SUPPORTING INFORMATION

Lagaspholones A and B: two new jatropholane-type diterpenes from

_Euphorbia lagascae_

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1. Experimental Section

1.1. General Experimental Procedures

Optical rotations were obtained using a Perkin Elmer 241 polarimeter. IR spectra were determined on a FTIR Nicolet Impact 400, and NMR spectra recorded on a Bruker ARX-400 NMR spectrometer (\textsuperscript{1}H 400 MHz; \textsuperscript{13}C 100.61 MHz), using CDCl\textsubscript{3} as solvent. MS were taken on a Kratos MS25RF spectrometer and HRSIMS on a Micromass Autospec spectrometer. Column chromatography was carried out on SiO\textsubscript{2} (Merck 9385). TLC were performed on precoated SiO\textsubscript{2} F\textsubscript{254} plates (Merck 5554 and 5744) and visualized under UV light and by spraying with sulphuric acid-acetic acid-water (1:20:4) or sulphuric acid-water (1:1) followed by heating. HPLC was carried out on a Merck-Hitachi instrument, with UV detection (254 nm), using a Merck LiChrospher 100 RP-18 (10 \textmu m, 250 \times 10 mm) column.

1.2. Plant material

\textit{Euphorbia lagascae} Spreng. was cultivated in Cova da Beira, Coimbra, Portugal, and identified by Dr. Teresa Vasconcelos (plant taxonomist) of Instituto Superior de Agronomia, University of Lisbon. A voucher specimen (nº 323) has been deposited at the herbarium of Instituto Superior de Agronomia.

1.3. Extraction and isolation

The air-dried powdered plant (5.9 Kg) was extracted with methanol (6 \times 12 L) at room temperature. Evaporation of the solvent (under \textit{vacuum}, 40 °C) from the crude extract yielded a residue of 284 g which was suspended in boiling MeOH and freezed, to give a precipitate (55 g), consisting mainly of waxes, that was eliminated by filtration. The filtrate
was evaporated, and the residue (233 g) resuspended on a MeOH/H₂O solution (1:2, 3 L), and extracted with Et₂O (6 x 2 L). The ether extract was dried (Na₂SO₄) and evaporated under vacuum, yielding a residue (68.3 g) that was chromatographed over SiO₂ (2 Kg), using mixtures of n-hexane/EtOAc (1:0 to 0:1) and EtOAc/MeOH (19:1 to 1:4) as eluents. According to differences in composition as indicated by TLC, eight crude fractions were obtained (Fr A - H). The more apolar crude fractions, containing mainly waxes and triterpenes, were not further investigated.

The crude Fr E (7.2 g) eluted with n-hexane/EtOAc (7:13 to 1:9) was subjected to CC on SiO₂ with mixtures of n-hexane/EtOAc. After TLC monitoring, chromatographic fractions were combined into five fractions (Fr EA - ED).

The residue of Fr E B (1.6 g, n-hexane/EtOAc, 7:3) was chromatographed by CC using gradients of n-hexane/EtOAc. Fractions eluted with n-hexane/EtOAc 7:3, were associated and submitted to preparative TLC (CHCl₃/MeOH, 49:1, 2 ×) yielding, after recrystallization (CH₂Cl₂/n-hexane), 17 mg of compound 7. Fractions eluted with n-hexane/EtOAc (13:7, 900 mg) were rechromatographed twice by CC, using mixtures of CH₂Cl₂/acetone and CH₂Cl₂/MeOH, to give four subfractions (EB₁A to EB₁D). Subfraction EB₁B (30 mg) was submitted to preparative TLC (CHCl₃) affording 13.6 mg of compound 5 and 7 mg of compound 6. Subfraction EB₁C (190 mg) showed the presence of two spots with a strong absorption at 254 nm, which were separated by preparative TLC (CHCl₃/MeOH, 19:1, 2 ×). The spot with < R_f was rechromatographed by preparative TLC (CH₂Cl₂/acetone, 9:1) and purified by HPLC yielding 10 mg of compound 3 (MeOH/H₂O, 13: 7; 4ml/min, t_R = 5 min), and 6 mg of compound 1 (MeOH/H₂O, 13: 7; 4ml/min, t_R = 15 min).
The residue of the crude fraction Ec (2.0 g), eluted with n-hexane/EtOAc (1:1), was submitted to three repeated column chromatography on SiO₂ with mixtures of n-hexane/EtOAc and n-hexane/CH₂Cl₂. The residue of the fraction eluted with CH₂Cl₂ (100 mg) crystallized from the elution solvent affording 50 mg of compound 4.

The crude fraction Ed (900 mg), eluted with n-hexane/EtOAc (2:3 to 0:1), was chromatographed on SiO₂ with mixtures of n-hexane/EtOAc of increasing polarity giving four fractions, after TLC monitoring.

The residue (60 mg) of the fraction eluted with n-hexane/EtOAc (1:1) showed the presence of a spot with a strong absorption at 254 nm, which was separated by preparative TLC (CHCl₃/MeOH, 17:3, 2 ×) and purified by HPLC to give 5 mg of compound 2 (MeOH/H₂O, 13:7; 4ml/min, tᵣ = 10 min).

