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Structure and Absolute Configuration of Diterpenoids from *Hymenaea stigonocarpa*

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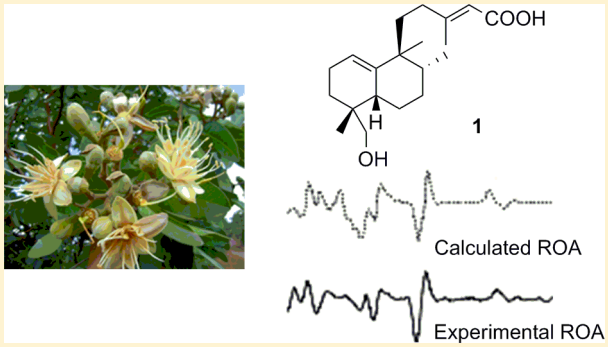
Supporting Information

ABSTRACT: Chemical investigations of the ethanolic extracts from the flowers and leaves of *Hymenaea stigonocarpa* Mart. ex Hayne afforded one new *ent*-halimane diterpenoid, 18-hydroxy-*ent*-halima-1(10),13-(*E*)-dien-15-oic acid (**1**), together with five known compounds (**2**−**6**). The structural elucidation was performed by means of NMR (COSY, HSQC, HMBC, and NOESY) and MS analyses. Complete ^{1}H and ^{13}C NMR data assignments are also reported for labd-13-en-8β-ol-15-oic (**2**) and labd-7,13-dien-15-oic (**3**) acids. The absolute configurations of **1** and **2** were established by comparison of experimental and calculated Raman optical activity spectra.



Hymenaea stigonocarpa Mart. ex Hayne (Fabaceae) is a medicinal plant species commonly found in the Brazilian savannah (Cerrado) and popularly known as jatobá-do-cerrado.[1,2] Ethnopharmacological data emphasize the therapeutic potential of the stem bark and fruits of this plant. These are widely used in Brazilian folk medicine as decoctions or infusions to treat gastric pain, ulcers, diarrhea, bronchitis, flu, and cough and as an anti-inflammatory.[3,4]

Previous chemical investigations of different parts of the *H. stigonocarpa* tree have revealed the presence of sesquiterpenoids, diterpenoids, coumarins, fatty acids, alkaloids, steroids, phenolic compounds, flavonoids, and proanthocyanidins. These metabolites were associated with antibacterial, antiherbivore, antitermitic, antioxidant, antidiarrheal, gastroprotective, cicatrizing, and anti-inflammatory activities.[1,2,5−11]

The chemical study of the ethanolic extract of the flowers of *H. stigonocarpa* led to the isolation of the new compound (+)-(4*R*,5*S*,8*R*,9*S*)-18-hydroxy-*ent*-halima-1(10),13-(*E*)-dien-15-oic acid (**1**). In addition, five known substances, (+)-(5*S*,8*S*,9*R*,10*S*)-labd-13-en-8β-ol-15-oic acid (**2**),[12] (+)-(5*S**,9*S**,10*S**)-labd-7,13-dien-15-oic acid (**3**),[12,13] (+)-(5*S**,9*S**,10*S**)-labd-7-en-15-oic acid (**4**),[14] *p*-hydroxybenzoic acid (**5**),[15] and 4′,5,7-trihydroxy-3′,5′-dimethoxyflavone (**6**),[16] were isolated from the ethanolic extract of the leaves. The absolute configuration of **1** was determined using Raman optical activity (ROA), and this technique was also used to examine the absolute configuration of diterpenoid **2**, for which complete ^{1}H and ^{13}C NMR assignments are reported.

ROA vibrational chiroptical spectroscopy represents a powerful method for the conformational and configurational analyses of chiral molecules in solution. It measures a small difference in the intensity of vibrational Raman scattering from chiral molecules in right- and left-circularly polarized incident light or, equivalently, the intensity of a small circularly polarized component in the scattered light by using incident light of fixed nonelliptical polarization.[17] ROA has been predominantly used to characterize the structure of macromolecules in aqueous solution with many examples reported for peptides,[18] proteins,[18,19] nucleic acids,[20] carbohydrates,[21] and even viral coat proteins.[22] However, only a few examples can be found for its application to natural product chemistry.[23−26] Herein we present the first report of the use of experimental and quantum chemical calculated ROA for determining the absolute configuration of diterpenoids.

