Isolation and Absolute Configuration of *ent*-Halimane Diterpenoids from *Hymenaea courbaril* from the Suriname Rain Forest[1]

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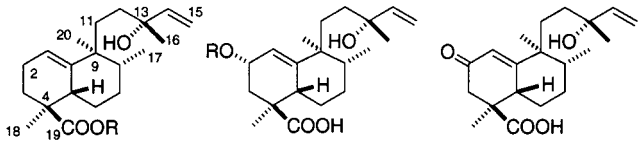
Bioactivity-directed fractionation of a methanol extract of *Hymenaea courbaril* afforded the three new diterpenoids (13*R*)-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid (**1**), (2*S*,13*R*)-2,13-dihydroxy-1(10),-14-*ent*-halimadien-18-oic acid (**2**), and (13*R*)-2-oxo-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid (**3**). The configurations of these compounds were determined from X-ray crystallography of **1**, circular dichroism of **2** and **3**, and spectral studies of prepared derivatives. Compound **1** exhibited weak cytotoxicity toward the 1138 mutant yeast strain and the A2780 human ovarian cancer cell line.

As part of an ongoing program to combine biodiversity conservation with drug discovery and economic development through an International Cooperative Biodiversity Group (ICBG),[2−4] we obtained an extract from *Hymenaea courbaril* L. (Caesalpinaceae), a large and widely distributed tropical tree, from the rain forest of Suriname. *H. courbaril* (also known as courbaril, the kerosene tree, and the West Indian locust) is useful for its timber; it has reportedly been employed as an anodyne, antiseptic, astringent, expectorant, laxative, purgative, sedative, stimulant, and tonic in folk medicine.[5] A previous phytochemical study resulted in the isolation of a number of diterpenes from *H. courbaril*.[6] A dried methanol extract of this plant exhibited a positive response to the 1138 mutant yeast strain, which has been shown to respond to topoisomerase II inhibiters,[3] and to the Sc-7 yeast strain, which responds to cytotoxic agents.[2] Bioactivity-guided fractionation of the methanol extract afforded three new diterpenoids: (13*R*)-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid (**1**), (2*S*,-13*R*)-2,13-dihydroxy-1(10),14-*ent*-halimadien-18-oic acid (**2**), and (13*R*)-2-oxo-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid (**3**).



1 R = H
4 R = Me

2 R = H
5 R = OCOPh

3

Results and Discussion

Partitioning of the methanol extract of *H. courbaril* between hexane and MeOH−H_2O (8:2) followed by dilution of the aqueous layer with H_2O to MeOH−H_2O (6:4) and extraction with $CHCl_3$ gave bioactive hexane and $CHCl_3$ fractions. Extraction of the hexane fraction with base followed by acidification and re-extraction with hexane gave a diterpene-enriched extract. This extract was subjected to chromatography on Si gel with elution with EtOAc−hexane; the third fraction gave good quality crystals of **1**.

The negative ion FABMS of **1** ($C_{20}H_{32}O_3$) showed a major signal at *m/z* 319 $(M - H)^-$. The positive ion FABMS did not display a molecular ion, but major fragment ions could be seen at *m/z* 303 $(M - OH)^+$, 257 $(M - H_2O - CO_2H)^+$, and 221 $(M - C_6H_{11}O)^+$. The ^1H and ^{13}C NMR data are shown in Table 1 and indicated that its structure and relative stereochemistry were similar to that of 13-hydroxy-1(10)-halimadien-18-oic acid methyl esters. Compound **1** has not been previously isolated in pure form, although a partially purified preparation was previously obtained and characterized as its methyl ester **4**.[7,8] Compound **4** was prepared from **1**, and comparison of its ^1H and ^{13}C NMR data with the literature data confirmed this assignment.[7,8] This structure was confirmed and its relative stereochemistry established unambiguously by an X-ray crystallographic structure determination. The thermal ellipsoid plot of **1** (Figure 1) clearly indicates the relative configuration.

