Cytotoxic *cis*-Fused Bicyclic Sesquiterpenoids from *Jatropha neopauciflora*

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Analysis of polar fractions of the bark extract of *Jatropha neopauciflora* provided two uncommon *cis*-fused bicyclic sesquiterpenoids, which were characterized as (1*R*,2*R*,5*S*,6*S*,7*S*,10*S*)*-5-*epi*-eudesm-4(15)-ene-1α,2*β*,6α-triol (**1**) and (1*R*,2*R*,5*S*,6*S*,7*R*,10*S*)*-ax-4(15)-ene-1α,2*β*,7*β*-triol (**2**). Their absolute configuration and biogenesis were derived by correlations with congeners of known absolute configurations. Biological investigation of less polar fractions of the bark extract led to the isolation of moderately cytotoxic triterpenes, calenduladiol (**3**) and (3*β*,16*β*)-16-hydroxylup-20-(29)-en-3-yl (*E*)-3-(4-hydroxyphenyl)prop-2-enoate (**4**).

Jatropha neopauciflora (Euphorbiaceae) is a Mexican endemic medicinal plant used in the treatment of wounds and buccal infections. Recent reports have described the antibacterial activity of latex extractions of the bark of *J. neopauciflora*,[1] as well as the isolation of uncommon sesquiterpenoids from the bark of this species.[2] Chemical constituents of the genus *Jatropha*, such as some jatrophane-type diterpenes, have shown relevant cytotoxic and antitumoral properties against leukemia and nasopharyngeal tumor cells.[3] Therefore, the structures of two unknown bark constituents (**1**, **2**) of *J. neopauciflora* were determined by spectroscopic methods and the cytotoxicity data of two isolates (**3**, **4**) are reported.

The CH_2Cl_2/MeOH extract from the bark of *J. neopauciflora* exhibited moderate cytotoxicity against the U251 human central nervous system tumor cell line (34.29 μg/mL ± 2.48) and the K562 leukemia cell line (43.12 μg/mL ± 3.63). The organic extract was fractionated, and its main fractions (F1−F13) were evaluated in a cytotoxicity assay using the sulforhodamine B colorimetric method.[4] Fractions F5 and F6 exhibited 91−100% of tumor cell growth inhibition at 50 μg/mL, when evaluated against U251 and K562 cell lines. A search for the cytotoxic compounds led to the isolation and identification of calenduladiol (**3**)[5] and (3*β*,16*β*)-16-hydroxylup-20(29)-en-3-yl (*E*)-3-(4-hydroxyphenyl)prop-2-enoate (**4**).[2] Compound **3** showed $IC_{50} = 48.44 \pm 4.31$ μM (21.41 μg/mL) and 31.44 ± 3.55 μM (13.89 μg/mL) against U251 and K562 cell lines, respectively. Compound **4** gave an $IC_{50} = 41.04 \pm 6.91$ μM (24.13 μg/mL) against U251. The positive control, adriamycin, showed $IC_{50} = 0.32 \pm 0.02$ and 0.28 ± 0.01 μM against U251 and K562, respectively, indicating moderate cytotoxicity for **3** and **4**. Chemical investigations of the more polar fractions F7 and F8 led to the isolation of an isomeric mixture of two hydroxylated terpenoids. Further separations by HPLC and characterizations by extensive spectroscopic studies, mainly 1D and 2D ^{1}H and ^{13}C NMR methods, led to two uncommon sesquiterpenes with an axane and *cis*-eudesmane skeleton. The absolute configurations of the new compounds **1** and **2** were derived by correlation with congeners, which had been obtained in a previous study of *J. neopauciflora*,[2] and the proposed biogenesis was in analogy with the biogenetic pathway of related sesquiterpenoids.[6] The isomeric mixture of **1** and **2** did not show activity when evaluated against the U251 and K562 cancer cell lines.

