Inhibition of squalene synthase of rat liver by abietane diterpenes derivatives


ABSTRACT
In the current study, chemical composition of cultivated Salvia canariensis L was determined. In this work, carnosol was the main product isolated, and the concentration of rosmannol is also significant. We prepared more lipophilic derivatives from carnosol, and both isolated and semisynthetic abietane diterpenes were evaluated in vitro as inhibitors of squalene synthase isolated from rat liver. Among the compounds tested, carnosol was the most potent inhibitor with an IC$_{50}$ value of 17.6 μM. These results highlight the great potential of this species for the commercial production of extracts of interest for their possible uses as ingredients in nutritional supplements for the treatment of hyperlipidemia.
1. EXPERIMENTAL

1.1 General experimental procedures

The NMR spectra were recorded on BrukerAvance 300 MHz and Bruker Avance 400 MHz spectrometers in CDCl₃, unless otherwise noted. Chemical shifts are given in ppm with TMS as the internal standard. IR spectra were obtained on a Bruker IFS 28/55 (FTIR) spectrometer and UV spectra on a JASCO V-560. Low resolution mass spectra were run on a VG Micromass ZAB-2F and high-resolution mass spectra on a VG Micromass ZAB-2F at 70 eV. Merck silica gel 60 Å 0.063-0.200 mm (70-230 mesh) was used for column chromatography. Analytical thin-layer chromatography (TLC) and preparative TLC were carried out on precoated Schleicher and Schüll plates.

1.2 Plant material

The aerial parts (leaves, flowers, stems) of S. canariensis L. (1.5 Kg) were harvested in the experimental fields of Monteflor S. A. (Gran Canaria, Spain) in July 2015. A voucher specimen (N° 25252) was deposited in the herbarium of the Department of Botany, Faculty of Biology, Universidad de La Laguna (Spain).

1.3 Extraction and isolation

The dried plant material was extracted with acetone (3.0 L) at room temperature for 3 days. The extract was filtered, and the solvent was evaporated. The extraction was repeated two more times. The combination of these three fractions give us an acetonic extract (52.4g, 7.0% of the plant dry weight), which was fractionated into n-hexane (7.96g), CH₂Cl₂ (25.3g), AcOEt (0.86g) and n-BuOH soluble materials (2.0g). The CH₂Cl₂ extract, the main diterpenic fraction, was subjected to flash chromatography. The column was packed with Merck silica gel 60 Å 0.063-0.200 mm (70-230 mesh). The extract was adsorbed onto silica gel, allowed to dry and subsequently applied on top of adsorbent layer. Then the column was eluted using n-hexane/ethyl acetate gradient (100:0 to 0:100) to provide 39 fractions (500mL each) which were analysed by TLC on silica gel and similar fractions were collected. Combined fractions 11-23 were further purified by Sephadex LH-20 column eluting with the isocratic mixture n-hexane/CHCl₃/MeOH (2:1:1) to give twenty fractions (50mL each). Carnosol 2 (220 mg) was obtained from the
combined 4-6 fractions by recrystallization with CH₂Cl₂ and acetone (1:1). Fractions 7-8 were re-chromatographed on a silica gel column using n-hexane/AcOEt (9:1) to furnish rosmanol 3 (64.9 mg), galdosol 6 (24.5 mg), rosmedial 7 (6.8 mg), epirosmanol 8 (1.5 mg), isorosmanol 9 (9.4 mg), isogaldosol 10 (1.7 mg) and demetilsalvicanol 11 (1.8 mg). Fraction 9 was submitted to preparative TLC (CH₂Cl₂:Acetone, 9:1) yielding 7α-methoxyrosmanol 4 (22.0 mg), 7α-ethoxyrosmanol 5 (12.7 mg) and 7β-methoxyrosmanol 12 (1.6 mg). All compounds were characterized by comparing their spectroscopic data with those reported in the literature.