Compound **2** was isolated from the bioactive $CHCl_3$ fraction, which was subjected to washing with base, reverse-phase column chromatography, and reverse-phase HPLC. The negative ion FABMS of **2** showed major fragment ions of *m/z* 335 $(M - H)^-$, 318 $(M - H_2O)^-$, and 290 $(M - HCO_2H)^-$. The positive FABMS did not show a molecular ion, but a sodiated ion at *m/z* 341 $(M - H_2O + Na)^+$ and major fragment ions at 318 $(M - H_2O)^+$ and 301 $(M - H_2O - OH)^+$ were observed. These data, together with the NMR data discussed below, indicated that **2** had the composition $C_{20}H_{32}O_4$. Its ^1H NMR spectrum clearly showed signals for four olefinic protons (δ 5.94, 5.82, 5.14, and 4.99), an allylic proton (δ 2.22), and four methyl groups (δ 1.20, 1.18, 0.92, and 0.83). The spectrum was quite similar to **1**; however the signal for H-2 was shifted downfield compared to its position in **1**. ^1H COSY and DQF-COSY spectra confirmed the position of H-2 ($^1H-^1H$ COSY with H-1), H-3 ($^1H-^1H$ COSY with H-2), H-6α and β ($^1H-^1H$ COSY with H-5), H-8 ($^1H-^1H$ COSY with H-17), H-11a

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**4**[a,b,c]

	1	**2**	**3**	**4**
1	δ 5.27, bs	5.94, d, J = 5.7	5.71, s	5.22, m
2	δ 2.05, m	4.76, ddd, J = 5.5, 4.5, <2		2.00, m
3α	δ 1.28, m	2.05, dd, J = 11.5, 5.3	2.69, d, J = 15.8	1.81, ddd, J = 12.9, 12.9, 4.8
3β	δ 1.40, m	2.14, dd, J = 11.3, 4.8	2.16, d, J = 15.8	1.45, m
5	δ 2.64, dd, J = 12.4, 2.3	2.22, dd, J = 12.4, 4.8	3.2, dd, J = 12.9, 4.2	2.67, dd, J = 13.5, 3.7
6α	δ 1.24 m	1.71, m	1.42 m	1.24 m
6β	δ 1.31 m	1.44, m	1.42 m	1.24 m
7α	δ 1.44 m	1.33, m	1.82 m	1.46 m
7β	δ 1.44 m	1.33, m	1.82 m	1.46 m
8	δ 1.56 m	1.64, m	1.77 m	1.54
11a	δ 2.22, ddd, J = 12.4, 12.4, <2	2.05, ddd, J = 12.6, 12.6, 3.9	2.34, ddd, J = 12.6, 12.6, 3.0	2.13, ddd, J = 12.3, 12.3, 3.0
11b	δ 1.33, m J = 12.2, 12.2, <2	1.35, ddd, J = 12.6, 12.6, 3.9	1.33 ddd, J = 12.4, 12.4, 3.0	1.42, m
12a	δ 0.99 m	1.16, ddd, J = 12.6, 12.6, 3.9	0.89, m	1.03, ddd, J = 12.3, 12.3, 6.4
12b	δ 0.99 m	1.09, ddd, J = 12.6, 12.6, 3.9	0.89, m	1.03, ddd, J = 12.3, 12.3, 6.4
14	δ 5.78, dd, J = 10.9, 17.5	5.82, dd, J = 10.9, 17.4	5.83, dd, J = 11.0, 17.4	5.80, dd, J = 10.6, 17.2
15A	δ 5.21, dd, J = 17.5, 0.7	5.14, dd, J = 17.5, 1.5	5.16, dd, J = 17.4, 1.6	5.19, dd, J = 17.6, 1.6
15B	δ 5.03, dd, J = 10.8, 0.7	4.99, dd, J = 10.8, 1.5	4.99, dd, J = 11, 1.6	4.99, dd, J = 10.6, 1.7
16	δ 1.28, s	1.20, s	1.25, s	1.26, s
17	δ 0.74, d, J = 6.8	0.83, d, J = 6.9	0.81, d, J = 7.1	0.71, d, J = 6.0
19	δ 1.16, s	1.18, s	1.18, s	1.09, s
20	δ 0.82, s	0.92, s	0.98, s	0.98, s
OMe				3.62, s

[a] At 500 MHz. [b] Compounds **1** and **4** in $CDCl_3$, **2** and **3** in CD_3OD. [c] J values in Hz.

