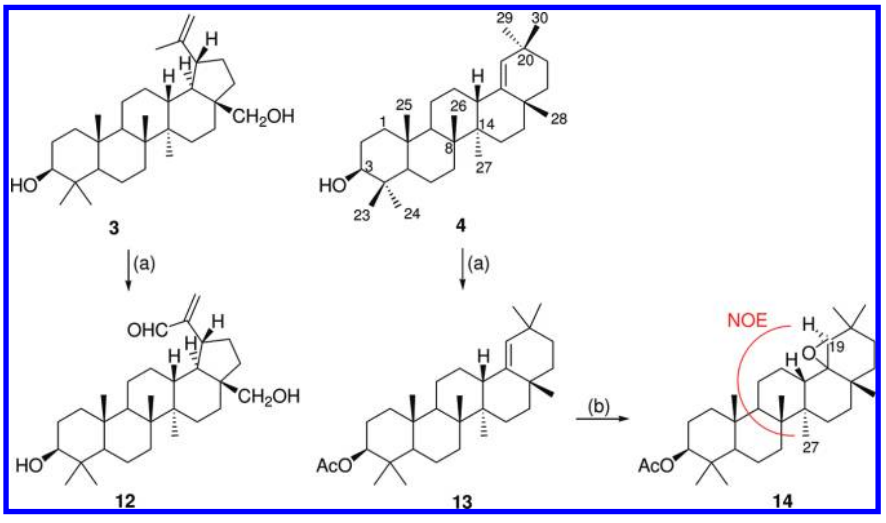
Scheme 1[a]



[a]Reagents and reaction conditions: (a) Jones reagent; (b) Ac_2O, py, DMAP; (c) SeO_2, EtOH; (d) MCPBA, $NaHCO_3$, DCM; (e) $NH_2OH.HCl$, NaOAc, $EtOH/H_2O$.

Scheme 2[a]



[a]Reagents and reaction conditions: (a) Ac_2O, py, DMAP; (b) MCPBA, $NaHCO_3$, DCM.

method is widely known to confidently assign the absolute configuration of natural products possessing one or more stereogenic centers.[21−25] Moreover, the configurational determination of diastereomeric sesquiterpene derivatives of pacifenol[26] and cedrol[27] has been successfully achieved, revealing the potential application of the VCD methodology in stereochemical assessments.

Epoxylupenone (**8**), obtained in two steps from lupeol (**1**), was considered to be the candidate of choice for this study since it has less conformational freedom than epoxylupeol or its O-acetyl derivative (**9**). In addition the single-bond C−O vibrations of this compound arise only from the epoxide bonds. Compound **8** has 80 atoms ($C_{30}H_{48}O_2$), 244 electrons, and 234 vibrational frequency modes that are active vibrations in the IR and VCD spectra.

Since the rotational strength modes of the vibrational frequency VCD spectra depend closely on molecular conformation, an initial search for the most stable conformers

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of the 20-(*R*) and 20-(*S*) epoxylupanone epimers was undertaken using the Monte Carlo MMFF94 molecular mechanics method, setting a 10 kcal/mol window in the Spartan'04 (Wavefunction, Irvine CA, USA) software. The energy and the population percentages of the four conformers are summarized in Table 1. To optimize the geometry of the

Table 1. Monte Carlo Calculated Relative Energies and Populations (%) and Relative Free Energies ($\Delta G°$) and Populations (%) at the B3LYP/DGDZVP Level of 20-(*R*)- and 20-(*S*)-20,29-Epoxylupan-3-one Conformers

epimer-conformer	ΔE_{MMFF94} (kcal/mol)[a]	$\%_{MMFF94}$	$\Delta\Delta G_{OPT}$ (kcal/mol)[a]	$\%_{OPT}$[b]
20-(*R*)-**a**	0.00[c]	91.1	0.39	34.1
20-(*R*)-**b**	1.85	8.0	0.00[e]	65.9
20-(*R*)-**c**	2.77	0.9		
20-(*R*)-**d**	4.56	0.0		
20-(*S*)-**a**	0.00[d]	90.4	0.33	30.8
20-(*S*)-**b**	1.69	5.2	0.86	12.6
20-(*S*)-**c**	1.83	4.1	0.00[f]	53.7
20-(*S*)-**d**	3.43	0.3	1.72	2.9

[a]Relative to the lowest energy conformer. [b]Calculated using the optimized free energies of the relevant conformers. [c]E_{MMFF} = 154.08 kcal/mol. [d]E_{MMFF} = 153.66 kcal/mol. [e]DFT ΔG_{OPT} = −829 538.61 kcal/mol. [f]DFT ΔG_{OPT} = −829 538.21 kcal/mol.