**Figure S1.** Abietane diterpenes detected in *S. canariensis* and their concentration expressed as miligrams of compound per 100 grams of dried weight sample (mg/100 g DW).
Spectroscopic data

Carnosol (2). Crystalline solid (DCM/Acetone, 8:2), mp: 232-235°C; \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) 0.86 (3H, s, Me-19), 0.90 (3H, s, Me-18), 1.22 (6H, d, \(J = 7.0 \) Hz, Me-16 and Me-17), 1.31 (1H, dd, \(J_1 = 3.5 \) Hz, \(J_2 = 14.0 \) Hz, H-3\(\alpha\)), 1.73 (1H, q, \(J = 5.5 \) Hz, H-5), 1.90 (1H, td, \(J_1 = 3.0 \) Hz, \(J_2 = 12.0 \) Hz, H-6\(\beta\)), 1.99 (1H, dt, \(J_1 = 3.9 \) Hz, \(J_2 = 13.7 \) Hz, H-2\(\alpha\)), 2.20 (1H, m, H-6\(\alpha\)), 2.39 (1H, td, \(J_1 = 4.3 \) Hz, \(J_2 = 14.0 \) Hz, H-1\(\alpha\)), 2.90 (1H, br d, \(J = 7.0 \) Hz, H-1\(\beta\)), 3.08 (1H, hept, \(J = 7.0 \) Hz, H-15), 5.26 (1H, br s, Ar-\(\text{OH}\)), 5.37 (1H, dd, \(J_1 = 1.4 \) Hz, \(J_2 = 4.0 \) Hz, H-7), 5.73 (1H, br s, Ar-\(\text{OH}\)), 6.64 (1H, s, H-14); \(^{13}\)C-NMR (125 MHz, CD\(_3\)OD) \(\delta\) 19.8 (c-2), 20.1 (c-1 7), 23.2 (C-16), 23.3 (C-19), 27.8 (C-15) 30.2 (C-I), 30.6 (C-6), 32.3 (C-18), 35.3 (C-4), 42.1 (C-3), 46.5 (C-5), 49.5 (C-10), 78.4 (C-7), 112.6 (C-14), 123.2 (C-9) 133.5 (C-8), 135.2 (C-13) 143.7 (C-12), 143.80 (C-1 I), 176.15 (C-20); EIMS m/z 330 [M]+ (22), 286 (100), 271 (9), 243 (7), 215 (30)

Rosmanol (3). Isolated as an amorphous solid, mp: 237-239°C; \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \(\delta\) 0.93 (3H, s, Me-19), 1.03 (3H, s, Me-18), 1.22, 1.23 (each 3H, d, \(J = 7.0 \) Hz, Me-16 and Me-17), 2.00 (1H, td, \(J_1 = 5.3 \) Hz, \(J_2 = 13.1 \) Hz H-1\(\alpha\)), 2.21(1H, s, H-5), 3.09 (1H, hept, \(J = 7.0 \) Hz, H-15), 3.16 (1H, br d, \(J = 10.6 \) H-1\(\beta\)), 4.57 (1H, d, \(J = 3.3 \) Hz, H-6), 4.74 (1H, d, \(J = 3.3 \) Hz, H-7), 6.87 (1H, s, H-14); \(^{13}\)C-NMR (125 MHz, CD\(_3\)OD) \(\delta\) 20.1 (C-2), 22.4 (C-17), 22.5 (C-19) 22.9 (C-16), 27.9 (C-15), 28.6 (C-1), 31.9 (C-18) 32.3 (C-4), 39.3 (C-3), 48.5 (C-10), 51.6 (C-5), 69.3 (C-7), 79.1 (C-6), 120.5 (C-14), 124.9 (C-9), 129.2 (C-8), 137.5 (C-13), 143.2 (C-12), 145.0 (C-11), 180.0 (C-20); EIMS m/z 346 [M]+ (48), 330 (26), 314 (11), 300 (34), 287 (44), 285 (42), 284 (100), 271 (44), 269 (80), 257 (26), 215 (99), 121 (44), 71 (29).