Compound **1** was obtained as a white, amorphous powder with $[\alpha]^{20}_{D}$ +89.7 (*c* 0.50, EtOAc). Its molecular formula was deduced to be $C_{20}H_{32}O_3$ based on the ^{13}C NMR spectroscopic data and the HRESIMS spectra in both negative- and positive-

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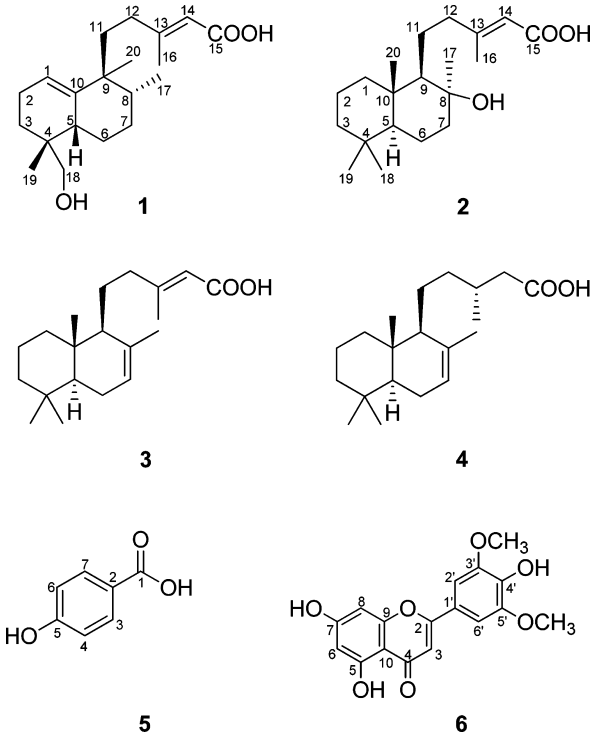

Figure 1. Chemical structures of the compounds isolated from *H. stigonocarpa*.

ion modes, which revealed an ion at m/z 319.2287 $[M - H]^-$ (calcd for $C_{20}H_{31}O_3$, 319.2279) and another ion at m/z 343.2241 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2231), respectively, indicating five indices of hydrogen deficiency. The IR absorption bands at 3384 and 1691 cm^{-1} indicated the presence of hydroxy and α,β-unsaturated carbonyl functionalities, respectively.

The ^1H NMR spectrum showed signals of an olefinic hydrogen at δ 5.34 (t, J = 3.8 Hz), two tertiary methyl groups at δ 0.94 (s) and 0.92 (s), and one secondary methyl group at δ 0.82 (d, J = 7.5 Hz), typical of halimane-type diterpenoids. The spectrum also indicated the presence of a hydroxymethylene group [δ 3.51 (d, J = 11.2 Hz) and 3.34 (d, J = 11.2 Hz)]. In addition, a one-hydrogen doublet at δ 5.66 (J = 1.5 Hz) and a methyl doublet at δ 2.15 (d, J = 1.5 Hz) suggested the presence of a side chain requiring an α,β-unsaturated carboxy group.

The ^{13}C NMR spectrum of **1** (Table 1) revealed 20 carbon signals, including four methyl groups (δ 15.5, 19.3, 21.7, and 21.9), seven methylenes (δ 22.5, 24.0, 26.5, 29.6, 36.2, 36.8, and 69.9), three methines (δ 40.5, 115.0, and 120.1), two quaternary carbons (δ 36.0 and 43.1), two fully substituted olefinic carbons (δ 140.7 and 164.8), and a hydroxycarbonyl carbon (δ 171.6).

The COSY spectrum of **1** suggested the presence of three individual spin systems: **I**, (CH-1)-(CH_2-2)-(CH_2-3); **II**, (CH-5)-(CH_2-6)-(CH_2-7)-(CH-8)-(CH_3-17); and **III**, (CH_2-11)-(CH_2-12)-(C-13)-(CH_3-16)-(CH-14) (Figure 2).