Compound **1** was a colorless oil ($[\alpha]^{25}_D$ −83.52). The molecular formula $C_{15}H_{26}O_3$ ($[M + H]^+$ *m*/*z* 255.1965; calcd 255.1960, $C_{15}H_{27}O_3$) was deduced from its HRFABMS, which indicated three degrees of unsaturation. The IR spectrum exhibited absorption bands at 3385 cm^{-1} (hydroxyls) and 1648, 1460, and 1049 cm^{-1} (double bond(s)). The ^{13}C NMR spectrum (Table 1) showed the presence of 15 resonances, which corresponded by DEPT analysis to six methines (three oxymethines), four methylenes (one sp^2), three methyls, and two quaternary carbons (one sp^2). The MS and NMR data suggested the presence of a sesquiterpenoid skeleton. The ^{1}H NMR spectrum (Table 1) revealed the presence of two vinylic protons at δ 5.07 (t, $J = 2.0$ Hz, H-15a) and 4.93 (t, $J = 2.0$ Hz, H-15b) attributed to the methylene carbon resonating at δ_C 116.06 (C-15), according to the HSQC data. The olefinic methylene signal exhibited ^3J cross-peak correlations with a methylene carbon at δ_C 38.1 (C-3) and its corresponding hydrogens resonating at δ 2.60 (ddd, $J = 13.0$, 5.0, 2.0 Hz, H-3α) and 2.30 (ddt, $J = 13.0$, 11.0, 2.0 Hz, H-3*β*). The H_2-3 showed ^{1}H−^{1}H correlations with an oxymethine proton at δ 3.74 (ddd, $J = 11.0$, 9.0, 5.0 Hz, H-2α), which in turn correlated with another oxymethine proton at δ 3.65 (d, $J = 9.0$ Hz, H-1*β*). The relative orientations of H-3α, H-3*β*, H-2α, and H-1*β* were defined according to the *trans*-diaxial coupling between H-3*β* and H-2α ($J = 11.0$ Hz), the axial−equatorial coupling between H-3α and H-2α ($J = 5.0$ Hz), and the *trans*-diaxial coupling between H-1*β* and H-2α ($J = 9.0$ Hz). The vinylic methylene signal exhibited cross-peak correlations with a methine carbon at δ 61.3 (C-5) and its corresponding hydrogen at δ 1.85 (d, $J = 10.0$ Hz, H-5α). Here H-5α showed ^{1}H−^{1}H correlations with H-6*β* (t, $J = 10.0$ Hz) at δ 3.55, which in turn correlated with H-7 (m) at δ 1.31−1.22. Multiplicity of H-6*β* (t, $J = 10.0$ Hz) indicated two *trans*-diaxial couplings with H-5α ($J = 10.0$ Hz) and with H-7 (m, δ 1.31−1.22), establishing its α-orientation. HSQC correlation of H-7α with the carbon signal at δ 49.0 exhibited cross-peaks with two methyl groups at δ 0.94 (d, $J = 7.0$ Hz, H_3-13) and 0.84 (d, $J = 7.0$ Hz, H_3-12), which correlated (COSY) with proton H-11 at δ 2.20 (dsept, $J = 7.0$, 2.0 Hz). Multiplicities of H-11, H_3-12, and H_3-13 indicated an isopropyl unit, which was confirmed by the fragment *m*/*z* 43 in the mass spectrum. The overlapped hydrogen signals at δ 1.31−1.22 showed HSQC correlations with a methylene carbon at δ 18.1 (C-8) and exhibited ^{1}H−^{1}H correlations with H-8 (δ 1.48). C-8 (δ 18.1) showed cross-peaks with a methylene group at δ_C 34.5 (C-9), δ_H 2.08 (dt, $J = 13.5$, 3.0 Hz, H-9*β*), and 1.05 (ddd, $J = 13.5$, 13.5, 3.5 Hz, H-9α). The coupling constants of H_2-9 established their axial and equatorial orientations. The connectivity of the (H-5α)-C5−C6(H-6*β*)−C7(H-7α)−C8(H_2-8)−C9(H_2-9) fragment was confirmed by the HMBC spectrum: H-5α (δ 1.85) correlated with C-6 (δ 67.4) and C-7 (δ 49.0); H-9α (δ 1.05) correlated with C-7 (δ 49.0) and C-1 (δ 73.8); H-9*β* (δ 2.08) correlated with C-5 (δ 61.3); and H-8α (δ 1.48) correlated with C-6 (δ 67.4). In addition, H-5α, H-9α, and H-9*β* exhibited cross-peak correlations with a quaternary carbon at δ 39.6 (C-10), which in turn correlated with an uncoupled methyl group (CH_3-14) resonating at δ_C 22.5 and δ_H 0.89. Additionally, CH_3-14 showed HMBC correlations with C-10 (δ 39.6), C-9 (δ 34.5), C-5 (δ 61.3), and C-1 (δ 73.8), establishing