Monte Carlo MM conformers, the structures were submitted to density functional theory (DFT) single-point calculations using the B3LYP functional and the 6-31G(d) basis set in the Spartan'04 suite, followed by geometry optimization using DFT at the B3LYP/DGDZVP level of theory employing the Gaussian 03 program (Gaussian Inc., Pittsburgh, PA, USA). A frequency analysis was then performed but only for the global minimum conformer and in those conformers that reside in a 2 kcal/mol limit above it. Thence, only two of the four conformers were considered for the 20-(*R*) epimer, representing 98.1% of the population (Figure 1), and four conformers for the 20-(*S*) diastereoisomer (Figure 2). The free energy ($\Delta G° = -RT \ln K$) at 25 °C and the conformer populations obtained for each epimer are listed in Table 1.

The free energies of conformers **a** and **b** of the 20-(*R*) epimer are only 0.39 kcal/mol apart in favor of **b**, while for the 20-(*S*) epimer the most populated conformer is **c**, followed by

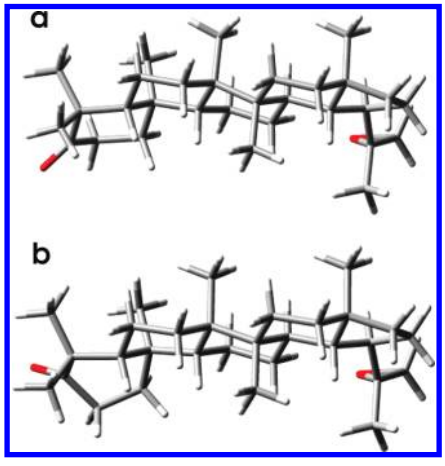

Figure 1. Geometry-optimized conformers of 20-(*R*)-epoxylupanone at the B3LYP/DGDZVP level of theory.

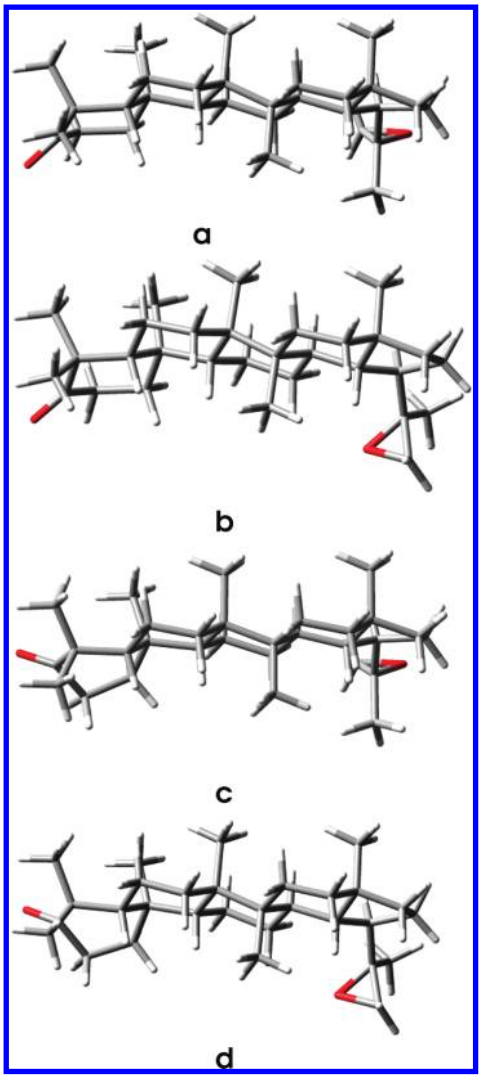

Figure 2. Geometry-optimized conformers of 20-(*S*)-epoxylupanone at the B3LYP/DGDZVP level of theory.

conformer **a**. The twist-boat conformation is preferred over the chair for ring A in both epimers. As expected, other portions of the lupane skeleton are quite rigid, displaying six-membered rings in chair conformations and the five-membered ring in an envelope conformation in which the C-19 substituent is in a pseudo-equatorial orientation. The rotameric conformation of the epoxide substituent is practically equal in the **a** and **b** conformers of the 20-(*R*) epimer, showing bond alternation with the C-19 methine; that is, the C-30 methyl group is *anti* to H-19_{ax} ($\omega_{30-20-19-H19} \approx 167°$), and C-29 and the epoxide-oxygen are synclinal to H-19_{ax}. The epoxide-oxygen is in close proximity to H-12_{eq} with a nonbonded O···H distance of 2.29 Å. Similarly in rotamers **a** and **c** of the 20-(*S*) epimer, H-19_{ax} is oriented *anti* to C-30 ($\omega_{30-20-19-H19} \approx 169°$), and in conformers **b** and **d** it is *anti* to the C-29 methylene ($\omega_{29-20-19-H19} \approx 178°$) with the epoxide oxygen close to H-12_{ax}, rendering a nonbonded O···H distance of ca. 2.45 Å. Some selected torsion angles for conformers **a** and **b** of 20-(*R*) and **a**–**d** of 20-(*S*) epimers are summarized in Table S1 in the Supporting Information. The IR and VCD calculated spectra were simulated from dipole and rotational strengths, respectively, using Lorentzian bandshapes with a bandwidth of ν = 6 cm^{-1}. The weighed VCD spectra of the 20-(*R*) and 20-

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(*S*) diastereoisomers, calculated according to the abundances reported in Table 1, are used for the spectroscopic comparison shown in Figure 1.