2. Physical and spectroscopic data of compounds 1 – 7

**Lagaspholone A** [1]: White crystals; mp 217 – 219 °C; [α]D²⁰ − 46.7 (CHCl₃, c 0.10); HRSIMS: m/z 317.2111 [M + 1]⁺, (calcd for C₂₀H₂₉O₃: 317.2117); IR (KBr), ν max: 3580, 3442, 1685, 1648, 1126, 1024, 887 cm⁻¹; FABMS m/z (rel. int): 339 [M + Na]⁺ (8), 317 [M + 1]⁺ (1), 316 [M]⁺ (20), 299 [M + 1 – H₂O]⁺ (18); ¹H and ¹³C NMR see Table 1 in the main manuscript.

**Lagaspholone B** [2]: Colourless oil; [α]D²⁰ − 30.0 (CHCl₃, c 0.11); HRSIMS: m/z 333.2068 [M + 1]⁺, (calcd for C₂₀H₂₉O₄: 333.2066); IR (CH₂Cl₂), ν max: 3580, 3376, 1702, 1637, 1447, 1370 cm⁻¹; FABMS m/z (rel. int): 356 [M + Na + 1]⁺ (< 1); EIMS m/z (rel. int): 332
[M]$^+$ (1), 314 [M – H$_2$O]$^+$ (2), 281 (3), 271 (8), 253 (6), 187 (10), 175 (15), 139 (23), 69 (18), 55 (21), 43 (100); $^1$H and $^{13}$C NMR see Table 1 in the main manuscript.

(+)-Dehydrovomifoliol [3]: colourless oil; $\left[\alpha\right]_D^{20} + 198$ (CHCl$_3$, 0.16); IR (CH$_2$Cl$_2$), $\nu_{\text{max}}$: 3456, 2965, 1663, 1625, 1365, 1427, 1254, 1128, 989 cm$^{-1}$. FAB-MS m/z (rel. int.): 245 (35) [M + Na]$^+$, 223 (100) [M + 1]$^+$, 154 (87), 136 (54), 89 (26), 77 (36). EI-MS m/z (rel. int.): 222 (14) [M]$^+$, 197 (7), 166 (32), 149 (15), 124 (100), 95 (4), 55 (8), 43 (62), 41 (29), 39 (21). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.77 (1H, d, $J = 15.6$ Hz, H-7), 6.40 (1H, d, $J = 15.6$ Hz, H-8), 5.89 (1H, t, $J = 1.2$ Hz, H-4), 2.43 (1H, d, $J = 17.6$ Hz, H-2a), 2.27 (1H, d, $J = 17.6$ Hz, H-2b), 2.24 (3H, s, Me-10), 1.82 (3H, d, $J = 1.2$ Hz, Me-13), 1.04 (3H, s, Me-12), 0.96 (3H, s, Me-11). $^{13}$C NMR (100.61 MHz, CDCl$_3$): $\delta$ 197.1 (C-3), 197.5 (C-9), 160.5 (C-5), 145.1 (C-7), 130.4 (C-8), 127.8 (C-4), 79.3 (C-6), 49.5 (C-2), 41.4 (C-1), 28.4 (C-10), 24.3 (C-11), 22.9 (C-12), 18.7 (C-13).

This compound was identified as (+)-dehydrovomifoliol by comparison of the obtained spectroscopic data with literature values.$^{1,2}$

Scopoletin [4]: yellow crystals (CH$_2$Cl$_2$): mp 207-209 °C (lit. 205-207 °C, ethanol), IR (KBr), $\nu_{\text{max}}$: 3323, 1710, 1625, 1596, 1560, 1291, 850, cm$^{-1}$; EI-MS m/z (rel. int.): 192 (M)$^+$ (100), 177 (81), 164 (61), 149 (79), 135 (5), 121 (51), 107 (7), 105 (6), 92 (10), 81 (14), 79 (46), 69 (67), 65 (19), 53 (17), 50 (26), 39 (12), 29 (6). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.60 (1H, d, $J = 9.6$ Hz, H-4), 6.92 (1H, s, H-8), 6.85 (1H, s, H-5), 6.27 (1H, d, $J = 9.3$ Hz, H-3), 3.96 (3H, s, O-CH$_3$).