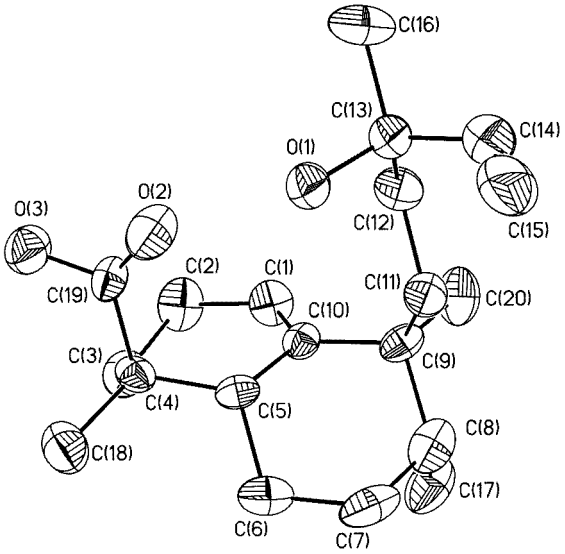

Figure 1. Thermal elipsoid plot (30% probability) of the molecular structure of one of the two crystallographically independent molecules of **1**. Hydrogen atoms have been omitted for clarity.

(^{1}H−^{1}H COSY with H-11b), H-12 and H-12′ (^{1}H−^{1}H COSY with H-11 and H-11′). The positions of H-11a and b were determined from comparison with the NMR spectrum of **1**. The ^{13}C NMR spectrum of **2** was very similar to that of **1**, with the exception of the C-1, C-2, and C-10 signals, whose shifts were those expected for an allylic alcohol. The shifts of C-12, C-13, C-14, C-15, and C-16 were similar to those of the corresponding carbons of **1** (in CD_3OD), indicating that the stereochemistry of the C-13 position is also *R*. HMQC was used for making the assignments of H-7α and H-7β (C-7, δ 25.9) and H-11 and H-11′ (C-11, δ 32.0), along with the assignments of the remaining carbon signals. 1D NOE and GOESY spectra showed nuclear Overhauser interactions between H-1 and H-2, H-1 and H-20, and H-18 with H-3α and H-5. The stereochemistry of the C-2 hydroxyl group was determined by homonuclear decoupling of the C-1 proton; the J values of the C-2 proton signal were determined to be 4.5 and <1.0 Hz. These results are indicative of equatorial−axial and equatorial−equatorial interactions with the C-3 protons; taking into account the X-ray structure of **1**, these data assigned the configuration at C-2 as α.

The absolute configuration of **2** was assigned by determining the CD spectrum of its benzoate derivative **5**. Compound **5** had a negative Cotton effect at 238 nm, consistent with a 2*S* stereochemistry by the benzoate chirality rule.[9] On the basis of this information, the similarity to **1**, and on biosynthetic considerations we have assigned the structure and absolute configuration as (2*S*,13*R*)-2,13-dihydroxy-1(10),14-*ent*-halimadien-18-oic acid.

Compound **3** was isolated in a manner similar to **2**. The negative ion FABMS of **3** showed major fragment ions at m/z 333 $(M - H^+)^-$ and 290 $(M - CO_2H)^-$. The positive ion FABMS did not show a molecular ion; instead a sodiated ion (m/z 357) was observed, along with a major fragment ion of 317 $(M - OH)^+$. These data, together with the NMR data discussed below, indicated that **3** had the composition $C_{20}H_{30}O_4$. The IR spectrum displayed the presence of an α,β-unsaturated ketone (1658 cm^{-1}). Its ^{1}H NMR spectrum clearly showed signals for four olefinic protons (δ 5.83, 5.71, 5.16, and 4.99), an allylic proton (δ 3.17), and four methyl groups (δ 1.25, 1.18, 0.98, and 0.81). The spectrum was quite similar to that of **1** or **2**, suggesting a close structural similarity between the three compounds. The H-1 olefin proton signal in **3** was a sharp singlet, compared to a broad singlet in **1** or a doublet in **2**; the H-5 proton signal was also shifted downfield (δ 3.18) compared to that of **1** (δ 2.64). ^{1}H COSY, DQF COSY, and J-coupling measurements, together with comparison to the spectrum of **1**, were used to assign the signals for H-3α and β (^{1}H−^{1}H COSY, J = 15.8 Hz), H-6 (^{1}H−^{1}H COSY with H-5), H-7 (^{1}H−^{1}H COSY with H-6), H-8 (^{1}H−^{1}H COSY with H-17), H-11a and b (^{1}H−^{1}H COSY with COSY, J = 12.6 Hz), and H-12α and β (^{1}H−^{1}H COSY with H-11). The ^{13}C NMR spectrum of **3** (Table 1) was very similar to that of **1** with the exception of the resonances for the C-1, C-2, and C-10 carbon signals, whose shifts (δ 124.3, 201.5, 172.7) were those expected for an α,β-unsaturated ketone. The shifts of the resonances for the C-12, C-13, C-14, C-15, and C-16 carbons were similar to those of the corresponding carbons in **1** (in CD_3OD), indicating that the stereochemistry of the C-13 position is also *R*. HMQC also helped to identify the resonances for H-3α and H-3β (C-3, δ 41.9), H-11 and H-11′