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** in $CDCl_3$ at 500 (^{1}H) and 125 MHz (^{13}C)[a]

	1		**2**	
position	^{1}H	^{13}C	^{1}H	^{13}C
H-1β	3.65, d (9.0)	73.8	3.46, d (10.0)	76.7
H-2α	3.74, ddd (11.0, 9.0, 5.0)	71.9	3.59, ddd (11.5, 10.0, 5.0)	72.4
H-3α	2.60, ddd (13.0, 5.0, 2.0)	38.1	2.56, ddd (13.0, 5.0, 2.0)	38.8
H-3β	2.30, ddt, (13.0, 11.0, 2.0)		2.38, ddt (13.0, 11.0, 2.0)	
4		143.0		147.2
H-5α	1.85, d (10.0)	61.3	2.23, d (11.0)	61.4
H-6β	3.55, t (10.0)	67.4	2.33, dddd (11.0, 10.0, 8.5, 7.5)	45.1
H-7α	1.31−1.22, m	49.0	3.37, dd (8.5, 3.5)	82.4
H-8β	1.31−122, m	18.1	1.91, m	25.9
H-8α	1.48, m		1.42, m	
H-9β	2.08, dt (13.5, 3.0)	34.5	2.03, ddd (12.0, 8.5, 2.5)	36.3
H-9α	1.05, ddd (13.5, 13.5, 3.5)		1.35, m	
10		39.6		48.6
H-11	2.20, dsept (7.0, 2.0)	26.4	1.72, dsept (7.0, 3.5)	31.1
H_3-12	0.84, d (7.0)	16.2	0.94, d (7.0)	20.2
H_3-13	0.94, d (7.0)	20.9	0.84, d (7.0)	15.1
H_3-14	0.89, s	22.5	0.94, s	19.1
H-15a	5.07, d (2.0)	116.1	5.02, t (2.0)	114.2
H-15b	4.93, d (2.0)		4.96, t (2.0)	
OH	1.88, br s		1.95, br s	

[a] δ in ppm and J in Hz.

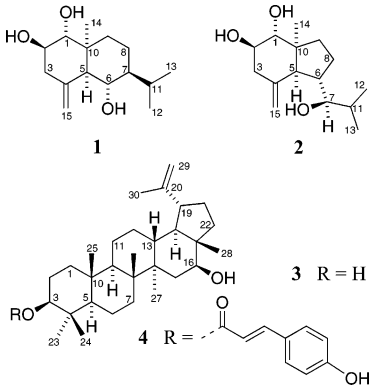


Figure 1. Terpenoids from *J. neopauciflora*.

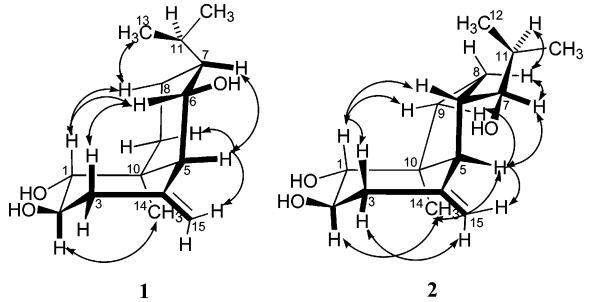


Figure 2. NOESY interactions of compounds **1** and **2**.