7α-methoxyrosmanol (4). White amorphous powder, mp: 182-183 °C. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) 0.94 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.23 (6H, d, \(J = 7.0 \) Hz, Me-16 and Me-17), 2.00 (1H, td, \(J_1 = 5.6 \) Hz, \(J_2= 13.8 \) Hz, H-1\(\alpha\)), 2.25 (1H, s, H-5), 3.07 (1H, hept, \(J = 7.0 \) Hz, H-15), 3.17 (1H, bd, \(J = 14.6 \) Hz, H-1), 3.66 (3H, s, -\(\text{OCH}_3\)), 4.27 (1H, d, \(J = 3.2 \) Hz, H-6), 4.71 (1H, d, \(J = 3.2 \) Hz, H-7), 5.48 (1H, bs, Ar-\(\text{OH}\)), 6.60 (1H, bs, Ar-\(\text{OH}\)), 6.80 (1H, s, H-14); \(^{13}\)C-NMR (125 MHz, CDCl\(_3\)) \(\delta\) 19.5 (C-2), 22.1 (C-19), 22.4 (C-16), 22.6 (C-17), 27.6 (C-15), 27.7 (C-1), 31.5 (C-4), 31.7 (C-18), 38.2 (C-3), 47.5 (C-10), 50.9 (C-5), 58.3 (-\(\text{OCH}_3\)), 74.9 (C-6), 77.8 (C-7), 120.8 (C-14), 124.5 (C-9), 126.8
(C-8), 135.1 (C-13), 142.3 (C-12), 142.3 (C-11), 179.2 (C-20); EIMS m/z 360 [M]+ (100), 314 (81), 29 (80), 284 (88), 269 (84), 245 (93), 228 (38), 215 (93).

7α-ethoxyrosmanol (5). Isolated as a gum that did not crystallize; 1H-NMR (300 MHz, CDCl3) δ 0.92 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.22 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.33 (3H, t, J = 7.0 Hz, -OCH2-CH3), 1.99 (2H, td, J1 = 5.3 Hz, J2= 13.6 Hz H-1α), 2.28 (1H, s, H-5), 3.06 (1H, hept, J = 7.0 Hz, H-15), 3.17 (1H, br d, J = 14.0 Hz, H-1β), 3.86 (2H, q, J = 3.4 Hz, -OCH2-CH3), 4.36 (1H, d, J = 3.20 Hz, H-6), 4.66 (1H, d, J = 3.20 Hz, H-7), 5.59 (1H, br s, Ar-OH), 6.15 (1H, br s, Ar-OH), 6.79 (1H, s, H-14); 13C-NMR (CDCl3) δ 16.0 (-OCH2-CH3), 19.0 (C-2), 22.0 (C-19), 22.2 (C-16), 22.3 (C-17), 27.2 (C-15), 27.3 (C-1), 31.1 (C18), 31.4 (C-4), 38.0 (C-3), 47.0 (C-10), 50.9 (C-5), 66.3 (-OCH2-CH3), 75.3 (C-6), 75.8 (C-7), 120.6 (C-14), 124.4 (C-9), 126.7 (C-8), 134.8 (C-13), 141.7 (C-11), 142.4 (C-11), 179.1 (C-20); EIMS m/z 374 [M]+ (100), 328 (87), 300 (29), 247 (17), 231 (24), 215 (68), 115 (24), 69 (40), 55 (51).

Galdosol (6). Isolated as an amorphous solid, mp: 148-150°C. 1H-NMR (300 MHz, CDCl3) δ 0.98 (3H, s, Me-19), 1.12 (3H, s, Me-18), 1.18 and 1.29 (each 3H, d, J = 7.0 Hz, Me-16 and Me-17), 1.50 (1H, m, H-3β), 1.64 (1H, m, H-2β), 1.73 (1H, m, H-2α), 2.07 (1H, m, H-1α), 2.46 (1H, s, H-5), 3.20 (1H, hept, J = 7.0 Hz, H-15), 3.26 (1H, m, H-1β), 4.72 (1H, s, H-6), 7.69 (1H, s, H-14); 13C-NMR (CDCl3) δ 19.1 (C-2), 22.2 (C-17), 22.3 (C-19), 22.5 (C-16), 27.5 (C-15), 27.6 (C-1), 31.6 (C-18), 32.5 (C-4), 38.0 (C-3), 49.8 (C-10), 60.6 (C-5), 81.2 (C-6), 120.8 (C-14), 122.4 (C-8), 128.8 (C-9), 134.8 (C-13), 141.4 (C-12), 149.1 (C-11), 177.7 (C-20), 189.9 (C-7); EIMS m/z 345 [M+1]+ (10), 327 (10), 299 (50), 281 (30), 275 (25), 257 (55), 231 (100), 157 (25), 85 (30).