A critical step for absolute configurational assignment is the reliable band-to-band comparison of the observed and calculated VCD spectra. A visual inspection of VCD spectra (Figure 3) to assign the 20-(*R*) or 20-(*S*) configuration to

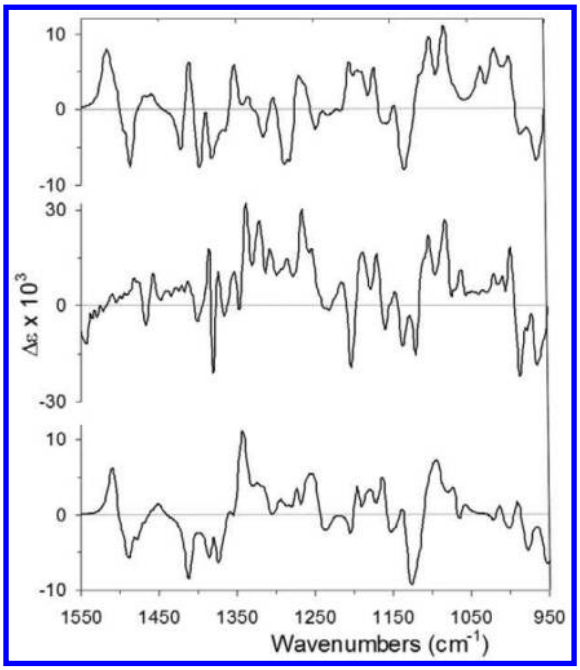

Figure 3. Comparison of the observed VCD spectrum of epoxylupanone (**8**) (center) with the conformationally averaged theoretical VCD spectra of the 20-(*R*) epimer (top) and the 20-(*S*) epimer (bottom) calculated at the B3LYP/DGDZVP level of theory.

epoxylupanone is not trivial, since much of their spectroscopic differences arises from the change of configuration of one of a total of 10 stereogenic centers. In addition, measured IR and VCD frequencies derive from an anharmonic force field, while calculated frequencies derive from a harmonic force field. As a consequence, the later data are normally scaled using an anharmonicity factor (anH) that frequently is set close to 0.97 from statistical one-to-one band comparison of the main bands in the experimental and calculated IR spectra,[28] as we have illustrated for eremophilanoids.[29] Manual extraction of vibrational frequencies and strengths is a time-consuming methodology, although reliable for absolute configuration assignment. Alternatively, a high level of confidence for absolute configuration assignments is provided by neighborhood similarity indexes in the Compare*VOA* algorithm,[30] a recently introduced software (BioTools Co., Jupiter, FL, USA) that we applied for the study of 1-*R*-(−)-myrtenal at several levels of theory.[31] The Compare*VOA* algorithm uses a correlation function to describe the integrated overlap of two patterns, the experimental and theoretical data, as a function of a relative vibrational frequency shift. The optimal shift calculated is the predicted anH factor. IR and VCD spectra of 20-(*R*) and 20-(*S*) diastereomers were *in silico* compared to the corresponding experimental ones; the results are summarized in Table 2. The anH factors are 0.979 for the 20-(*R*) and 0.974 for the 20-(*S*) diastereomers. The IR spectroscopic similarity index (S_{IR}) suggests the C-20 absolute configuration of epoxylupanone (**8**)

Table 2. Confidence Level Data for the IR and VCD Spectra of 20-(*R*)- and 20-(*S*)-20,29-Epoxylupan-3-one

epimer	anH[a]	S_{IR}[b]	S_E[c]	S_{-E}[d]	ESI[e]	C[f]
20*R*	0.979	70.1	60.6	26.6	34.0	46
20*S*	0.974	79.7	72.2	14.8	57.4	100

[a]Anharmonicity factor. [b]IR spectoscopic similarity. [c]VCD spectroscopic similarity for the correct enantiomer. [d]VCD spectroscopic similarity for the incorrect enantiomer. [e]Enantiomer similarity index, calculated as the $S_E - S_{-E}$ difference. [f]Confidence level for the stereochemical assignment.

to be *S* since its value of 79.7 is higher than 70.1 for the 20-(*R*) epimer. Moreover, data revealed by VCD spectroscopic similarity indexes S_E and S_{-E} and the enantiomer similarity index (ESI) reinforce the C-20 (*S*) assignment: S_E for the correct 5*R*,8*R*,9*R*,10*R*,13*R*,14*R*,17*R*,18*S*,19*R*,20*S* enantiomer is 72.2, and the S_{-E} value for its antipode is 14.8, while for the 5*R*,8*R*,9*R*,10*R*,13*R*,14*R*,17*R*,18*S*,19*R*,20*R* epimer these values are 60.6 and 26.6, respectively. The ESI values determined as the difference between S_E and S_{-E} indexes are 34.0 for 20-(*R*) but 57.4 for 20-(*S*), a considerably higher value, which confirmed the 20-(*S*) assignment. In addition, the calculated confidence level for the 20-(*R*) assessment is only 46%, in contrast to 100% for the 20-(*S*) diastereomer.

