Identification of New Diterpenoids from *Euphorbia calyptrata* Cell Cultures

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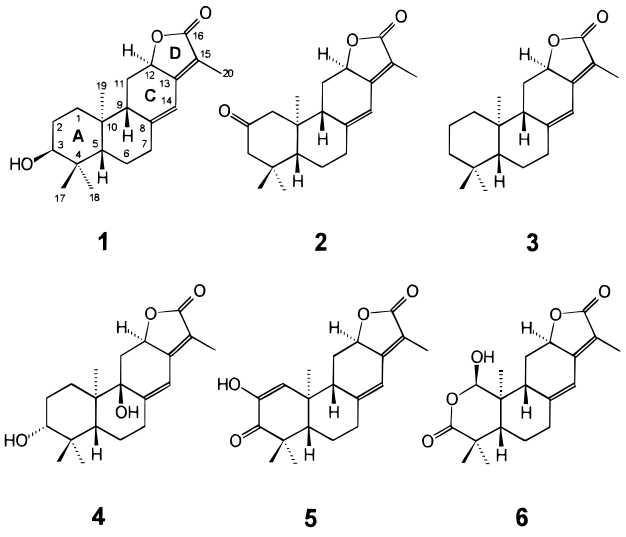
Four new diterpenoids, helioscopinolides F (**2**), H (**4**), I (**5**), and L (**6**), were isolated from three different cultured cell lines of *Euphorbia calyptrata* var. *involucrata*. Helioscopinolide B (**1**) and jolkinolide E (**3**), previously found in *Euphorbia helioscopia* and *Euphorbia jolkini*, respectively, were also isolated from the same cultures. Structures were identified by spectroscopic methods.

Euphorbia helioscopia[1,2] and *Euphorbia jolkini*[3] produce diterpenes as secondary metabolites named helioscopinolides and jolkinolides, respectively. These diterpenes, characterized by an α,β-unsaturated γ-lactone with a conjugated double bond at the β position, have the same carbon skeleton but different oxidation patterns. Helioscopinolides bear oxidations only on rings A and B and jolkinolides only on rings C and D.

Euphorbia calyptrata Coss and Dur var. *involucrata* (Euphorbiaceae), a rare shrub growing in the Sahara desert, also produces helioscopinolides.[4] Four main products, helioscopinolides A, C, D, and E, were isolated from its suspension cultures.[5] Other helioscopinolides present either in the plant or in the cultures were also detected by HPLC analysis, but in amounts too low to be isolated.

Therefore, a study was undertaken to select cell lines by hormonal manipulation from genetically variable wild *Euphorbia calyptrata* parent cells that produced the minor helioscopinolides in sufficient quantities for structure elucidation. Three cell lines were obtained from which six helioscopinolides (**1**−**6**) were isolated. In fact, these cell lines produced at least 10 times the quantity of the desired metabolites in comparison to the parent cultures.

The structures of the isolated products have been investigated by MS, ^{1}H-NMR, ^{13}C-NMR, and, if necessary, by homonuclear (DQFCOSY, NOESY) and heteronuclear correlation (HETCOR) experiments. From inspection of their NMR spectra, compounds **1**−**6** showed the same general structure of the previously reported helioscopinolides with modifications only in the A ring; thus, we will discuss only the special features of each compound. The ^{1}H- and ^{13}C-NMR spectra are reported in the Experimental Section.



Helioscopinolide B (**1**) had a molecular formula of $C_{20}H_{28}O_3$ (316 daltons), and its ^{1}H-NMR spectrum corresponded to reported data.[1]

Helioscopinolide F (**2**) had the same molecular weight (314 Da) as helioscopinolide E[5] but differed in the carbonyl group position (at C-2 instead of C-3). The ketone showed a ^{13}C-NMR signal at 209.4 ppm, and its position was established by the coupling pattern of the four protons at C-1 and C-3. The two equatorial protons at C-1 and C-3 (2.55 and 2.21 ppm, respectively) were long-range coupled (J_w = 2.2 Hz) in addition to showing geminal coupling. Geminal protons in positions C-1 and C-2 were distinguished by NOE studies: H-1eq showed NOESY cross peaks with H-11eq and Me-19, while H-3eq had cross peaks with Me-18 and Me-17. Similarly, cross peaks between H-1ax/H-9 and H-3ax/CH_3-17 differentiated H-1ax from H-3ax.