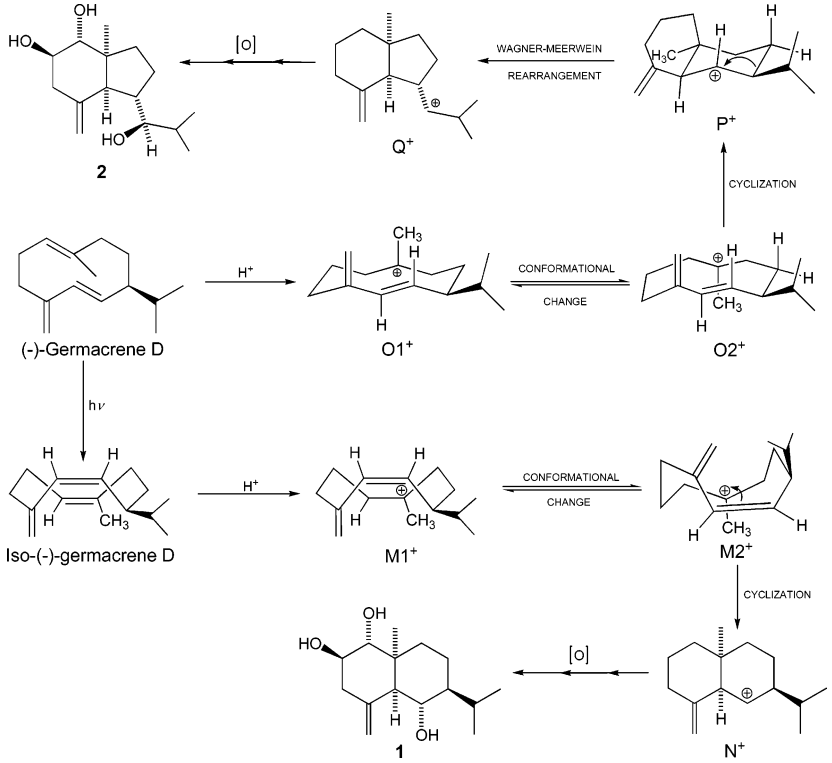
a eudesmane-type sesquiterpene skeleton (**1**). The relative configuration of compound **1** was confirmed by NOESY experiments, which exhibited interactions between H-1β and H-3β, H-8β, and H-6β and between H-6β and H-3β and H-8β (which interacted with H_3-13). The observed interactions between H-5α and H-7α, H-9α, H-15a, and H_3-14 established the *cis*-fusion of the eudesmane-type sesquiterpene **1**. The latter was supported by the NOESY interaction between H-2α and H_3-14, which in turn correlated with H-9α (Figure 2).

Compound **2**, an optically active colorless oil ($[\alpha]^{25}_D$ −29.78) possessed the same molecular formula as **1** by HRFABMS ([M + H]$^+$ *m*/*z* 255.1971; calcd 255.1960, $C_{15}H_{27}O_3$). The IR and the ^{1}H and ^{13}C NMR spectra (Table 1) exhibited functional group absorptions similar to those described for **1**. However, the proton and carbon chemical shifts were not identical, indicating another arrangement of the sesquiterpene skeleton. The main differences were found in the oxymethine protons resonating at δ 3.59 (ddd, J = 11.5, 10.0, 5.0 Hz, H-2α), 3.46 (d, J = 10.0 Hz, H-1β), and δ 3.37 (dd, J = 8.5, 3.5 Hz, H-7α). Protons at δ 3.59 and 3.46 exhibited COSY correlations, and according to their observed *J*-values, their vicinal positions and relative configurations were established as indicated in **1**. The multiplicity of H-7α indicated two spin−spin couplings, which were confirmed by COSY correlations between H-7α and H-6β (δ 2.33, dddd, J = 11.0, 10.0, 8.5, 7.5 Hz) and H-11 (δ 1.72, d sept, J = 7.0, 3.5 Hz). The δ 1.72 resonance was attributed to a methine proton of an isopropyl unit due to its COSY correlations with two doublets (J = 7.0 Hz) at δ 0.94 (H_3-12) and 0.84 (H_3-13). Additionally, H-6β showed COSY correlation with a doublet at δ 2.23 (J = 11.0 Hz, H-5α), thus establishing a *trans*-diaxial coupling. Also, H-6β exhibited ^{1}H−^{1}H correlations with a methylene signal resonating at δ 1.42 (H-8α) and 1.91 (H-8β). The observed COSY correlations for H-6β and H-7α established the axane skeleton for compound **2** with connectivities being confirmed by analysis of its HMBC spectrum. The relative configuration of **2** was determined by the NOESY spectrum analysis with main interactions found between H-1β and H-3β, H-9β, and H-6β, between H6-β and H-8β, and between H-8α and H-11. Additionally, relevant interactions were observed between H-5α and H-7α, H-9α, H_3-14α, and H-15a, between H_3-14α and H-2α, and between H-3α and H-15b. These data were in accord with a *cis*-fused axane-type sesquiterpene with structure **2**, as shown in Figure 2.