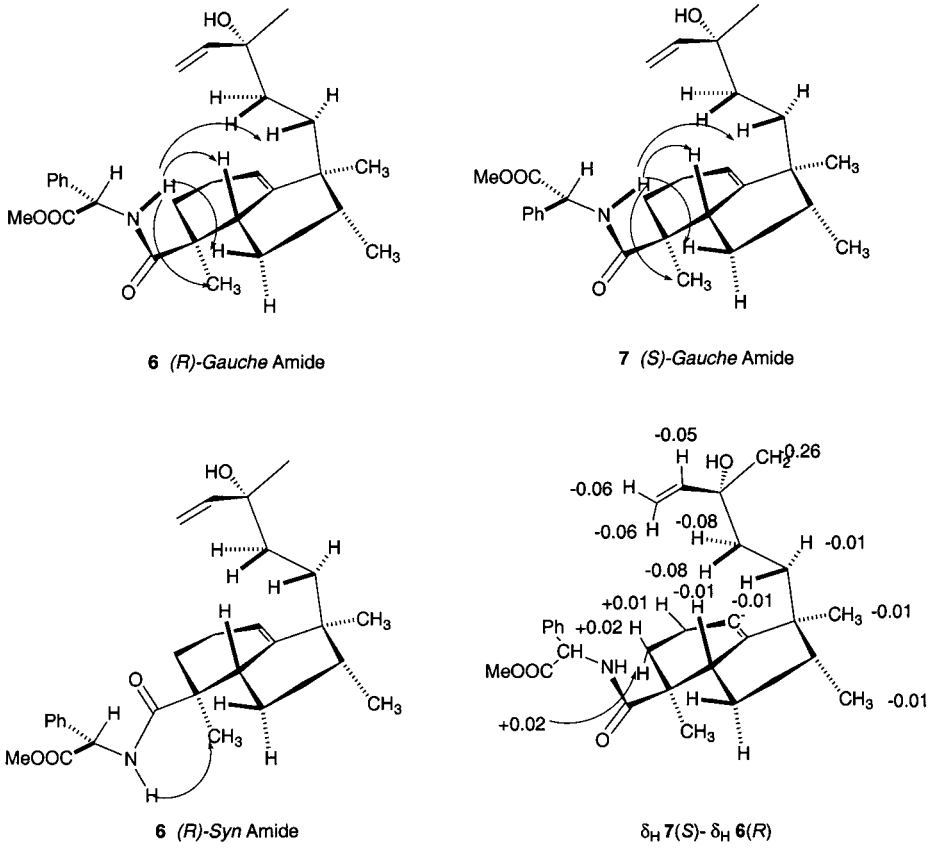

Figure 2. Conformations of PGME amides and resulting $\Delta\delta$ values.

(C-11, δ 33.0), C-5 (δ 44.1). NOESY and 1D NOE experiments indicate interactions between H-1 and H-20, H-8 and H-20, H-7β and H-17, H-15b and H-17, and H-5 and H-7α; these interactions, the absence of other significant interactions, and the agreement of the NOE information with the crystal structure of **1** leads us to assign the structure and absolute configuration of **3** as (13*R*)-2-oxo-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid. A final proof of stereochemistry was obtained from the CD spectrum of **3**, which showed a positive Cotton effect. This is only consistent with a 9*S* stereochemistry, since molecular modeling indicated that only the 9*S* isomer has an excess of atoms in the positive quadrants, and should thus give a positive Cotton effect by the octant rule.[10]

The absolute configuration of 13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid methyl ester from *Halimium viscosum*[8] has been determined through semisynthesis and single-crystal X-ray analysis.[11,12] This work, in conjunction with the establishment of the identity of **4** (obtained from **1**) by the agreement of its NMR spectrum with that of a previously reported compound,[8] and the establishment of the absolute configuration of **2** and **3** by circular dichroism, permitted us to assign the absolute configuration of **1**.