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Compound **3** had a molecular weight of 300 Da and an ^{1}H-NMR spectrum (all protons of the A ring resonated in the crowded region between 1.0 and 2.0 ppm) that suggested a structure without substituents in ring A. A DQFCOSY NMR experiment showed a six-proton spin-system with a coupling pattern characteristic of three consecutive methylenes in a six-membered ring in the chair conformation. The H_2-1 protons were distinguished from the H_2-3 protons, similarly to compound **2**, by their NOESY cross peaks (H-1ax/H-9, H-1eq/H-11eq, H-1eq/Me-19, H-3eq/Me-17, H-3eq/Me-18, and H-3ax/Me-17). The HETCOR experiment established the ^{13}C-NMR chemical shifts of C-1, C-2, and C-3 (39.7, 19.1, and 42.0, respectively). Compound **3** was reported in a previous work[7] as helioscopinolide G, but later it was discerned as being identical to jolkinolide E, found in *E. jolkini*.[8] Because jolkinolides and helioscopinolides differ only in the position of the oxidations of the same carbon skeleton, compound **3** can be assigned either to the jolkinolide or the helioscopinolide group because it is not oxidized.

The ^{1}H- and ^{13}C-NMR spectral data of helioscopinolide H (**4**) clearly showed, as in the case of helioscopinolide D,[5] the presence of a hydroxyl group at C-9 (H-9 was missing and C-9 was observed at 77.2 ppm). The molecular weight (332 Da) suggested the presence of an additional hydroxyl in the A ring, that the ^{1}H-NMR and NOESY spectra assigned to the 3-equatorial position. The carbinol proton appeared as a double doublet at 3.30 ppm with coupling constants (J = 4.1, 11.4 Hz) typical of an axial proton and its occurrence at the C-3 position was established by NOESY cross peaks between Me-17/H-3 and H-1eq/H-11eq. The two hydroxyl protons appeared at 5.00 ppm (singlet HO-9) and at 4.30 ppm (doublet J = 5.0 Hz, HO-3) in the ^{1}H-NMR spectra in DMSO-d_6 (data not reported).

The molecular weight determined for helioscopinolide I (**5**) was 328 daltons. In addition to the signals of H-5, Me-17, and Me-18, the ^{1}H-NMR spectrum showed, for the A ring, a broad D_2O-exchangeable signal at 6.1 ppm and a singlet at 6.40 ppm. The ^{13}C-NMR spectrum, after assignment of the known portion of the molecule (carbon C-4 to C-20), displayed chemical shifts at 123.4, 145.2, and 200.2 ppm for the three remaining carbons. The last two were quaternary (no correlation in the HETCOR experiment), and one (200 ppm) was assigned a carbonyl. The first one was protonated and correlated with the signal at 6.40 ppm in the ^{1}H-NMR spectrum. Thus, the molecule had a ketoenolic group, consistent with the observed experimental data. The vinylic proton (6.40 ppm) was positioned at C-1, because of observed correlations with H-11eq, H-9, and Me-19 in the NOESY spectrum, so C-3 must be the location of the carbonyl group.

Inspection of the ^{1}H- and ^{13}C-NMR spectra indicated several characteristic features in the molecule of helioscopinolide L (**6**). One of the A-ring carbons, C-1 through C-3, was missing, while the other two were a quaternary carbon (177.5 ppm) and a methine carbon (98.8 ppm with its hydrogen as a singlet at 5.62 ppm in the ^{1}H-NMR spectrum). The former carbon was a lactone or ester carbonyl. Therefore, in combination with the observed molecular weight (332 daltons), a hemiacetal structure depicted by **6** was proposed. The hydrogen at 5.62 ppm was assigned to H-1eq from its NOESY cross peaks with H-11eq and Me-19. The hydroxyl proton, not observable in $CDCl_3$, appeared as a broad signal at 8.0 ppm in DMSO-d_6 (data not reported).