The HMBC correlations from CH-1 (δ_H 5.34) to C-2 (δ_C 22.5), C-3 (26.5), C-5 (40.5), and C-9 (43.1); from CH_2-3 (δ_H 1.34 and 1.10) to C-1 (δ_C 120.1), C-2 (22.5), C-4 (36.0), C-5 (40.5), and C-18 (69.9), and from both CH_3-19 (δ_H 0.94) and CH_2-18 (δ_H 3.51 and 3.34) to C-3 (δ_C 26.5), C-4 (36.0), and C-5 (40.5) indicated that C-4 connected units **I** and **II**. This spectroscopic information delineated several structural fragments corresponding to the A and B rings of the halimane-type diterpenoid.[27]

Table 1. NMR Spectroscopic Data (600 or 500 MHz, $CDCl_3$) for 1

position	δ_C, type	δ_H (J in Hz)	HMBC
1	120.1, CH	5.34, t (3.8)	2, 3, 5, 9
2	22.5, CH_2	2.08, m	
3a	26.5, CH_2	1.34, m	1, 2, 4, 5, 18
3b		1.10, m	
4	36.0, C		
5	40.5, CH	1.80, m	
6a	24.0, CH_2	1.60, m	
6b		1.30, m	
7a	29.6, CH_2	2.00, m	
7b		1.37, m	
8	39.5, CH	1.56, m	
9	43.1, C		
10	140.7, C		
11a	36.8, CH_2	2.15, m	
11b		1.24, m	
12a	36.2, CH_2	2.10, m	11, 13, 14, 16
12b		1.83, m	
13	164.8, C		
14	115.0, CH	5.66, d (1.5)	12, 13, 15,16
15	171.6, C		
16	19.3, CH_3	2.15, d (1.5)	12, 13, 14, 15
17	15.5, CH_3	0.82, d (7.5)	7, 8, 9, 20
18a	69.9, CH_2	3.51, d (11.2)	3, 4, 5, 19
18b		3.34, d (11.2)	
19	21.7, CH_3	0.94, s	3, 4, 5, 18
20	21.9, CH_3	0.92, s	8, 9, 10, 11

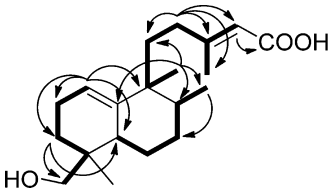

Figure 2. Key ^1H–^1H COSY (bold) and HMBC (arrow) correlations for **1**.

Other key HMBC correlations were observed from CH_3-20 (δ_H 0.92) to C-8 (δ_C 39.5), C-9 (43.1), C-10 (140.7), and C-11 (36.8), which suggested that C-10 connected units **I** and **II**, thus completing the decalin ring fusion, and that the connectivity between moieties **II** and **III** involved C-8 and C-9. The acid side chain was confirmed by the respective HMBC correlations shown in Figure 2.

The structure elucidation was supported by the MS/MS data of **1** in the positive-ion mode (Figure S3, Supporting Information), which showed consecutive losses of water molecules (m/z 303.2252 and m/z 285.2146). Further fragments were observed for the elimination of CO (m/z 257.2188) followed by the loss of a C_4H_6 fragment, resulting in a substituted decalin ion (m/z 203.1740). Opening of this ring system resulted in the ion at m/z 135.1137.

The α-orientations of CH_3-17, CH_3-20, and CH_2-18 were deduced from the NOESY correlations between CH_3-20 (δ_H 0.92) and CH_3-17 (δ_H 0.82). The cross-peaks between H-5β and CH_3-19β confirmed that they were cofacially oriented, and, hence, CH_2-18 must be α-oriented. Correlations between H-8

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(δ_H 1.56) and CH_2-11 (δ_H 1.24) demonstrated that these were β-oriented.

Compound **1** is a new *ent*-halimane diterpenoid containing a C-4 hydroxymethylene group. A similar compound with the same skeleton, an ester of a dicarboxylic acid diterpenoid, has been identified from *H. courbaril*.[28]

Compounds **2** and **3** were previously reported, respectively, from *H. oblongfolia*[12,29] and *H. courbaril*.[12] However, physical data comparisons with literature data revealed inconsistencies in the NMR data for compounds **2** and **3**, hence initiating a detailed spectroscopic study for these substances on the basis of 1D and 2D NMR experiments, including COSY, HSQC, HMBC, and NOESY. The analyses resulted in complete ^1H and ^{13}C NMR assignments for both molecules as collated in the Experimental Section and compiled in Tables (T1 and T2) in the Supporting Information.