Previous structural studies have shown that axane- and *cis*-eudesmane-type sesquiterpenes are not common in Nature.[7−10] The absolute configurations of the axane-type sesquiterpenes from the sponge *Axinella cannabina* establishing the *cis*-β fusion of the perhydroindane skeleton were established by chemical correlations and circular dichroism experiments.[7] In addition, an axane sesquiterpene isolated from the plant *Teclea nobilis* (Rutaceae) has also been reported; however, its absolute configuration was not established.[8] There are two additional reports describing the presence of one *cis*-eudesmane sesquiterpene in the higher plants *Litsea verticillata* (Laureaceae)[9] and *Caragana intermedia* (Leguminosae),[10] whose absolute configuration remains undetermined.

The absolute configurations of **1** and **2** may be hypothesized by taking into account the previous chemical study of *J. neopauciflora*[2] and biogenetic pathways proposed by Bülow and König, who considered (−)-germacrene D as the biogenetic precursor.[6] Thus, biosynthesis of **1** implies the isomerization of (−)-germacrene D into iso-(−)-germacrene D, which could be protonated to form the

Scheme 1. Proposed Biogenesis of Sesquiterpenoids **1** and **2**.



germacrenyl cation **M1**. Thus, the conformational change of germacrenyl cation **M1** to **M2** allows a cyclization reaction to produce the *cis*-eudesmyl cation **N**, which could form compound **1** through several oxidative steps (Scheme 1). Furthermore, the biogenetic pathway of compound **2** implies a variant of the mechanism of the reaction reported for (1*R*,2*R*)-dihydroxycycloax-4(15)-ene.[2] Following protonation of (−)-germacrene D to form the germacrenyl cation **O1**, a conformational change to the germacrenyl cation **O2** allows a *cis*-cyclization reaction to form the *cis*-eudesmyl cation **P**, which could form the perhydroindane skeleton of type **2** by a Wagner−Meerwein rearrangement to allow the biogenesis of compound **2** by consecutive oxidative reactions (Scheme 1).

On the basis of the proposed absolute configuration for **2**, it could be suggested that one enantiomeric series is produced by higher plants, whereas the other is formed in marine organisms.[7]

Experimental Section

General Experimental Procedures. Column chromatography (CC) was performed using silica gel (70−230 mesh). HPLC separations were carried out on a Waters apparatus equipped with a diode-array detector and with Lychrosorb 5 RP-18 (Phenomenex) of 5 μm, 250 × 4.00 mm. The gradient elution was carried out with a mixture of CH_3CN/H_2O at room temperature. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 341 polarimeter. IR ($CHCl_3$) spectra were recorded on a Nicolet Magna FT-IR 750 spectrometer. ^1H (499.89 MHz) and ^{13}C (125.71 MHz) NMR spectra were recorded on a Varian Unity Plus 500 spectrometer. EI and HRFAB mass spectra were measured on Jeol JMS-AX505HA and JMX-SX102A mass spectrometers, respectively.

Plant Material. *J. neopauciflora* was collected in Totomuchil, Estado de México, in April 2003 and authenticated by Dr. Jaime Jiménez Ramírez (Facultad de Ciencias, UNAM). A voucher specimen was deposited at the Herbario Facultad de Ciencias, UNAM; registration number 089165.