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This compound was identified as scopoletin by comparison of the obtained spectroscopic data with literature values.3

**Dehydrodiconiferyl diacetate [5]:** yellowish oil; \([\alpha]_D^{20} = 6.0\) (CHCl\(_3\), c 0.30), (lit. \([\alpha]_D^{25} = 2.0,\) CH\(_2\)Cl\(_2\), c 1.20); IR (CH\(_2\)Cl\(_2\)), \(\nu_{max}\) 3429, 2938, 1736 1604, 1233 1031, 964 cm\(^{-1}\). EIMS m/z 442 [M]*\(^+\) (18), 382 [M – CH\(_3\)COOH]*\(^+\) (8), 368 (6), 322 (6) [M – 2 x CH\(_3\)COOH]*\(^+\), 291 (12), 161 (14), 137 (20), 43 (100). FABMS m/z 465 (5) [M + Na]*\(^+\), 442 (17) [M]*\(^+\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.88 (5H, m, H-2, H-5, H-6, H-2’, H-6’), 6.60 (1H, d, \(J = 15.8\) Hz, H-7’), 6.15 (1H dt, \(J = 6.6, 15.8\) Hz, H-8’), 5.65 (1H, s, OH), 5.47 (1H, d, \(J = 7.32\) Hz, H-7), 4.71 (1H, d, \(J = 6.44,\) H-9’), 4.43 (1H, dd, \(J = 5.5, 11.2\) Hz, H-9a), 4.30 (1H, dd, \(J = 7.4, 11.0\) Hz, H-9b), 3.90 (3H, s, OCH\(_3\)), 3.86 (3H, s, OCH\(_3\)), 3.78 (1H, dd, \(J = 3.0, 12.6\) Hz, H-8), 2.10 (3H, s, COCH\(_3\)), 2.03 (3H, s, COCH\(_3\)); \(^{13}\)C NMR (CDCl\(_3\), 100.61 MHz) \(\delta\): 170.6 (2 \(\times\) COCH\(_3\)), 147.9 (C-4’), 146.6 (C-3), 145.9 (C-4), 144.2 (C-3’), 134.4 (C-7’), 132.4 (C-1), 130.3 (C-1), 127.0 (C-5’), 121.2 (C-8’), 119.6 (C-6), 115.3 (C-6’), 114.3 (C-5), 110.6 (C-2’), 108.6 (C-2), 88.8 (C-7), 65.3 (C-9, C-9’), 56.0 (OCH\(_3\)), 50.3 (C-8), 20.8 (2 \(\times\) COCH\(_3\)).

This compound was identified as dehydrodiconiferyl diacetate, by comparison of the obtained spectroscopic data with literature values.4

**3-Indolcarbaldehyde [6]:** colourless crystals (CH\(_2\)Cl\(_2\)): mp 207 – 210 °C; IR (KBr), \(\nu_{max}\) 3157, 1629, 1611, 1571, 1517, 1446, 1245, 787, 762 cm\(^{-1}\); EIMS m/z 145 [M]*\(^+\) (90), 144 [M – H]*\(^+\) (100), 116 [M – CHO]*\(^+\) (33), 89 (20), 63 (12); \(^1\)H RMN (400 MHz, CDCl\(_3\)) \(\delta\) 10.08 (1H, s, CHO), 8.77 (1H, br s, N-H), 8.33 (1H, m, H-4), 7.86 (1H, d, \(J = 2.8\) Hz, H-2),

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7.45 (1H, m, H-7), 7.33 (2H, m, H-5, H-6); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 185.1 (CHO), 135.2 (C-2), 124.4 (C-5), 123.1 (C-6), 122.0 (C-4), 111.5 (C-7).

This compound was identified as 3-indolcarbaldehyde by comparison of the obtained spectroscopic data with literature values.$^{5,6}$

**4-hydroxy-3,5-dimetoxibenzaldehyde [7]**: yellowish crystals (CH$_2$Cl$_2$/n-hexane): mp 112 – 114 °C; IR (KBr), $\nu$$_{\text{max}}$ 3285, 1671, 1608, 1331, 1252, 1207, 1140, 1105, 831, 728 cm$^{-1}$; EIMS m/z 183 (11), 182 [M]$^+$ (100), 181 (61), 111 (12), 96 (12), 93 (12), 43 (10); $^1$H NMR (CDCl$_3$, 400 MHz) δ 9.82 (1H, s, CHO), 7.15 (2H, s, H-2, H-6), 6.08 (1H, s, OH), 3.97 (6H, OCH$_3$).

This compound was identified as 4-hydroxy-3,5-dimetoxibenzaldehyde by comparison of the obtained spectral data with literature values.$^7$

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3. Chemical structures of the known compounds 3 -7.

4. Copies of spectra
$^1$H NMR spectrum of lagaspholone A (1)
$^{13}$C NMR spectrum of lagaspholone A (1)
DEPT spectrum of lagaspholone A (1)
$^1$H-$^1$H COSY spectrum of lagaspholone A (1)
HMQC spectrum of lagaspholone A (1)
HMBC spectrum of lagaspholone A (1)
NOESY spectrum of lagaspholone A (1)
$^{1}$H NMR spectrum of lagaspholone B (2)
$^{13}$C NMR spectrum of lagaspholone B (2)
DEPT spectrum (part 1) of lagaspholone B (2)
DEPT spectrum (part 2) of lagaspholone B (2)
$^1$H–$^1$H COSY spectrum of lagaspholone B (2)
HMQC spectrum of lagaspholone B (2)
HMBC spectrum of lagaspholone B (2)
NOESY spectrum of lagaspoholone B (2)