The structure and stereochemistry of compound **2** were established by Hugel and co-workers[30] in 1966 on the basis of its spectroscopic properties and chemical correlations. In 1968, the specific rotation of compound **3** ($[\alpha]_D$ −26, $CHCl_3$) was determined and its stereochemistry was related to that of copalic acid. This correlation was further supported by the hydrogenation of its ester, which afforded a levorotatory derivative.[31] Therefore, based on their negative optical rotation values (in $CHCl_3$), compounds **2** and **3** were assigned to the *ent*-labdane stereochemical series. Compound **4** had its absolute configuration recently assigned by Gárcia-Sanchez and co-workers as (5*S*,9*S*,10*S*,13*S*).[14]

Although the optical rotation of labdanes has been extensively used to provide information about their stereochemistry, both enantiomers can occur in a single plant species, hence requiring their absolute configuration to be assigned unambiguously.[32] Additionally, comparison of optical rotation at a single wavelength may be risky, since it is not uncommon to find closely related natural compounds possessing the same absolute configuration, but opposite optical rotation.[33,34]

In order to unambiguously determine the absolute configuration of compounds **1** and **2**, experimental and calculated ROA spectra were compared. The agreement between the experimental ROA and calculated data for the average of their lowest-energy conformers permitted the assignment of the absolute configuration of (+)-**1** as 4*R*,5*S*,8*R*,9*S* and that of (+)-**2** as 5*S*,8*S*,9*R*,10*S* (Figure 4). These assignments were based mainly on the couplets centered at 1460 cm^{-1} for **1** (sequentially negative at low and positive at high) and at 1470 cm^{-1} for **2** (positive at low and negative at high), which arise from CH_3, CH_2, and CH deformation modes involving most of the molecular framework. Vibrational circular dichroism (VCD) has successfully been used to determine the absolute configuration of labdanes and *ent*-labdanes;[35,36] however, this is the first report of the use of ROA for configurational studies of diterpenoids. These results confirm that *H. stigonocarpa* produces labdanes belonging to the *normal* enantiomeric series.

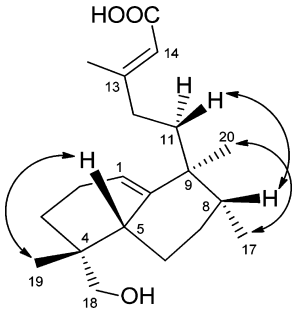

Figure 3. Key NOESY correlations for **1**.

In conclusion, one new diterpenoid (**1**) and five known compounds (**2**–**6**) were isolated from the flowers and leaves of *H. stigonocarpa*, respectively. Compounds **2** and **3** were previously described from *Hymenaea* plants,[12,29] but there were no reports of compounds **4**, **5**, and **6** from this genus. The absolute configurations of diterpenoids **1** and **2** were determined by ROA, and complete ^1H and ^{13}C NMR assignments for compounds **2** and **3** were also reported.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded in EtOAc solutions on a Schimidt-Haensch (Berlin, Germany) Polartronic H-100 polarimeter using quartz cells of 1 dm path length at 20 °C. IR spectra were acquired on an FT-IR Vertex 70 Bruker spectrometer in ATR mode. Raman and ROA spectra were recorded using a BioTools (Jupiter, FL, USA) ChiralRaman spectrometer operated via Critical Link (Syracuse, NY, USA) LLC software. The instrument was set up with a scattered circular polarization strategy in backscattering geometry using a Spectra-Physics (Neath, UK) Millenium Pro Nd–VO_4 laser with an excitation wavelength of 532 nm, laser power of 600 mW at the sample, spectral resolution of 7 cm^{-1}, sample concentration at 30 mg/mL in DMSO-d_6, and spectral acquisition time of 16 h for **1** and 33 h for **2**. NMR spectra were acquired at 400, 500, or 600 MHz for ^1H and 100 or 125 MHz for ^{13}C with Bruker DRX 400, DRX 500, or Avance 600 spectrometers, respectively. $CDCl_3$, methanol-d_4, or DMSO-d_6 (Sigma-Aldrich, St. Louis, MO, USA) was used as solvent and TMS as internal reference. Chemical shift values are reported in parts per million, and coupling constants (J) are in hertz. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained on a Bruker (Billerica, MA, USA) micrOTOF-QII system in both positive- and negative-ion modes, using TFA-Na^+ (sodiated CF_3CO_2H) as internal standard. HPLC analyses were performed on an analytical Agilent (Santa Clara, CA, USA) 1200 Series Purification System with a DAD detector (set at 264 and 360 nm), and semipreparative HPLC was carried out on a preparative Agilent 1200 Series System with an MWD detector. Both instruments were individually coupled with a Rheodyne injector and controlled by ChemStation software. The HPLC columns used were packed in-house using Luna C18 (10 μm) bulk media (Phenomenex, Torrance, CA, USA) as follows: analytical (h = 15.0 cm, φ = 0.46 cm) and semipreparative (h = 25.0 cm, φ = 0.70 cm). Silica gel (70–230 and 230–400 mesh) from Merck (Darmstadt, Germany) and Sephadex LH-20 from Amersham Pharmacia Biotech (Little Chalfout, UK) were used as stationary phases for column chromatography. The solvents employed were AR and HPLC grades (Sigma-Aldrich and Panreac).