In order to gain independent evidence for the stereochemical outcome of the epoxidation in the lupeol skeleton and hoping to obtain crystals suitable for X-ray diffraction analysis, a sample of 3-epilupeol was treated with *m*-chloroperbenzoic acid in dichloromethane. Epoxyepilupeol was purified and characterized via its HRMS and NMR data. The presence of the epoxide was evident from the ^{1}H NMR spectrum, showing two doublets (J = 4.7 Hz) at δ 2.66 and 2.61 for the C-29 methylene protons and in the ^{13}C NMR spectrum exhibiting the C-20 quaternary carbon at δ 60.4 and the C-29 methylene at δ 57.6. Full assignment of the ^{13}C NMR spectrum was assisted by the reported spectra of lupeol derivatives.[32] Particularly useful for the assignment of the signals of rings A and B were those reported for epilupeol,[33] while for rings D and E those of epoxylupanone (**8**) were used.

The solid-state structure of epoxyepilupeol obtained by single-crystal X-ray diffraction is shown in Figure 3. The molecular architecture corroborated that epilupeol epoxidation proceeded diastereoselectively to produce the 20-(*S*) epimer. Furthermore, slow evaporation of a solution of **8** in $CDCl_3$ left after NMR measurements also provided suitable crystals for X-ray diffraction, which reconfirmed the 20-(*S*) absolute configuration (Figure 4). These stereochemical results are in line with a prediction made for the C-20 relative configuration in epoxy derivative **9**, based on molecular model inspections.[34]

Compounds **1**−**14** were tested in order to establish their capacity to perturb X4- and R5-tropic HIV-1-envelope (Env)-mediated fusion membrane, in a cell-to-cell fusion model.[19,20] The transformations predominantly involved the C-3 hydroxy group, the C-20−C-29 double bond, and the C-30 allylic carbon in order to study their roles in the activity. As shown in Table 3, they exhibited low antifusogenic activity against X4- or R5-tropic HIV-1-Env in the HeLa-based cellular model. From these results some structure−activity relationships may be derived. When **1** was converted into the corresponding acetyl derivative (**5**), an increase in the activity was observed. The 3-oxo derivative (**2**) was inactive, while the oxime derivative (**10**) showed low antifusogenic activity. We also tested if the

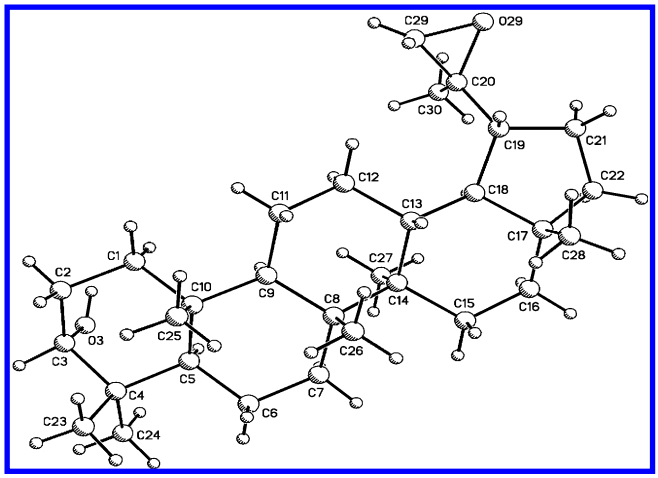

Figure 4. Single-crystal X-ray structure of 20-(*S*),29-epoxylupan-3α-ol.

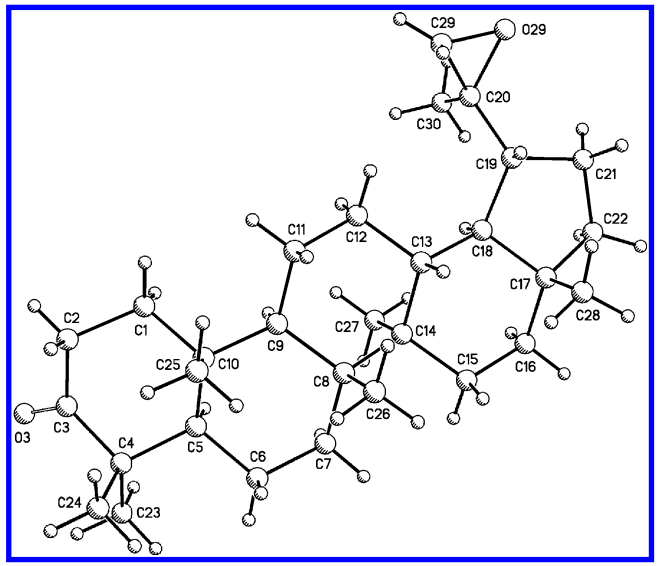

Figure 5. Single-crystal X-ray structure of 20-(*S*),29-epoxylupan-3-one (**8**).

