Supporting information

Bicyclo[4.4.0]decane oxygenated sesquiterpenes from *Eryngium maritimum* essential oil

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General experimental procedures

**GC analysis**

Analyses were carried out using a Perkin-Elmer Clarus 600 GC apparatus equipped with a dual flame ionisation detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol) (60 m x 0.22 mm i.d.; film thickness 0.25 µm). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min; helium was employed as a carrier gas (1 mL/min). The injector and detector temperatures were maintained at 280 °C, and samples were injected (0.2 µL of pure oil) in the split mode (1:50). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the Van den Dool and Kratz equation with the aid of software from Perkin-Elmer (TotalChrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without application of correction factors.

**GC-MS (EI) conditions**

Samples were analysed with a Perkin-Elmer Turbo mass detector (quadrupole) coupled to a Perkin-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C (35 min); helium was employed as a carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 L of pure oil; injector temperature, 280 °C; split, 1:80; ion source temperature, 150 °C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s.

**GC-MS (CI) conditions**
PCI and NCI mass spectra were recorded on the same apparatus equipped with an Rtx-Wax column and specific ionisation chemical source. The oven temperature was programmed as above and the following conditions were employed: injection volume 0.2 µL of pure oil; ionising gas methane or ammonia; ion source temperature, 150 °C; source pressure, 0.2 mbar; ionisation energy, 70 eV; MS (CI) acquired over the mass range, 60–350 Da; scan rate, 1 s.

**NMR analysis conditions**

The structure elucidation of 1, 2, 3 and 4 was carried out by $^1$H- and $^{13}$C-NMR, DEPT and 2D-NMR (HMBC, HSQC, COSY and NOESY). Spectra were measured in deuterated chloroform using a Bruker Avance 400 Fourier Transform spectrometer operating at 100.13 MHz for $^{13}$C-NMR and 400.52 MHz for $^1$H-NMR and equipped with a 5-mm probe. All shifts were referred to the internal standard tetramethylsilane (TMS). $^{13}$C-NMR spectra were recorded with the following parameters: pulse width, 4 s (flip angle, 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/pt. The number of accumulated scans was 3000–5000 for each sample depending on the amount of product. The $^1$H-NMR spectra were recorded with the following parameters: flip angle, 30°; acquisition time, 2.56 s for 32 000 data table with a spectral width of 7000 Hz (17.5 ppm). 2D-NMR sequences were recorded using Bruker micro-programs.

**Isolation of single components**

10 g of essential oil were fractioned by CC on silica gel (ICN 200-500 µm, 150 g, diameter 2.5 cm, length 40 cm, flow rate 7.5 mL/min, fractions of 25 mL) and successively gave three fractions (A-C, 500 mL each) with a gradient n-pentane/diethyl ether as the mobile phase.
aldehyde-rich fraction B (1: 30.9 % and 2: 59.1 %) were submitted to LiAlH₄ reduction and gave 3450 mg of a mixture of 3 (30.2 %) and 4 (64.3 %). 3 (96.8 % in B1: 60 mg) and 4 (99.1% in B2: 269 mg) were separated by four successive CCs on silica gel (ICN 63-200, 60 g diameter 2 cm, length 60 cm, flow 2.5 mL/min, fractions of 10 mL) using a gradient of n-pentane/diethyl oxide 94/6 (v/v) (2 L) as the mobile phase. 42 mg of B1 and 247 mg of B2 were submitted to pyridinium chlorochromate oxidation in order to obtain after CC purification (ICN 63-200, 10 g, diameter 2 cm, length 5 cm, flow 2.5 mL/min, fractions of 10 mL) using a gradient of n-pentane/diethyl oxide (v/v): 95/5 (100 mL), 0/100 (100 mL) as the mobile phase: 1 (91.5 % in B’1: 18 mg) and 2 (95.5 % in B’2: 123 mg). Each step of the isolation process has been controlled by GC(FID), CPG/SM(IE) and ¹³C-NMR.

Reduction of fraction B and oxidation of B1 and B2 fractions

The fraction B (3620 mg) obtained by CC was dissolved in dry diethyl ether (40 mL) and carefully added to a suspension of lithium aluminium hydride (900 mg) in dry diethyl ether (60 mL) at 0 °C. The mixture was stirred at room temperature and then refluxed for 3 h. The reaction mixture was hydrolyzed by the addition of 15 % sodium hydroxide solution (2 mL) and cold water. The organic layer was separated, washed with water to neutrality, dried over sodium sulphate and concentrated under vacuum. The reduction displayed a mixture (3450 mg) of 3 (30.2 %) and 4 (64.3 %) as major components.

42 mg of fraction B1 and 247 mg of fraction B2 dissolved in 2 mL of CHCl₃ was separately added to a suspension of pyridinium chlorochromate (15 mg and 85 mg, respectively). The mixture was first stirred at 0 °C for 3 h and then at room temperature for 3 h. The reaction mixture was filtered and the solvent was removed under reduced pressure.
Compounds data

4βH-muurol-9-en-15-al (1): 1β-isopropyl-4-methyl-1,2,4a,5,6,7,8,8a-cis-octahydronaphthalene-7α-carbaldehyde

Amorphous white oil ; R.I. _Rtx-1_: 1684 ; R.I. _Rtx-wax_: 2163 ; $^1$H-NMR (400.1 MHz, CDCl$_3$) and $^{13}$C-NMR (100.1 MHz, CDCl$_3$) data, see Table 1; MS (EI, 70 eV), $m/z$ (%) = 202 (40), 187 (16), 177 (20), 159 (55), 149 (51), 147 (43), 135 (20), 133 (24), 131 (36), 123 (37), 122 (51), 121 (36), 119 (34), 117 (33), 107 (90), 105 (80), 95 (40), 94 (39), 93 (52), 91 (51), 90 (36), 81 (86), 79 (59), 69 (58), 67 (74), 65 (40), 57 (40), 55 (58), 53 (35), 43 (60), 41 (100); MS (PCI-CH$_4$, 70 eV), $m/z$ (%,[type of ion]) = 221 (40, [M+H]$^+$), 203 (100, [M+H-H$_2$O]$^+$); MS (NCI-NH$_3$, 70 eV), $m/z$ (%,[type of ion]) = 219 (100, [M-H]$^-$).