The availability of **1** with a known absolute configuration and an α,α,α-trisubstituted acetic acid functionality provided an opportunity to determine whether Kusumi's method[13] for determining the absolute stereochemisty of carboxylic acids can be applied to this type of functionality. Phenylglycine methyl ester amides (PGME amides) are a relatively recent addition to the collection of commonly used chiral derivatizing agents for stereochemical studies, joining the well-known Mosher reagents[14] and other reagents in this area. They were introduced by Nagai and Kusumi[13] and differ from most other reagents of this type in that they are applied to the determination of the absolute configuration of carboxylic acids as opposed to secondary alcohols; to date they have been used primarily for the stereochemical determination of α,α-disubstituted acetic acids and β,β-disubstituted propionic acids. As with all such reagents, a reliable prediction of their effect depends on an accurate knowledge of the preferred conformation of the derivative and thus of its resulting anisotropic effects.

Both (*R*)- and (*S*)-PGME amides of **1** (**6** and **7**, respectively) were prepared by standard chemistry, and their predominant conformations were determined by careful NOE experiments. Although the amides studied previously adopted a predominant *syn* coplanar conformation with α,α-disubstituted acids,[13] amides **6** and **7** were found to exist primarily in a *gauche* conformation (Figure 2). The key finding that demanded this conformation was the observation of a strong NOE correlation between the NH proton and the C-11a, C-6, and C-5 protons; these correlations are only possible for the *gauche* conformation shown.

The $\Delta\delta$ values for compounds **6** and **7** (δ(**7***S*) $-$ δ(**6***R*)) are shown in Figure 2 and are completely consistent with the model for the absolute stereochemistry shown. This work thus provides both a confirmation of the absolute stereochemistry of **6** (and thus of **1**), and it also demonstrates that the PGME method can be applied to α,α,α-trisubstituted acids provided that the conformation of the derivative can be established by an independent method.

Compound **1** was found to be weakly active in the 1138 mutant yeast strain[2,3] (IC_{12} = 925 μg/mL) and the A2780 human ovarian cell line (IC_{50} > 40 μg/mL),[15] but this activity was insufficiently great to warrant further development. Compound **3** and the PGME amides **6** and **7** were found to be inactive in both cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thermolyne apparatus equipped with a microscope and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were measured on a Midac Inc. M series FTIR. All NMR spectra were recorded at either 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra on a Varian Unity 400 instrument or 500 MHz for ^{1}H and 125 MHz for ^{13}C spectra on a JEOL instrument. MS were obtained on either a VG7070 E-HF (magnetic sector) or a Fison's VG Quattro (Quadropole) instrument. Gel filtration chromatography used Sephadex LH-20 (25−100 μM) and column chromatography employed Si gel 60 (230−400 mesh) (for normal phase) or Varian 5g C18 SPE (for reversed-phase). HPLC separation was performed on a Rainin Dynamax 60 Å 10 mm column monitored at 254 nm. A2780 cytotoxicity measurements were measured on a Cytofluor 2300 fluorescence measurement system from Millipore. All solvents were removed on a rotary evaporator with a bath temperature of 40 °C or less. Phenylglycines [(*R*) and (*S*)] and 1-HOBt were obtained from the Aldrich Chemical Co., and PyBOP was obtained from Novabiochem.

Plant Material. The leaves, stems, and twigs of *Hymenaea courbaril* L. (Caesalpinaceae) were collected near Asindopo village in central Suriname in July 1997 and January 1998 and asigned collection numbers 973DeK05943 and 977DeK-09928. Voucher specimens are deposited at the National Herbarium of Suriname, Paramaribo, Suriname.

Extract Preparation. The plant samples were dried, ground, and extracted with EtOAc to give extracts E970379 and E980037 and then with MeOH to give extracts M970379 and M980037.

Yeast Bioassay. The yeast bioassay was performed using yeast strains Sc-7 and 1138 as previously reported.[3] Extract M970379 gave an IC_{12} value of 2000 μg/mL against the 1138 strain, and extract M980037 gave IC_{12} values of 2000 μg/mL against the 1138 strain and 3000 μg/mL against the Sc-7 strain.