Experimental Section

General Experimental Procedures. Suspension cultures were added to an equal volume of EtOH, homogenized, and centrifuged. The clear supernatant was directly analyzed by HPLC. Analyses were performed using a Beckman Gold apparatus equipped with a photodiode-array detector. Two RT 250-4 Hibar RP-18 columns (Merck) assembled in series were utilized and eluted in isocratic conditions with the following solvent system: NaH_2PO_4 2 g/L−MeCN (45:55); flow rate 1 mL/min. All NMR spectra were recorded at 27 °C in $CDCl_3$ on a Varian VXR-400-S spectrometer at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, the standard Varian pulse sequences and processing software for 1D and 2D experiments were used. A mixing time of 1.0 s in the NOESY spectra was used. Sample concentration was 20 mg/mL. Field-desorption mass spectra were recorded on a Varian MAT 311-A with a combined FI/FD/EI ion source and benzonitrile-activated emitters. The total potential difference between the field emitter (anode) and the cathode was 9 kV. The emitter heating current was in the range of 0−25 mA, and the source temperature was 120 °C. HREIMS spectra have been obtained on a VG 70-70 EQ-HF instrument with manual peak-matching technique, using the appropriate PKF peaks as internal reference.

Cell Cultures. Three different *E. calyptrata* var. *involucrata* cell lines (EC6/201, EC9/115, and EC0/28) were cultured in Gamborg medium with different hormonal compositions as described elsewhere.[7] Each produced all the known helioscopinolides but in different amounts and with different internal rates. Line EC6/201 produced high percentages of helioscopinolides H (**4**) and I (**5**); line EC9/115 produced helioscopinolides F (**2**) and G (**3**); and line EC0/28 produced helioscopinolides B (**1**) and L (**6**). Three lots of 200 Erlenmayer flasks each, containing 50 mL of Gamborg medium without hormones,[9] were inoculated with one of the three above-reported cell lines and fermented for two weeks.

Extraction and Isolation. The 600 flasks were harvested and pooled. The pool was mixed with an equal volume of EtOH, homogenized, and centrifuged. The sediment was reextracted twice with EtOH and the pooled extracts concentrated under reduced pressure to 3 L volume. The resulting aqueous phase was extracted twice with EtOAc. The total amount of helioscopinolides present in the pooled extracts, determined by UV spectrum, corresponded to 3.88 g expressed as helioscopinolide E.[5] The EtOAc extract was concentrated under reduced pressure and loaded on a Si gel Si 60 (Merck) column (7 × 50 cm) in *n*-hexane. The column was eluted with *n*-hexane (2 L), *n*-hexane−CH_2Cl_2 1:1 (2 L), CH_2Cl_2 (1 L), CH_2Cl_2−0.5% EtOH (2 L), CH_2Cl_2−2% EtOH (2 L), CH_2Cl_2−10% EtOH (2 L), and finally EtOH (1 L). Separation and purification of each compound were achieved by repeated TLC on analytical Si gel F_{254}

plates. The following solvent systems were used: (A) Et_2O, (B) CH_2Cl_2−EtOH 95:5, (C) CH_2Cl_2−*i*-PrOH 92:8, (D) CH_2Cl_2−*n*-hexane 1:1, (E) Et_2O−EtOH 9:1, and (F) Et_2O−*n*-hexane 1:1. Final purification was always performed by preparative TLC on Si gel Teflon sheets without binders (Empore Si 60 F_{254}, Analytical International, Varian Associated, Inc., Harbor City, CA), in solvent system A. The *n*-hexane fraction of the column yielded compound **3** (30.2 mg) after purification by TLC [solvents D (R_f 0.65), and F (R_f 0.75)] and Empore sheets. The fraction eluted with *n*-hexane−CH_2Cl_2 1:1 yielded, after purification by TLC [solvents D (R_f 0.60) and F (R_f 0.55)] and Empore sheets, compound **2** (28.8 mg). The fraction eluted with CH_2Cl_2−0.5% EtOH gave compound **1** (9.3 mg), after purification by TLC [solvents A (R_f 0.75) and B (R_f 0.65)] and Empore sheets. Compounds **5** and **6** were present in the fraction eluted with CH_2Cl_2−2% EtOH. They were purified by TLC [solvents A (R_f 0.70 and 0.55, respectively) and B (R_f 0.60 and 0.40, respectively)] and Empore sheets; yields: 20.8 mg and 6.8 mg, respectively. Compound **4** (56.4 mg), eluted in the CH_2Cl_2−10% EtOH fraction, was purified by TLC [solvents A (R_f 0.60) and B (R_f 0.35)] and Empore sheets.