Extraction and Isolation. Bark of *J. neopauciflora* (500 g) was processed as previously described.[2] Polar fractions F7 and F8 (2.15 g) were fractionated to furnish a subfraction (66 mg, CC, $CHCl_3$/MeOH, 85:15) that contained a mixture of two hydroxylated terpenenoids, as deduced from the ^1H NMR spectra. This subfraction was purified by HPLC using the following conditions. Flow rate: 1 mL/min; gradient elution: CH_3CN/H_2O, 7:3 for 7 min; then, increasing proportions of CH_3CN/H_2O from 7:3 to 9:1 for 8 min. This process led to the purification of **1** (8 mg) and **2** (8 mg). Compounds **3** and **4** were isolated as previously described.[2]

(1*R*,2*R*,5*S*,6*S*,7*S*,10*S*)*-5-*epi*-Eudesm-4(15)-ene-1α,2β,6α-triol (1): colorless oil; $[\alpha]^{25}_D$ −83.52 (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3385, 2958, 2933, 2873, 1648, 1460, 1049, 757; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 254 $[M]^+$ (3), 236 $[M - H_2O]^+$ (22), 218 $[236 - H_2O]^+$ (24), 123 (100), 107 (42), 95 (77), 43 (26), 41 (31); HRFABMS *m*/*z* 255.1965 (calcd for $C_{15}H_{27}O_3$, 255.1960).

(1*R*,2*R*,5*S*,6*S*,7*R*,10*S)-Ax-4(15)-ene-1α,2β-7β-triol (2):** colorless oil; $[\alpha]^{25}_D$ −29.78 (*c* 0.235, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3385, 2956, 2872, 1645, 1465, 1052, 759; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 254 $[M^+]$ (2), 255 $[M + H]^+$ (1), 236 $[M - H_2O]^+$ (8), 218 $[236 - H_2O]^+$ (7), 211 $[M - C_3H_7]^+$ (62), 200 $[218 - H_2O]^+$ (9), 182 $[M - C_4H_8O]^+$ (62), 164 $[236 - C_4H_8O]^+$ (56), 147 (57), 123 (45), 122 (100), 107 (43), 93 (32), 81 (30), 55 (32), 43 (31); HRFABMS *m*/*z* 255.1971 (calcd for $C_{15}H_{27}O_3$, 255.1960).

Cytotoxicity Assay. Human tumor cell lines of leukemia (K562, CML) and central nervous system (U251, Glia) were supplied by the National Cancer Institute (NCI). The cytotoxicity of the test compounds against tumor cells was determined using the protein-binding dye sulforhodamine B (SRB) in a microculture assay to measure cell growth.[4] The cell lines were cultured in RPMI-1640 (Sigma Chemical Co., Ltd., St. Louis, MO), supplemented with 10% fetal bovine serum, 2 μM L-glutamine, 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, and 0.25 μg/mL amphotericin B (Gibco). They were maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. For the assay, 5×10^4 cells/mL (K562, MCF-7) and 7.5×10^4 cells/well (U251, PC-3), with 100 μL/well of the cell suspensions, were seeded in 96-well microtiter plates and incubated to allow cell attachment. After 24 h, 100 μL of each test compounds and the positive controls were added to each well. After 48 h, adherent cell cultures were fixed in situ by adding 50 μL of cold 50% (w/v) aqueous trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed three times with H_2O and then air-dried. Cultures fixed with TCA were stained for 30 min with 100 μL of 0.4% SRB solution. Protein-bound dye was extracted with 10 μM unbuffered Tris base, and the optical densities were read on an Ultra microplate reader (Elx 808, BIO-TEK Instruments, Inc.), at a wavelength of 515 nm. Results were expressed as concentration giving 50% inhibition (IC_{50}). The data were calculated according to the protocol of Monks,[4] where

a dose−response curve was plotted for each compound; the IC_{50} values were estimated from linear regression equations. The IC_{50} values (mean ± standard error) for **3** and **4** and the positive control, adriamycin, are reported in the text.

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