Cytotoxicity Bioassay. In vitro antitumor cytoxicity assays were performed using the A2780 human ovarian cell line. RPMI (200 μL) was dispensed to the column 12 well in a 96-well tissue culture plate. RPMI media (20 μL) was added to column 11. All wells in columns 1−11 were seeded with 180 μL of 2.7×10^{-5} c/mL A2780 DDP-S (Platinol-Sensitive) cells (5×10^{-4} cells/well). Plates were incubated for 3 h in 5% CO_2 at 37 °C to allow cells to adhere. The diluted compound (20 μL in up to 50% aqueous DMSO) was added to the wells. Column 12 was used for media control. Actinomycin D was the positive control and was run at four dilutions with an IC_{50} ~1−3 ng/mL in column 11. RPMI (20 μL) was added to the last four rows of column 11 as a negative control. The plate was incubated 48 h at 37 °C in a 5% CO_2 incubator. The media was removed from the plates. Fresh RPMI (200 μL/well) was added plus 10% FCS containing 1% Alamar Blue solution. The plate was incubated for 4 h at 37 °C and at 5% CO_2. The plates were read on a Cytofluor at an emission of 530 nm and an excitation of 590 nm with a gain of 40, and IC_{50} values were calculated by a curve-fitting program..

Bioactivity-Guided Fractionation and Isolation of 1. The dried bioactive methanol extract M980037 (16 g) was partitioned between hexane and MeOH−H_2O (8:2). Water was added to the MeOH−H_2O fraction to give a MeOH−H_2O (6:4) solution, which was thoroughly extracted with $CHCl_3$. Evaporation of the solvents gave bioactive hexane and $CHCl_3$ fractions (4.1 and 10.0 g, respectively). The hexane fraction was diluted in 100 mL of $CHCl_3$ and extracted with 50 mL of aqueous 5% Na_2CO_3 (three times); the aqueous fractions were combined and neutralized with aqueous 1% HCl and re-extracted with $CHCl_3$. Solvent removal provided 1.6 g of acidic substances. This acidic fraction was chromatographed over silica gel with elution with EtOAc−C_6H_{12} (2:8). Eleven fractions were collected, of which fractions 3−5 yielded **1** (719 mg total) after evaporation. Fraction 3 (212 mg) was a syrupy material which slowly crystallized over a one-week period to give crystals of **1**; one large crystal was removed and submitted for X-ray analysis.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**4**[a,b]

position	1	2	3	4
1	119.9	123.5	124.3	119.8
2	23.3	73.0	201.5	23.3
3	28.4	35.9	41.9	29.7
4	44.6	45.0	45.1	45.0
5	37.4	41.4	44.1	37.8
6	24.0	22.0	23.7	23.7
7	29.1	25.9	28.2	28.8
8	39.9	39.4	41.2	39.6
9	43.4	42.2	45.1	43.3
10	141.8	149.7	172.7	141.9
11	32.0	32.0	33.0	32.2
12	36.6	36.9	36.1	36.6
13	73.6	72.4	72.5	72.7
14	143.4	145.2	144.4	144.4
15	111.7	111.0	110.4	111.4
16	29.6	27.9	27.6	29.7
17	15.7	14.4	14.5	15.5
18	21.8	18.0	20.6	22.1
19	182.5	183.4	182.7	178.8
20	23.1	21.0	21.8	22.4
OMe				51.9

[a] At 125 MHz. [b] **1** and **4** in $CDCl_3$, **2** and **3** in CD_3OD.

A small portion (330 mg) of the initial chloroform partition (10.0 g) was dissolved in CH_2Cl_2 (200 mL) and extracted with 0.1 M $NaHCO_3$ (200 mL). Evaporation of the CH_2Cl_2 fraction gave neutral material (89 mg), which was subjected to reverse-phase chromatography on a Varian C-18 SPE column (5 g size) using MeOH−H_2O (8:2) as eluent and evaluation of the fractions by ^{1}H NMR spectroscopy. Fraction 5 (out of 9 fractions) (17 mg) was subjected to reverse-phase HPLC using a C18 column and MeOH−H_2O (85:15) as eluent to give **2** (3.2 mg). The aqueous $NaHCO_3$ phase was acidified with aqueous 10% HCl and extracted with 200 mL of CH_2Cl_2. Solvent was removed, and the product (67 mg) was subjected to reverse-phase HPLC (twice) using a C18 column and MeOH−H_2O (85:15 and 70:30) as eluents to yield **3** (1.9 mg).