Helioscopinolide B (1): colorless oil; UV (MeOH) λ max (log ϵ) 278 (4.28) nm; FDMS (EHC = 0 mA) *m/z* 317 $[MH]^+$ (54), 316 $[M]^+$ (100), 299 $[M - OH]^+$ (23), 273 $[M - CO_2]^+$ (11); HREIMS *m/z* $[M]^+$ 316.2030 ($C_{20}H_{28}O_3$ requires 316.2038); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, s, H_3-18), 0.93 (3H, s, H_3-19), 0.98 (3H, s, H_3-17), 1.41 (1H, dddd, J = 12.9, 12.9, 12.9, 4.1 Hz, H-6ax), 1.50 (1H, ddd, J = 13.5, 13.5, 8.8 Hz, H-11ax), 1.6−1.7 (2H, m, H-1ax, H-1eq), 1.64 (1H, dd, J = 12.9, 2.6 Hz, H-5ax), 1.67 (1H, m, H-2eq), 1.72 (1H, m, H-6eq), 1.81 (3H, d, J = 1.5 Hz, H_3-20); 1.95 (1H, m, H-2ax), 2.22 (1H, ddd, J = 5.2, 13.5, 12.9, H-7ax), 2.29 (1H, d, J = 8.8 Hz, H-9ax), 2.48 (1H, m, H-3eq), 2.49 (1H, ddd, J = 13.5, 4.1, 2.2 Hz, H-7eq), 2.56 (1H, dd, J = 6.1, 13.5 Hz, H-11eq), 4.86 (1H, ddq, J = 13.5, 6.1, 1.5 Hz, H-12ax), 6.25 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.2 (C-20), 16.7 (C-19), 22.2 (C-18), 23.4 (C-6), 25.7 (C-2), 27.5 (C-11), 28.7 (C-17), 32.1 (C-1), 37.1 (C-7), 37.8 (C-4), 41.3 (C-10), 48.4 (C-5), 51.6 (C-9), 75.6 (C-3), 76.0 (C-12), 114.1 (C-14), 116.4 (C-15), 152.0 (C-8), 156.0 (C-13), 175.2 (C-16).

Helioscopinolide F (2): amorphous white powder; UV (MEOH) λ max (log ϵ) 276 (4.29) nm; FDMS (EHC = 0 mA) *m/z* $[MH]^+$ 315 (38), $[M]^+$ 314 (100), $[M - CO_2]^+$ 270 (14); HREIMS *m/z* $[M]^+$ 314.1889 ($C_{20}H_{26}O_3$ requires 314.1882); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, s, H_3-18), 0.93 (3H, s, H_3-19), 1.09 (3H, s, H_3-17), 1.47 (1H, dddd, J = 4.0, 12.5, 13.2, 13.2 Hz, H-6ax), 1.56 (1H, ddd, J = 8.8, 13.4, 13.7 Hz, H-11ax), 1.73 (1H, dd, J = 2.5, 12.5 Hz, H-5ax), 1.83 (3H, d, J = 1.7 Hz, H_3-20); 1.95 (1H, dddd, J = 2.5, 2.5, 5.2, 13.2 Hz, H-6eq), 2.21 (1H, dd, J = 2.2, 13.1 Hz, H-3eq), 2.27 (1H, ddd, J = 5.2, 13.2, 13.6 Hz, H-7ax), 2.29 (1H, d, J = 12.5 Hz, H-1ax), 2.36 (1H, d, J = 13.1 Hz, H-3ax), 2.41 (1H, dd, J = 6.3, 13.7 Hz H-11eq), 2.45 (1H, d, J = 8.8 Hz, H-9ax), 2.55 (1H, dd, J = 2.2, 12.5 Hz, H-1eq), 2.57 (1H, ddd, J = 2.5, 4.0, 13.6 Hz, H-7eq), 4.83 (1H, ddq, J = 1.7, 6.3, 13.4 Hz, H-12ax), 6.33 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.2 (C-20), 23.0 (C-18), 23.6 (C-6), 27.6 (C-11), 33.5 (C-17), 36.4 (C-7), 38.7 (C-4), 46.2 (C-10), 51.3 (C-9), 54.0* (C-3), 54.5 (C-5), 55.9* (C-1), 75.3 (C-12), 114.9 (C-14), 117.3 (C-15), 149.5 (C-8), 155.0 (C-13), 17.3 (C-19), 174.7 (C-16), 209.4 (C-2) (*interchangeable).