Table 3. Inhibition (%) of the R5-Tropic Env-Mediated Cell Fusion Process and X4-Tropic Env-Mediated Cell Fusion Process by Lupanes at 5 μM[a]

compound	R5-tropic-Env	X4-tropic-Env
3	17	34
5	31	15
7	41	6
9	26	6
10	7	23
11	28	44
12	26	10

[a]Compound **8** was not tested; compounds **1**, **2**, **4**, **6**, and **14** were not active.

presence of a C-20−C-29 epoxy function could lead to active derivatives since this moiety could be susceptible to attack by nucleophilic residues. The best percentages of inhibition were achieved with the compounds obtained by allylic oxidation using SeO_2 (compounds **7**, **11**, and **12**). Only compound **6** with a formyl moiety at C-30 and a hydroxy group at C-3 was inactive, which indicated the importance of the C-3 acetyl group in this series of compounds (**6** vs **7**). These results are in agreement with our previous work,[7] in which we reported the activity of calenduladiol [lup-20(29)-ene-3β,16β-diol] derivatives and the importance of an α,β-unsaturated formyl group for the antiviral activity. Moreover, our results indicate that a β-hydroxy group at C-16 is essential for inhibitory activity, since compound **6** with identical structure to calenduladiol but devoid of the 16-OH group was inactive. The presence of a hydroxy group in the E ring at C-28 instead of at C-16 (compound **12**) also led to low activity.

In summary, a set of lupane derivatives of lupeol, lupenone, and betulin, together with three germanicanes, were tested for anti-HIV activity. These results and comparison with previous results indicated the importance of the 20(30)-en-29-al functionality and the hydroxy group at C-16 in the lupane series. The olean-18-ene derivatives were inactive.

In addition, the absolute configuration of C-20 was established as 20-(*S*) in 20,29-epoxylupanes by application of VCD on epoxylupanone (**8**). This configuration was confirmed by single-crystal X-ray diffraction of 20,29-epoxyepilupeol and 20,29-epoxylupenone (**8**).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 341 polarimeter. NMR measurements were performed in $CDCl_3$ with TMS as internal standard at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury spectrometer and Bruker Avance 400 MHz for ^{1}H and 100 MHz for ^{13}C. The HR-MS data were recorded on an Agilent LC-TOF instrument at the UCR Mass Spectrometry Facility, University of California, Riverside, and a VG Micromass ZAB-2F. The IR and VCD measurements were performed on a Bio-Tools Chiral*IR* FT-VCD spectrophotometer equipped with dual photoelastic modulation. A sample of 9.0 mg of epoxylupanone was dissolved in 150 μL of 100% atom-D $CDCl_3$ and placed in a BaF_2 cell with a path length of 100 μm, and data were acquired at 4 cm^{-1} during 4 h. Single-crystal X-ray data were collected on a Bruker Nonius CAD4 diffractometer equipped with Cu Kα radiation (λ = 1.54184 Å) at 293(2) K in the ω−2θ scan mode.

Plant Material. Leaves of *Maytenus spinosa* (Griseb) Lourteig et O'Donel (Celastraceae) were collected in Osma, Argentina, at 1250−1300 m above sea level. A voucher specimen, MCNS 12712, identified by B.Sc. Lazaro Novara in December 2006 is on file at the Herbarium of Botánica of the Universidad Nacional de Salta, Salta, Argentina

Extraction and Isolation. The dried leaves of *M. spinosa* (910 g) were extracted with EtOH in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure gave 105 g of extract, which was repeatedly chromatographed on Sephadex LH-20, silica gel, preparative TLC, and a Chromatotron to afford lupeol (**1**) (2.87 g), germanicol (**4**) (97.0 mg), lupenone (**2**) (97.0 mg), betulin (**3**) (101.0 mg), and several fractions enriched in β-dihydroagarofurans.

Lup-20(29)-en-3-one (2). To 100 mg of lupeol (**1**) dissolved in 3 mL of acetone was added slowly Jones reagent at 0 °C. The reaction color changed from colorless to orange, and the reaction mixture was stirred for 30 min, quenched with 2-propanol, filtered through Florisil, and concentrated under vacuum. The residue was purified by preparative TLC using hexanes/EtOAc (4:1) to yield 60.7 mg (61%) of **2**, which showed identical spectroscopic data to those reported.[35]

Lup-20(29)-en-3β-ol Acetate (5). To 500 mg of **1** dissolved in the minimum amount of pyridine was added cat. DMAP and an excess of Ac_2O. The mixture was stirred at room temperature for 48 h until disappearance of the starting material. Water was added, and the mixture was extracted several times with DCM. The combined organic extracts were washed with a saturated solution of $CuSO_4$, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography using mixtures of hexanes/EtOAc of increasing