(13*R*)-13-Hydroxy-1(10),14-*ent*-halimadien-18-oic Acid (1): colorless crystals, mp 94−96 °C, $[\alpha]^{25}_D$ +22° (*c* 0.6, MeOH); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 209 (−0.13), 220 (+0.24), 250 (+0.05), 261 (+0.04), 275 (+0.03); IR(neat film) λ_{max} 3403, 2972, 2936, 2875, 1704, 1693, 1463, 1377, 1284, 1243, 1189 cm^{-1}; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; FABMS (negative ion) *m*/*z* 320 ($M^{-\bullet}$, 22), 319 (M^- − H, 100) FABMS (positive ion) *m*/*z* 303 (M^+ − 17, 17), 257 (20), and 221 (53); HRFABMS (negative ion) *m*/*z* 319.2273 (M^- − H, calcd for $C_{20}H_{31}O_3$, 319.2275).

X-ray Crystallography of (13*R*)-13-Hydroxy-1(10),14-*ent*-halimadien-18-oic Acid (1).[16] A clear colorless block was crystallized as described above. The crystal was cut (ca. 0.5 × 0.5 × 0.5 mm^3), mounted on a glass fiber with epoxy, and placed on a Siemens (Bruker) P4 diffractometer. Unit cell parameters were determined by least squares refinement of 39 reflections that have been automatically centered on the diffractometer.[17] The Laue symmetry and systematic absences were consistent with the orthorhombic space groups $P2_12_12_1$. The structure was solved by direct methods and refined using the SHELXTL-NT v5.10 program package.[18] The asymmetric unit of the structure comprises two crystallographically independent molecules. As there were no heavy atoms, the absolute configuration could not be determined from the Friedel pairs; the Friedel pairs were therefore merged for the final refinement. The absolute configuration was assigned based on previous literature (see above) which confirmed the *R* configuration at the C(13) center. The final refinement model involved anisotropic displacement parameters for all non-hydrogen atoms and a riding model for all hydrogen atoms. The hydrogen attachments of the carboxylic acid groups were assigned to oxygen with the longest C−O bond length. All bond

lengths and angles were within the expected ranges. The XP subroutine of the program package SHELXTL-NT was used for the ensuing molecular graphics generation.

Crystal data: $C_{20}H_{32}O_3$ (MW = 320.46), T = 20 °C, orthorhombic, space group $P2_12_12_1$, a = 11.6830(15) Å, b = 12.4363(18) Å, c = 27.115(3) Å (the 90° angles are omitted and the *P* is in italics), V = 3939.7(9) Å^3, Z = 8, density$_{calc}$ 1.081 g/cm^3, absorption coefficient 0.071 mm^{-1}, $F(000)$ = 1408, cryst size 0.5 × 0.5 × 0.5 mm^3, θ range for data collection 1.50 to 20.00 °, index ranges $-11 \le h \le 0$, $-11 \le k \le 0$, $-26 \le l \le 0$, and $0 \le h \le 11$, $0 \le k \le 11$, $0 \le l \le 26$, reflections collected 4235, independent reflections 2120 [R(int) = 0.0453], completeness to θ = 20.00° = 100.0% (Mo Kα), absorption correction none, refinement method full-matrix least squares on F^2, data/restraints/parameters 2120/0/428, goodness-of-fit on F^2 0.840, final R indices [$I > 2\sigma(I)$] R1 = 0.0352, wR2 = 0.0529, R indices (all data) R1 = 0.0746, wR2 = 0.0621, extinction coefficient 0.0047(2), largest difference between peak and hole 0.110 and −0.104 e$^-$ Å^{-3}.

(2*S*,13*R*)-2,13-Dihydroxy-1(10),14-*ent*-halimadien-18-oic acid (**2**)**:** colorless amorphous matrix, $[\alpha]_D$ +45° (*c* 0.4, MeOH); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 218 (−0.06), 245 (+0.18), 257 (+0.14), 268 (+0.05), 280 (+0.04); IR (neat film) λ_{max} 3465, 2966, 2929, 2869, 1762, 1446, 1379 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; FABMS (negative ion) *m*/*z* (rel int) 336 (M$^-$, 5), 335 (24), 334 (40) 333 (44), 319 (47), 318 (100), 290 (11), 289 (12), 275 (15), 255 (88); FABMS (positive ion) *m*/*z* (rel int) 341 (MNa$^+$ − 18, 22), 318 (6), 317 (10), 301 (47), 273 (15), 257 (100), 255 (37).