Jolkinolide E (3): colorless oil; UV (MeOH) λ max (log ϵ) 277 (4.25) nm; FDMS (EHC = 0 mA) *m/z* $[MH]^+$ 301 (38), $[M]^+$ 300 (100), $[M\text{-}CO_2]^+$ 256 (3); HREIMS *m/z* $[M]^+$ 300.2079 ($C_{20}H_{28}O_2$ requires 300.2083); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.83 (3H, s, H_3-18), 0.89 (3H, s, H_3-17), 0.90 (3H, s, H_3-19), 1.08 (1H, ddd, J = 4.5, 12.6, 12.6 Hz, H-1ax), 1.14 (1H, dd, J = 2.4, 12.8 Hz, H-5ax) 1.18 (1H, ddd, J = 4.6, 13.0, 13.0 Hz, H-3ax), 1.36 (1H, dddd, J = 4.1, 12.8, 13.3, 13.3 Hz, H-6ax), 1.4−1.6 (2H, m H-2ax, H-2eq), 1.43 (1H, m H-3eq), 1.46 (1H, ddd, J = 8.7, 13.5, 13.5 Hz, H-11ax), 1.80 (3H, d, J = 1.4 Hz, H_3-20); 1.81 (1H, dddd, J = 2.4, 2.4, 5.1, 13.3 Hz, H-6eq), 1.90 (1H, d, J = 12.6 Hz, H-1eq), 2.18 (1H, d, J = 8.7 Hz, H-9ax), 2.18 (1H, ddd, J = 5.1, 13.3, 13.4 Hz, H-7ax), 2.47 (1H, ddd, J = 2.4, 4.1, 13.4 Hz, H-7eq), 2.55 (1H, dd, J = 6.3, 13.5 Hz, H-11eq), 4.85 (1H, ddq, J = 1.4, 6.3, 13.5 Hz, H-12ax), 6.24 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.2 (C-20), 16.8 (C-19), 19.1 (C-2), 21.7 (C-18), 23.9 (C-6), 27.6 (C-11), 33.6 (C-4), 33.8 (C-17), 37.2 (C-7), 39.7 (C-1), 41.6 (C-10), 42.0 (C-3), 52.0 (C-9), 55.3 (C-5), 76.0 (C-12), 113.9 (C-14), 116.2 (C-15), 152.2 (C-8), 156.2 (C-13), 175.2 (C-16).

Helioscopinolide H (4): amorphous white powder; UV (MeOH) λ max (log ϵ) 271 (4.29) nm; FDMS (EHC = 8 mA) *m/z* $[MH]^+$ 333 (65), $[M]^+$ 332 (100), $[M - OH]^+$ 315 (6), $[M - H_2O]^+$ 314 (4), $[M - H_2O - OH]^+$ 297 (46); HREIMS *m/z* $[M]^+$ 332.1994 ($C_{20}H_{28}O_4$ requires 332.1988); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.84 (3H, s, H_3-18), 0.97 (3H, s, H_3-19), 1.05 (3H, s, H_3-17), 1.32 (1H, dd, J = 13.5, 13.0 Hz, H-11ax), 1.41 (1H, dddd, J = 12.9, 13.5, 13.5, 4.1 Hz, H-6ax), 1.62 (1H, m, H-1ax), 1.65 (1H, m, H-1eq) 1.77 (1H, m, H-2eq), 1.79 (1H, m, H-6eq), 1.83 (3H, d, J = 1.8 Hz, H_3-20); 1.88 (1H, m, H-1ax), 1.90 (1H, dd, J = 2.6, 12.9 Hz, H-5ax), 2.30 (1H, ddd, J = 13.5, 4.1, 2.6 Hz, H-7eq), 2.70 (1H, dddd, J = 13.5, 13.5, 5.3, 2.0 Hz, H-7ax), 3.08 (1H, dd, J = 6.1, 13.5 Hz, H-11eq), 3.30 (1H, dd, J = 4.1, 11.4 Hz, H-3ax) 4.87 (1H, ddq, J = 13.0, 6.1, 1.8 Hz, H-12ax), 6.35 (1H, d, J = 2.0 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.4 (C-20), 16.0 (C-18), 17.5 (C-19), 23.0 (C-6), 27.3 (C-2), 28.9 (C-17), 29.9 (C-1), 32.7 (C-7), 39.0 (C-4), 39.7 (C-11), 44.2 (C-10), 45.4 (C-5), 77.1 (C-12), 77.2 (C-9), 78.3 (C-3), 115.7 (C-14), 117.9 (C-15), 152.6 (C-8), 154.7 (C-13), 174.7 (C-16).