Plant Material. *H. stigonocarpa* specimens were collected during the flowering stage on February 13, 2014, from the Cerrado of the central-western region of Brazil, in Catalão-GO, and were identified by Prof. Hélder Nagai Consolaro from Unidade Acadêmica Especial de Biotecnologia-UFG/Regional Catalão. A voucher specimen (No. GD 046) is deposited at the Herbarium of EMBRAPA-Recursos Genéticos e Biotecnologia.

Extraction and Isolation. Flowers (1.2 kg) and leaves (1.5 kg) from *H. stigonocarpa* were individually macerated with EtOH (3 × 9 L, 3 days each) at room temperature and filtered. The filtered materials were concentrated to yield the crude flower (177 g) and leaf (190 g) extracts. A total of six fractions (A–F) were obtained from the separation of the flower extract (10 g) on a silica gel (70–230 mesh) column (5.0 × 11.0 cm), which was eluted with an *n*-hexane/EtOAc/MeOH (1:0:0; 8:2:0; 1:1:0; 2:8:0; 0:1:0; 0:1:1; 0:0:1) gradient system. Fr. B (1.2 g) was washed with hexane to provide compound **1** (16.2 mg) as an insoluble fraction. A portion (10 g) of the leaf extract was

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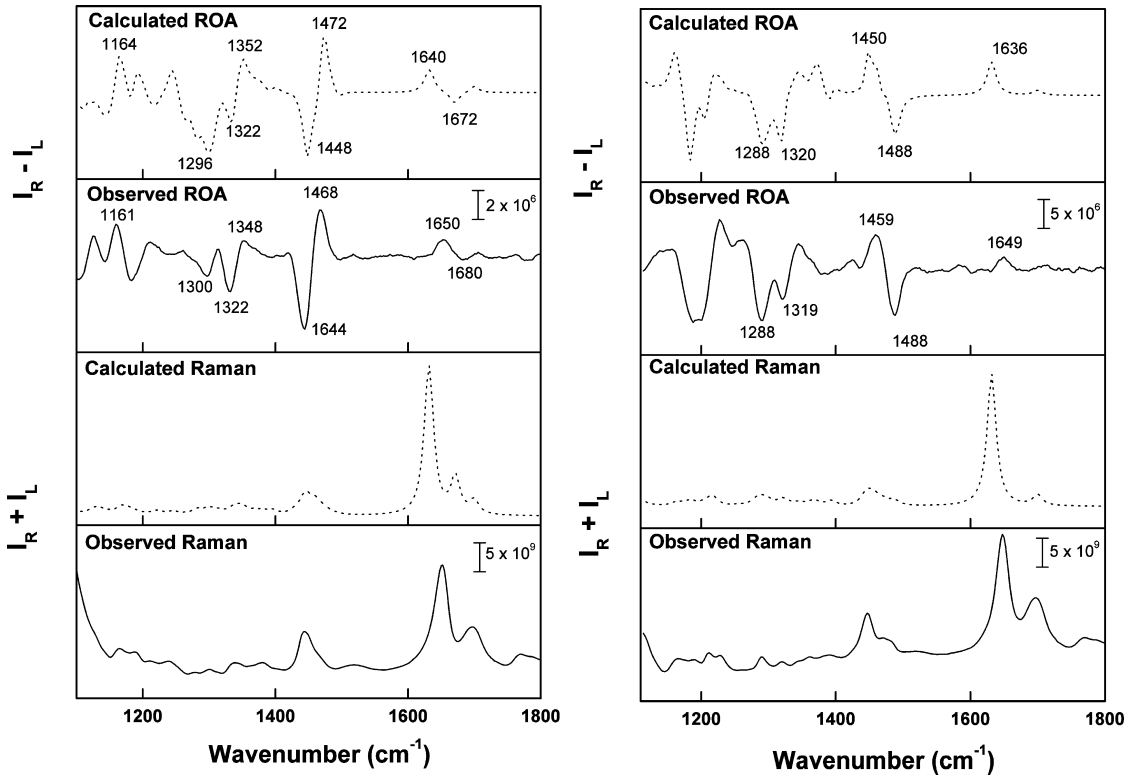