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polarity to yield 280 mg (51%) of **5**, which showed identical spectroscopic data to those reported.[36]

Lup-20(30)-en-3β-ol-29-al (6). To 50 mg (0.117 mmol) of **1** dissolved in 4 mL of EtOH was added 1.2 equiv of SeO_2 (15.6 mg). The reaction mixture was refluxed for 48 h, the solvent was evaporated, and the residue was purified by preparative TLC using hexanes:/EtOAc (4:1) to afford 23.0 mg (45%) of **6**, which showed identical spectroscopic data to those reported.[37]

Lup-20(30)-en-3β-ol-29-al Acetate (7). To 100 mg (0.214 mmol) of **5** dissolved in 5 mL of EtOH was added 47.5 mg (2.0 equiv) of SeO_2. The reaction mixture was refluxed for 72 h until disappearance of the starting material. The solvent was evaporated, and the residue was dissolved in EtOAc and washed with a saturated solution of brine. The aqueous phase was extracted several times with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (9:1) to yield 36.0 mg (35%) of **7**, which showed identical spectroscopic data to those reported.[38]

20*S*,29-Epoxylupan-3-one (8). To 112 mg (0.264 mmol) of **2** dissolved in 5 mL of DCM were added 19.4 mg (1.2 equiv) of MCPBA and 97.6 mg of $NaHCO_3$ (1.2 equiv). The reaction mixture was stirred at room temperature for 5 h and then treated with a saturated solution of sodium thiosulfate (5 mL). The aqueous phase was extracted with DCM (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (17:3) to yield 43.4 mg (37%) of **8**, which showed identical spectroscopic data to those reported.[39,40]

20*S*,29-Epoxylupan-3β-ol acetate (9). To 77 mg (0.165 mmol) of lupeol acetate (**5**) disolved in 5 mL of DCM were added 85.2 mg of MCPBA (1.5 equiv) and 60.8 mg of $NaHCO_3$ (4.4 equiv). The reaction mixture was stirred at room temperature for 3 h until disappearance of the starting material and then treated with a saturated solution of sodium thiosulfate (5 mL). The aqueous phase was extracted with DCM (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (17:3) to yield 35.2 mg (44%) of **9**, which showed identical spectroscopic data to those reported.[34]

Lup-20(29)-en-3-oxime (10). To 60.7 mg of compound **2** dissolved in 2 mL of EtOH were added 29.3 mg of hydroxylamine hydrochloride (3 equiv) and a solution of 23.5 mg of sodium acetate (2 equiv) in 5 mL of H_2O. The reaction mixture was left at room temperature for 36 h, and then the EtOH was evaporated. The residue was treated with H_2O and extracted with DCM (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (4:1) to yield 22.1 mg (35%) of **10**, which showed identical spectroscopic data to those reported.[12]

Lupa-1,20(30)-dien-29-al-3-one (11). To 83 mg (0.196 mmol) of **2** in 5 mL of EtOH was added 43.5 mg (2.0 equiv) of SeO_2. The reaction mixture was refluxed for 72 h, the solvent was evaporated, and the residue was redissolved in EtOAc and treated with a saturated solution of NaCl. The organic layer was separated, and the aqueous phase was extracted with AcOEt (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (9:1) to yield 16.7 mg (20%) of **11** as an amorphous, white solid: $[\alpha]^{20}_D$ +16.1 (c 0.64, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 245 (2,87) nm; IR γ_{max} (film) 2942, 1726, 1691, 1457, 1382, 1232, 1068, 944, 823, 737, 521 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.54 (1H, s, H-30), 7.08 (1H, d, J = 10,2 Hz, H-1), 6.31 (1H, s, H-29a), 5.96 (1H, s, H-29b), 5.79 (1H, d, J = 10,2 Hz, H-2), 2.79 (1H, m, H-12a), 2.17 (1H, m, H-11a), 1.14 (3H, s, Me-23), 1.11 (3H, s, Me-26), 1.09 (3H, s, Me-27), 1.07 (3H, s, Me-28), 0.95 (3H, s, Me-25), 0.86 (3H, s, Me-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.5 (s, C-3), 195.1 (d, C-30), 159.7 (s, C-20), 159.7 (d, C-1), 133.0 (t, C-29), 125.1 (d, C-2), 53.4 (d, C-5), 51.1 (d, C-19), 44.7 (s, C-4), 44.2 (d, C-9), 44.2 (d, C-18), 43.3 (s, C-17), 42.9 (s, C-14), 41.7 (s, C-10), 39.5 (s, C-8), 39.9 (t, C-22), 37.9 (d, C-13), 35.3 (t, C-16), 33.7 (t, C-7), 32.5 (t, C-11), 27.7 (q, Me-23), 27.4 (t, C-21), 27.2 (t, C-15), 21.1 (t, C-12), 18.9 (t, C-6), 21.4 (q, Me-24), 19.1 (q, Me-28), 17.8 (q, Me-26), 16.4 (q, Me-25), 14.3 (q, Me-27); EIMS m/z (%) 436 ([M]$^+$ (69), 421 (9), 407 (3), 243 (39), 203 (28), 189 (15), 175 (12), 161 (17); 150 (100), 137 (85), 121 (30), 107 (31), 95 (32), 81 (32), 69 (35); HR-EIMS m/z 436.3353 (calcd for $C_{30}H_{44}O_2$ 436.3341).