Helioscopinolide I (5): amorphous white powder, UV (MeOH) λ max (log ϵ) 275 (4.30) nm; FDMS (EHC = 0 mA) *m/z* $[M]^+$ 328 (100), $[M - CO_2]^+$ 284 (5); HREIMS *m/z* $[M]^+$ 328.1662 ($C_{20}H_{24}O_4$ requires 328.1674); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (3H, s, H_3-18), 1.13 (3H, s, H_3-19), 1.24 (3H, s, H_3-17), 1.59 (1H, dddd, J = 12.6, 13.2, 13.2, 3.8 Hz, H-6ax), 1.65 (1H, ddd, J = 13.8, 13.5, 8.8 Hz, H-11ax), 1.83 (3H, d, J = 1.6 Hz, H_3-20); 1.84 (1H, m, H-6eq), 1.98 (1H, dd, J = 2.6, 12.6 Hz, H-5ax), 2.30 (1H, ddd, J = 13.2, 13.5, 5.2 Hz, H-7ax), 2.46 (1H, d, J = 3.8 Hz, H-9ax), 2.60 (1H, ddd, J = 13.5, 3.8, 2.3 Hz, H-7eq), 2.67 (1H, dd, J = 6.4, 13.8 Hz, H-11eq), 4.86 (1H, ddq, J = 13.5, 6.4, 1.6 Hz, H-12ax), 6.1 (broad signal), 6.37 (1H, s, H-14), 6.40 (1H, s, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 2.0 (C-18), 8.3 (C-20), 19.4 (C-19), 23.2 (C-6), 23.4* (C-1), 27.0 (C-17), 27.6 (C-11), 36.7 (C-7), 41.7* (C-10), 44.0* (C-4), 48.3 (C-9), 52.6 (C-5), 75.4 (C-12), 116.0 (C-14), 117.7 (C-15), 145.2 (C-2),

148.9 (C-8), 154.9 (C-13), 174.9 (C-16), 200.2 (C-3) (*interchangeable).

Helioscopinolide L (6): amorphous white powder; UV (MeOH) λ max (log ϵ) 274 (4.30) nm; FDMS (EHC = 25 mA) *m/z* $[MH]^+$ 333 (64) $[M - CO_2]^+$ 288 (3), $[M]^+$ 332 (100), $[M - OH]^+$ 315 (5); HREIMS *m/z* $[M]^+$ 332.1630 ($C_{19}H_{24}O_5$ requires 332.1624); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (3H, s, H_3-19), 1.24 (3H, s, H_3-18), 1.36 (3H, s, H_3-17), 1.50 (1H, m, H-6ax), 1.53 (1H, ddd, J = 13.5, 13.5, 8.5 Hz, H11-ax), 1.81 (1H, m, H-6eq), 1.84 (3H, d, J = 1.5 Hz, H_3-20); 2.24 (1H, ddd, J = 13.2, 13.2, 5.0 Hz, H-7ax), 2.39 (1H, dd, J = 3.0, 12.9 Hz, H-5ax), 2.44 (1H, ddd, J = 13.2, 3.9, 2.4 Hz, H-7eq), 2.56 (1H, dd, J = 13.5, 6.2 Hz, H-11eq), 2.87 (1H, d, J = 8.5 Hz, H-9ax), 4.95 (1H, ddq, J = 13.5, 6.2, 1.5 Hz, H-12ax), 5.72 (1H, s, H-1), 6.36 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.2 (C-20), 14.4 (C-19), 24.1 (C-6), 24.1 (C-18), 27.2 (C-11), 29.6 (C-17), 36.0 (C-7), 40.4* (C-4), 42.8 (C-5), 42.9* (C-10), 43.4 (C-9), 76.0 (C-12), 98.8 (C-1), 115.1 (C-14), 117.1 (C-15), 150.6 (C-8), 156.1 (C-13), 175.8 (C-16), 177.5 (C-3) (*interchangeable).

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