4βH-cadin-9-en-15-al (2): 1β-isopropyl-4-methyl-1,2,4a,5,6,7,8,8a-trans-octahydronaphthalene-7α-carbaldehyde

Amorphous white oil ; R.I. _Rtx-1_: 1684 ; R.I. _Rtx-wax_: 2173 ; $^1$H-NMR (400.1 MHz, CDCl$_3$) and $^{13}$C-NMR (100.1 MHz, CDCl$_3$) data, see Table 2; MS (EI, 70 eV), $m/z$ (%) = 220 (40), 177 (16), 176 (20), 160 (95), 158 (61), 149 (35), 143 (26), 135 (24), 133 (19), 131 (43), 121 (40), 119 (33), 117 (35), 109 (25), 107 (90), 105 (95), 95 (55), 93 (65), 91 (51), 82 (25), 81 (40), 78 (53), 77 (67), 69 (58), 67 (74), 65 (40), 57 (60), 55 (78), 53 (45), 43 (80), 41 (100); MS (PCI-CH$_4$, 70 eV), $m/z$ (%,[type of ion]) = 221 (45, [M+H]$^+$), 203 (100, [M+H-H$_2$O]$^+$); MS (NCI-NH$_3$, 70 eV), $m/z$ (%,[type of ion]) = 219 (100, [M-H]$^-$).

4βH-muurol-9-en-15-ol (3): 1β-isopropyl-4-methyl-1,2,4a,5,6,7,8,8a-cis-octahydronaphthalene-7α-methanol

Amorphous yellow oil ; R.I. _Rtx-1_: 1734 ; R.I. _Rtx-wax_: 2422 ; $^1$H-NMR (400.1 MHz, CDCl$_3$) and $^{13}$C-NMR (100.1-MHz, CDCl$_3$) data, see Table 1; MS (EI, 70 eV), $m/z$ (%) = 222 (20), 179
4βH-cadin-9-en-15-ol (4): 1β-isopropyl-4-methyl-1,2,4a,5,6,7,8,8a-trans-octahydronaphthalene-7α-methanol

Amorphous yellow oil; R.I. \textsubscript{Rtx-1}: 1742; R.I. \textsubscript{Rtx-wax}: 2452; \textsuperscript{1}H-NMR (400.1 MHz, CDCl\textsubscript{3}) and \textsuperscript{13}C-NMR (100.1 MHz, CDCl\textsubscript{3}) data, see Table 2; MS (EI, 70 eV), \textit{m/z} (%) = 222 (20), 179 (100), 161 (85), 149 (15), 135 (15), 133 (22), 121 (21), 119 (45), 109 (20), 107 (29), 105 (90), 95 (40), 93 (85), 91 (75), 82 (28), 81 (60), 79 (53), 77 (41), 69 (31), 67 (41), 57 (20), 55 (52), 53 (20), 43 (39), 41 (70); MS (PCI-CH\textsubscript{4}, 70 eV), \textit{m/z} (%) = 221 (45, [M-H]\textsuperscript{+}), 205 (100, [M+H-H\textsubscript{2}O]\textsuperscript{+}); MS (NCI-NH\textsubscript{3}, 70 eV), \textit{m/z} (%) = 221 (100, [M-H]).

Antibacterial activity

The oil fraction obtained by CC, rich in 1, 2, 3 and 4, was tested against seven bacteria: three gram-positive bacteria: \textit{Bacillus cereus}, \textit{Staphylococcus aureus} and \textit{Listeria monocytogenes} and four gram-negative bacteria: \textit{Echerichia coli}, \textit{Citrobacter freundii}, \textit{Enterococcus faecalis} and \textit{Klebsiella pneumoniae}. All bacteria were isolated from the medical devices (catheters and vesicle probes) in the surgery service at the CHU of Tlemcen and their identification was achieved with API 20 test strips (Biomerieux). All the strains were grown on Mueller-Hinton agar (MHA). Antimicrobial activities were carried out using the paper disc diffusion [15]. The agar plate containing the appropriate medium was spread with the inoculums containing \textit{10}\textsuperscript{8} CFU/mL. The filter paper dishes (6 mm in diameter) were impregnated with 3 μL of the oil
and then placed onto agar plates. In addition, negative reference discs without any oil and positive reference discs with gentamicin 15 μg were used for comparison. After incubation at 37 °C ± 1 °C for 18 to 24 h, the diameters of the inhibition zones and the sensitivity were measured with a caliper (less than 6 mm for negative controls, 18 mm for positive controls). Minimal inhibitory concentrations (MICs) were determined using the dilution agar method [16]. Serial dilutions of the oil were carried out in Mueller Hinton agar medium. Appropriate volumes of every dilution were added to this medium to obtain the required concentration range and a final concentration of Tween 80 at 10% (v/v). Negative controls without any oil and positive controls with gentamicin were used for comparison. Each dish contained a sterile solution of Tween 80 and the culture medium. After incubation at 37 ± 1 °C for 24 h, the MICs were defined as the lowest concentration of the oil at which the microorganism did not demonstrate visible growth (no MIC observed for negative controls, MIC are 4 μg.mL⁻¹ for positive controls).