Lup-20(30)-en-3β,28-diol-29-al (12). To 20 mg (0.045 mmol) of betulin (**3**) dissolved in 4 mL of EtOH was added 10.0 mg of SeO_2 (2.0 equiv). The reaction mixture was heated under reflux for 36 h, the solvent was evaporated, and the resulting residue was treated with 5 mL of H_2O and extracted with DCM (3 × 5 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC using mixtures of hexanes/EtOAc (7:3) to yield 5.4 mg (26%) of **12**, which showed identical spectroscopic data to those reported.[41]

18,19-Epoxyolean-3β-ol acetate (14). To 77 mg (0.165 mmol) of germanicol acetate (**13**) dissolved in 5 mL of DCM were added 85.2 mg (1.5 equiv) of MCPBA and 60.8 mg (4.4 equiv) of $NaHCO_3$. The reaction mixture was stirred at room temperature for 3 h and treated with a saturated solution of sodium thiosulfate. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated to yield 53.0 mg of **14** as an amorphous white solid: $[\alpha]^{20}_D$ = +2.9 (c 0.42, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 (2.51) nm; IR (neat) ν_{max} 2931, 1729, 1453, 1371, 1250, 1023, 987, 835, 518 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.52 (1H, m, H-3), 2.74 (1H, s, H-19), 2.19 (1H, dd, J = 2.9, 12.6 Hz, H-13), 2.07 (1H, s, Me-2′), 1.08 (3H, s, Me-26), 1.07 (3H, s, Me-29), 1.05 (3H, s, Me-28), 1.01 (3H, s, Me-30), 0.96 (3H, s, Me-27), 0.91 (3H, s, Me-25), 0.87 (3H, s, Me-24), 0.86 (3H, s, Me-23); ^{13}C NMR ($CDCl_3$) δ 170.0 (s, C-1′), 80.9 (d, C-3), 66.1 (s, C-18), 65.0 (d, C-19), 55.6 (d, C-5), 51.4 (d, C-9), 43.0 (s, C-14), 40.9 (s, C-8), 38.6 (t, C-1), 37.8 (s, C-4), 37.2 (d, C-13), 37.1 (s, C-10), 34.1 (t, C-7), 33.7 (s, C-17), 33.1 (t, C-21), 31.7 (t, C-16), 31.6 (t, C-22), 29.3 (q, Me-29), 29.1 (s, C-20), 27.9 (q, Me-23), 26.7 (t, C-15), 24.8 (q, Me-30), 23.7 (t, C-2), 23.3 (q, Me-28), 21.3 (t, C-11), 21.0 (q, Me-2′), 20.8 (t, C-12), 18.1 (t, C-6), 16.8 (q, Me-26), 16.5 (q, Me-24), 16.1 (q, Me-25), 15.2 (q, Me-27); EIMS m/z 484 [M]$^+$ (60), 466 (12), 451 (9), 426 (3), 409 (6), 391 (4), 409 (6), 402 (16), 279 (8), 221 (100), 203 (25), 193 (26), 189 (59), 175 (21), 161 (17); 152 (47), 135 (42), 121 (42), 109 (38), 95 (46), 81 (40), 69 (57); HR-EIMS m/z 484.3916 (calcd for $C_{32}H_{52}O_3$, 484.3938).

20*S*,29-Epoxylupan-3α-ol. A solution of MCPBA (45 mg, 0.261 mmol) in 5 mL of DCM was added dropwise to a solution of epilupeol (**15**) (45 mg, 0.106 mmol) in 5 mL of DCM, and the mixture was stirred at room temperature for 16 h. A saturated solution of K_2CO_3 (10 mL) was added, and the organic layer was extracted with DCM (2 × 5 mL). The combined organic extract was washed with a saturated solution of NaCl (10 mL), dried with anhydrous Na_2SO_4, filtered, and evaporated. The solid residue was purified by successive recrystallization from hexanes (R_f: 0.29 in 4:1 hex/EtOAc) to obtain 23.2 g (49.7%) of white crystals. Further recrystallyzation from MeOH gave good-quality crystals for X-ray diffraction analysis: mp 194−196 °C; $[\alpha]^{20}_D$ = −2.66 (c 0.75, $CHCl_3$); IR (CCl_4) ν_{max} 3629, 2944, 2865, 1553, 1457, 1383, 1256, 1218, 1112, 1070, 1006, 995, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (1H, dt, J = 2.6, 5.6 Hz, H-3), 2.66 (1H, d, J = 4.7 Hz, H-29a), 2.61 (1H, dd, J = 4.7, 0.6 Hz, H-29b), 1.24 (3H, s, Me-30), 1.03 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.94 (3H, s, Me-24), 0.86 (3H, s, Me-25), 0.84 (3H, s, Me-23), 0.73 (3H, s, Me-28); ^{13}C NMR ($CDCl_3$, 75.14 MHz) δ 76.2 (C-3), 60.4 (C-20), 57.6 (C-29), 50.0 (C-9), 49.5 (C-19), 49.0 (C-5), 46.5 (C-18), 43.4 (C-17), 42.9 (C-14), 41.0 (C-8), 39.7 (C-22), 37.5 (C-4), 37.3 (C-10), 37.2 (C-13), 35.5 (C-16), 34.1 (C-7), 33.3 (C-1), 28.2 (C-23), 27.1 (C-15), 26.9 (C-12), 25.9 (C-21), 25.4 (C-2), 22.1 (C-11), 20.9 (C-24), 18.3 (C-6), 18.1 (C-30), 17.9 (C-28), 15.9 (2C, C-25 and C-26), 14.5 (C-27); HR-EIMS m/z 460.4155 (calcd for $C_{30}H_{50}O_2$ + NH_4^+, 460.4155).