Rosmadial (7). Isolated as a gum that did not crystallize; 1H-NMR (300 MHz, CDCl3) δ 1.27 (6H, d, J = 7.0Hz, Me-16 and Me-17), 1.28 (3H, s, Me-19), 1.50 (3H, s, Me-18), 1.55-2.27 (6H, overlapping signal, H-1α, H-1β, H-2α, H-3α, H-3β), 3.35 (1H, hept, J = 7.0Hz, H-15), 4.11 (1H, s, H-5), 6.84 (1H, br. s, Ar-OH), 7.41 (1H, s, H-14), 9.67 (1H, s, H-6), 9.78 (1H, s, H-7); 13C-NMR (CDCl3) δ 16.6 (C-2), 22.3 (C-17), 22.4 (C-16), 24.0 (C-19), 27.0 (C-15), 31.6 (C-1), 33.3 (C-18), 34.3 (C-4), 40.2 (C-3), 48.4 (C-10), 61.3 (C-5), 124.0 (C-8), 131.4 (C-14), 132.1 (C-9), 135.9 (C-13), 141.6 (C-11), 142.8 (C-12),
177.2 (C-20), 192.1 (C-7), 202.1 (C-6); EIMS m/z 344 [M]+ (38), 316 (15), 287 (100), 273 (20), 231 (16).

Epirosmanol (8). Isolated as a gum that did not crystallize. 1H-NMR (300 MHz, CDCl3) δ 0.95 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.23 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.97 (1H, s, H-5), 3.08 (1H, hept, J = 7.0 Hz, H-15), 3.19 (1H, dd, J = 14.0 Hz, H-1β), 4.77 (2H, overlapping signal, H-6 and H-7), 5.50 (1H, Ar-OH), 6.18 (1H, Ar-OH), 7.04 (1H, s, H-14); EIMS m/z 346 [M]+ (100), 302 (43), 287 (51), 284 (57), 273 (43), 269 (47), 231 (94), 215 (75).

Isorosmanol (9). Isolated as a gum that did not crystallize. 1H-NMR (300 MHz, CDCl3) δ 0.93 (3H, s, Me-19), 1.06 (3H, s, Me-18), 1.25 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.34 (1H, d, J = 3.6 Hz, H-5), 2.38 (1H, dd, J1 = 4.5 Hz, J2 = 13.0 Hz, H-1α), 2.87 (1H, dd, J = 14.6 Hz, H-1β), 3.07 (1H, hept, J = 7.0 Hz, H-15), 4.35 (1H, t, J = 4.5 Hz, H-6), 5.20 (1H, d, J = 4.5 Hz, H-7), 5.29 (1H, bs, Ar-OH), 5.78 (1H, bs, Ar-OH), 6.79 (1H, s, H-14); 13C-NMR (CDCl3) δ 19.6 (C-2), 21.4 (C-19), 22.9 (C-16), 23.2 (C-17), 27.5 (C-15), 29.8 (C-1), 32.5 (C-18), 34.8 (C-4), 41.9 (C-3), 48.7 (C-10), 56.3 (C-5), 69.0 (C-6), 80.8 (C-7), 116.0 (C-14), 122.9 (C-9), 129.9 (C-8), 134.9 (C-13), 143.6 (C-12), 143.8 (C-11), 175.2 (C-20); EIMS m/z 346 [M]+ (100), 302 (77), 300 (71), 273 (40), 257 (29), 245 (35), 233 (50), 232 (57), 231 (64), 219 (45), 205 (44).