Figure 4. (Left) Comparison of the Raman and ROA spectra of experimental (+)-**1** with the calculated [B3LYP/TZVP//B3LYP/TZVP in DMSO using PCM] Raman and ROA spectra of the average of the four lowest-energy conformers of the corresponding (4*R*,5*S*,8*R*,9*S*)-**1**. The comparison establishes the absolute configuration as (+)-(4*R*,5*S*,8*R*,9*S*)-**1**. (Right) Comparison of the Raman and ROA spectra of experimental (+)-**2** with the calculated [B3LYP/TZVP//B3LYP/TZVP in DMSO using PCM] Raman and ROA spectra of the average of the two lowest-energy conformers of the corresponding (5*S*,8*S*,9*R*,10*S*)-**2**. The comparison establishes the absolute configuration as (+)-(5*S*,8*S*,9*R*,10*S*)-**2**. Numbers represent selected vibrational transitions. I_R and I_L are the intensities of scattered right- and left-circularly polarized light, respectively.

chromatographed on a silica gel (230−400 mesh) column (5.0 × 9.5 cm) with an *n*-hexane/EtOAc/MeOH (1:0:0; 1:1:0; 0:1:0; 0:1:1; 0:0:1) gradient as eluent to yield four fractions (G−J). Fr. G (5.79 g) was separated into 46 fractions (Fr. G.1−G.46) on a silica gel (230−400 mesh) column (3.0 × 35.4 cm) eluted with *n*-hexane/EtOAc/MeOH (from 9:1:0 to 2:8:0; 0:1:0; 0:7:3; 0:1:1; 0:0:1), and then Fr. G.25 (325.2 mg) was subjected to a Sephadex LH-20 column (1.7 × 40.0 cm) eluted with EtOAc/MeOH (4:6 by volume) to provide compound **2** (101.8 mg). Fr. H (1.8 g) was applied to a silica gel (230−400 mesh) column (2.2 × 36.0 cm) using an *n*-hexane/EtOAc gradient as eluent (from 1:1 to 2:8) to afford 12 fractions (H.1−H.12). Fr. H.9 (200 mg) was subjected to the same type of chromatography (1.4 × 19.6 cm) employing an *n*-hexane/EtOAc gradient system (from 1:0 to 0:1) to afford compounds **3** (10.1 mg) and **4** (6.3 mg). Fr. I (1.33 g) was subjected to silica gel (230−400 mesh) column (3.0 × 21.0 cm) chromatography eluted with a hexane/EtOAc/MeOH (from 9:1:0 to 2:8:0; 0:1:0; 0:1:1; 0:0:1) gradient system to afford 15 fractions (I.1−I.15). Fr. I.11 (332.8 mg) was chromatographed on a Sephadex LH-20 column (2.2 × 42 cm) eluted with EtOAc/MeOH (3:7 by volume) to give 10 fractions (I.11.1−I.11.10). Fr. I.11.9 (20.4 mg) was separated by semipreparative HPLC employing a MeOH/3% aqueous HOAc gradient system (from 0:1 to 7:3; flow rate 4.0 mL/min; run time 47 min) to give compounds **5** (2.8 mg) and **6** (3.0 mg).

(+)-(4R,5S,8R,9S)-18-Hydroxy-ent-halima-1(10),13-(E)-dien-15-oic acid (1): white, amorphous powder; $[\alpha]^{20}_{D}$ +89.7 (*c* 0.50, EtOAc); IR ν_{max} 3384, 1691 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 319.2287 $[M - H]^-$ (calcd for $C_{20}H_{31}O_3$, 319.2279) and *m*/*z* 343.2241 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2231).