Single-Crystal X-ray Diffraction Analysis of 20-(*S*),29-Epoxylupan-3α-ol and 20-(*S*),29-Epoxylupan-3-one (8). Unit cell refinements using 25 machine-centered reflections were done using the CAD4 Express v2.0 software. Crystal data for 20-(*S*),29-epoxylupan-3α-ol were $C_{30}H_{50}O_2$, M = 442.70, orthorhombic, space

group $P2_12_12_1$, a = 6.804(2) Å, b = 14.327(2) Å, c = 26.580(4) Å, V = 2591.2(8) Å^3, Z = 4, ρ = 1.14 mg/mm^3, μ(Cu Kα) = 0.515 mm^{-1}, total reflections = 2127, unique reflections 2024 (R_{int} 0.01%), observed reflections 1786, final R indices [$I > 2\sigma(I)$] R1 = 4.9%, wR2 = 14.4%, and those for **8** were $C_{30}H_{48}O_2$, M = 440.68, orthorhombic, space group $P2_12_12_1$, a = 10.822(3) Å, b = 13.780(2) Å, c = 17.376(4) Å, V = 2591.2(9) Å^3, Z = 4, ρ = 1.13 mg/mm^3, μ(Cu Kα) = 0.515 mm^{-1}, total reflections = 1983, unique reflections 1885 (R_{int} 0.01%), observed reflections 1546, final R indices [$I > 2\sigma(I)$] R1 = 4.7%, wR2 = 12.4%. The structures were solved by direct methods using the SIR2004 and SHELXS-97 programs included in the WinGX v1.70.01 crystallographic software package. For the structural refinement, the non-hydrogen atoms were treated anisotropically, and the hydrogen atoms, included in the structure factor calculation, were refined isotropically. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk. The CCDC deposition number of **8** is 863409 and that for 20-(*S*),29-epoxylupan-3α-ol is 863410.

Molecular Modeling and VCD Calculations. Geometry optimizations for both C-20 diastereoisomers of epoxylupanone were performed using MMFF94 force-field calculations as implemented in the Spartan'04 program. A Monte Carlo search protocol was carried out considering an energy cutoff of 10 kcal/mol, providing four conformers for each diastereoisomer. The four conformers were optimized by DFT calculations at the B3LYP/DGDZVP level of theory[42−44] employing the Gaussian 03W program. The minimized structures were used to calculate the thermochemical parameters and the IR and VCD frequencies at 298 K and 1 atm. Molecular visualization was carried out in the GaussianView 3.0 program. Calculations required between 82 and 87 h of computational time per conformer when using a desktop personal computer with 2 Gb RAM operated at 3 GHz.

HIV-1-Env-Mediated Cell-to-Cell Fusion Assay. A β-galactosidase cell fusion assay was performed as previously described.[19,20] Briefly, HeLa ADA cells were mixed with HeLa-P5 cells, in 96-well plates, in a 1:1 ratio (20 000 total cells), in the absence or presence of 5 μM of the different molecules assayed. These cocultures were kept at fusion for 16 h at 37 °C. The fused cells were washed with Hanks' balanced salt solution and lysed, and the enzymatic activity was evaluated by chemiluminescence (β-galactosidase reporter gene assay; Roche Diagnostics, Germany). Anti-CD4 neutralizing mAb (5 μg/mL was preincubated in HeLa-P5 cells for 30 min at 37 °C before coculture with Env+ HeLa cells) was used as a control for the blockage of cell fusion.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of **11**, **14**, and 20-(*S*),29-epoxylupan-3α-ol. Single-crystal X-ray diffraction coordinates of **8** and 20-(*S*),29-epoxylupan-3α-ol. Dihedral angles of the optimized conformers of (20*S*)-**8** and its 20*R* diastereomer. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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