(13*R*)-2-Oxo-13-hydroxy-1(10),14-*ent*-halimadien-18-oic acid (3): colorless amorphous matrix, $[\alpha]_D$ +15° (*c* 0.3, MeOH); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 231 (sh, +0.57), 239 (sh, +0.67); 252 (+0.84); IR (neat film) λ_{max} 3404, 2953, 2923, 2869, 1730, 1658, 1603, 1463, 1372 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; FABMS (negative ion) *m*/*z* (rel int) 334 (M$^-$, 100), 333 (83), 311 (14) 290 (51), 289 (49), 265 (20), 255 (20); FABMS (positive ion) *m*/*z* (rel int) 357 (7), 318 (6), 317 (8), 277 (6), 242 (11), 223 (20), 207 (21); HRFABMS (negative ion) *m*/*z* 333.2042 (M$^-$ − H, calcd for $C_{20}H_{29}O_4$, 333.2066).

Preparation of (13*R*)-13-Hydroxy-1(10),14-*ent*-halimadien-18-oic Acid Methyl Ester (4). Compound **1** (50 mg) was dissolved in 2 mL of DMF; 200 mg (10 equiv) of K_2CO_3 and 100 μL (10 equiv) of CH_3I were added. The mixture was allowed to react at room temperature for 16 h. H_2O and $CHCl_3$ (50 mL each) were added and well shaken. The organic layer was dried and then purified by Si gel PTLC (EtOAc−hexane) to yield 25.4 mg (49%) of **4**. Compound **4:** oil, $[\alpha]^{25}_D$ +90.3° (*c* 0.214, $CHCl_3$), +37.3° (c 0.061, MeOH); IR(neat film) 3530, 2951, 2926, 1716, 1456, 1378, 1271, 1242, 1196, 995, 915 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; FABMS (positive ion) *m*/*z* (rel int) 357 (MNa$^+$, 100); HRFABMS (positive ion) *m*/*z* 357.2406 (calcd for $C_{21}H_{34}O_3Na$ 357.2406).

Preparation of (*R*)- and (*S*)-Phenylglycine Methyl Ester (PGME) Amides of 1. Phenylglycine methyl esters were prepared from phenylglycine by the literature procedure.[13] Compound **1** (20 mg) and 14.6 mg of (*S*)-PGME were dissolved in 1 mL of DMF and cooled to 0 °C. A 37.9 mg sample of PyBOP, 10 mg of 1-HOBt, and 23 μL of *N*-methylmorpholine were added in order, and the mixture was stirred at 0 °C for 1.5 h. Benzene (15 mL) and EtOAc (30 mL) were then added, the mixture was washed with aqueous 5% HCl, aqueous saturated $NaHCO_3$, and brine, the organic layer was dried with Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by Si gel PTLC, with development first with $CHCl_3$−MeOH, 97:3, and then with $CHCl_3$ to yield the (*S*)-PGME amide **7** (12.3 mg, 42%). The (*R*)-PGME amide **6** (13.8 mg, 47%) was prepared in a similar fashion from (*R*)-PGME.

Preparation of the 2-Benzoate of (2*S*,13*R*)-2,13-Dihydroxy-1(10),14-*ent*-halimadien-18-oic Acid (5). Approximately 140 μg of **2** was placed in 200 μL of pyridine and allowed to stir at room temperature. Benzoyl chloride (20 μL) was added along with a small quantity of DMAP, and the mixture was allowed to react overnight. The pyridine was removed in a stream of argon, water (1 mL) and $CHCl_3$ (1 mL) were added, and the mixture was shaken. The organic layer was removed by pipet and dried by rotary evaporation and then under high vacuum for 8 h. The sample was prepared for CD by dissolving it in 2 mL of methanol and passing it through a nylon filter to provide a clear colorless solution: CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 216 (sh, −0.38), 224 (−0.40), 238 (−0.38), 249 (−0.40), 268 (−0.45), 277 (sh, −0.52), 286 (sh, −0.62), 293 (−0.68), 312 (−0.64).

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Note Added in Proof. The isolation of two new diterpenoids from *Hymenaea courbaril* var. *stilbocarpa* with related structures to those of **1**−**3** has recently been reported.[19]

References and Notes

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