(+)-(5R,8R,9R,10S)-Labd-13-en-8β-ol-15-oic acid (2): white, amorphous powder; $[\alpha]^{20}_{D}$ +7.3 (*c* 0.49, EtOAc); IR ν_{max} 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.71 (1H, bs, H-14), 2.33 (1H, m, H-12a), 2.24 (1H, m, H-12b), 2.18 (3H, s, H-16), 1.90 (1H, m, H-7a), 1.67 (1H, m, H-1a), 1.65 (1H, m, H-11a), 1.63 (1H, m, H-2a), 1.61 (1H, m, H-6a), 1.46 (1H, m, H-2b), 1.44 (1H, m, H-6b), 1.42 (1H, m, H-7), 1.41 (1H, m, H-3a), 1.29 (1H, m, H-11b), 1.18 (1H, m, H-3b), 1.17 (3H, s, H-17), 1.09 (1H, bt, H-9), 0.97 (1H, m, H-1b), 0.92 (1H, m, H-5), 0.88 (3H, s, H-19), 0.80 (3H, s, H-18), 0.79 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 171.6 (C, C-15), 163.9 (C, C-13), 114.6 (CH, C-14), 74.4 (C, C-8), 61.3 (CH, C-9), 56.1 (CH, C-5), 44.6 (CH_2, C-7), 44.5 (CH_2, C-12), 41.9 (CH_2, C-3), 39.8 (CH_2, C-1), 39.2 (C, C-10), 33.4 (CH_3, C-19), 33.2 (C, C-4), 24.0 (CH_3, C-17), 23.5 (CH_2, C-6), 21.5 (CH_3, C-18), 20.5 (CH_2, C-11), 19.4 (CH_3, C-16), 18.4 (CH_2, C-2), 15.4 (CH_3, C-20); HRESIMS *m*/*z* 321.2443 $[M - H]^-$ (calcd for $C_{20}H_{33}O_3$, 321.2435) and *m*/*z* 345.2403 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2395);

(+)-(5S,9S*,10S*)-Labd-7,13-dien-15-oic acid (3):* yellow, amorphous powder; $[\alpha]^{20}_{D}$ +14.5 (*c* 0.35, EtOAc); IR ν_{max} 1691 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 5.71 (1H, bd, *J* = 0.9 Hz, H-14), 5.42 (1H, bs, H-7), 2.40 (1H, m, H-12a), 2.20 (3H, bd, *J* = 0.9 Hz, H-16), 2.13 (1H, m, H-12b), 2.00 (1H, m, H-6a), 1.88 (1H, m, H-6b), 1.85 (1H, m, H-1a), 1.71 (3H, bs, H-17), 1.65 (1H, m, H-9), 1.63 (1H, m, H-11a), 1.56 (1H, m, H-2a), 1.47 (1H, m, H-2b), 1.42 (1H, m, H-3a), 1.36 (1H, m, H-11b), 1.19 (1H, m, H-5), 1.18 (1H, m, H-3b), 0.97 (1H, m, H-1b), 0.89 (3H, s, H-19), 0.87 (3H, s, H-18), 0.77 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 171.9 (C, C-15), 163.6 (C, C-13), 134.6 (C, C-8), 122.8 (CH, C-7), 115.0 (CH, C-14), 54.4 (CH, C-9), 50.1 (CH, C-5), 43.6 (CH_2, C-12), 42.2 (CH_2, C-3), 39.2 (CH_2, C-1), 36.8 (C, C-10), 33.1 (CH_3, C-18), 33.0 (C, C-4), 25.3 (CH_2, C-11), 23.8 (CH_2, C-6), 22.2 (CH_3, C-17), 21.8 (CH_3, C-19), 19.3 (CH_3, C-16), 18.8 (CH_2, C-2), 13.6 (CH_3, C-20).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables of NMR assignments for **2**−**6**. ^{1}H, ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, HMBC, NOESY, IR, and HRESIMS spectra of

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1. ^{1}H and ^{13}C NMR spectra of **2** and **3**. Calculations of Raman optical activity spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00166.

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Notes

The authors declare no competing financial interest.

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