Isogaldosol (10). Isolated as a gum that did not crystallize. 1H-NMR (300 MHz, CDCl3) δ 0.93 (3H, s, Me-18), 1.21 (6H, d, J = 7.0 Hz, Me-16 and Me-17), 1.22 (3H, s, Me-19), 2.15 (1H, s, H-5), 2.40 (1H, dd, J1 = 4.0 Hz, J2 = 12.0 Hz, H-1α), 2.89 (1H, dd, J = 12.8 Hz, H-1β), 3.05 (1H, hept, J = 7.0 Hz, H-15), 5.16 (1H, s, H-7), 5.49 (1H, bs, Ar-OH), 5.86 (1H, bs, Ar-OH), 6.80 (1H, s, H-14); EIMS m/z 344 [M]+ (84), 316 (33), 300 (80), 285 (32), 273 (30), 257 (25), 233 (77), 231 (34), 205 (28), 152 (63), 120 (76), 95 (25), 83 (100), 71 (53) 57 (92).

Demetilsalvicanol (11). Isolated as a gum that did not crystallize. 1H-NMR (300 MHz, CDCl3) δ 0.85 (3H, s, Me-19), 0.92 (3H, s, Me-18), 1.21, 1.24 (3H each, d, J = 7.0 Hz, Me-16 y Me-17), 2.57, 3.03 (1H each, d, J = 14.4 Hz, H-20), 2.68 (2H, m, H-7), 3.16 (1H, hept, J = 7.0 Hz, H-15), 5.66 (1H, bs, Ar-OH), 6.43 (1H, bs, Ar-OH), 6.57 (1H, s,
H-14); EIMS m/z 318 [M]+ (9), 300 (54), 285 (7), 257 (6), 231 (17), 218 (8), 204 (17), 192 (100), 179 (30), 177 (21), 165 (8), 163 (11).

7β-methoxyrosmanol (12). Isolated as a gum that did not crystallize. 1H-NMR (300 MHz, CDCl3) δ 0.96 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.10, 1.17 (3H each, d, J = 7.0 Hz, Me-16 and Me-17), 1.96 (1H, s, H-5), 3.01 (1H, hept, J = 6.8 Hz, H-15), 3.21 (1H, bd, J = 14.2 Hz, H-1β), 3.58 (3H, s, -OCH3), 4.42 (1H, d, J = 2.0 Hz, H-6), 4.93 (1H, d, J = 2.0 Hz, H-7), 5.69 (1H, bs, Ar-OH), 5.95 (1H, bs, Ar-OH), 6.83 (1H, s, H-14); 13C-NMR (CDCl3) δ 18.9 (C-2), 22.0 (C-19), 22.1 (C-18), 22.7 (C-17), 27.2 (C-15), 27.3 (C-1), 31.7 (C-4), 32.3 (C-18), 38.0 (C-3), 47.9 (C-10), 55.4 (C-5), 56.0 (-OCH3), 74.7 (C-6), 78.2 (C-7), 118.9 (C-14), 122.9 (C-9), 126.2 (C-8), 135.2 (C-13), 142.1 (C-12), 142.3 (C-11), 178.5 (C-20); EIMS m/z 361 [M + 1]+ (7), 347 (21), 300 (21), 271 (60), 255 (74), 246 (66), 231 (50), 201 (46), 177 (40), 137 (59), 128 (44), 105 (37), 69 (100).

1.4 Synthesis

Oxidation of Carnosol (2) or Rosmanol (3) with Ag2O. Freshly prepared Ag2O (2 eq) was added to a solution of carnosol (2) or rosmanol (3) in diethyl ether (5 mL). After being stirred for 2 h, the solution was filtered, the solvent was evaporated and the product was chromatographed over silica gel using CH2Cl2 as eluent to yield carnosol quinone (13) (93.5 %) or rosmquinone (14) (99%) respectively.

Carnosol quinone (13). 1H-NMR (300 MHz, CDCl3) δ 0.87 (3H, s, Me-19), 0.89 (3H, s, Me-18), 1.12 (6H, d, J = 7.0 Hz, Me-16 y Me-17), 1.23 (1H, m, H-3α), 1.53 (1H, bd, J = 13.3 Hz, H-3β), 1.65 (2H, overlap, H-2 and H-5), 1.87 (1H, dt, H-2α), 1.97 (1H, m, H-6β), 2.20 (1H, m, H-6α), 2.30 (1H, td, J1 = 4.6 Hz, J2 = 14.0 Hz, H-1α), 2.68 (1H, bd, J = 14.4 Hz, H-1β), 2.97 (1H, hept, J = 7.0 Hz, H-15), 5.19, 5.20 (1H, dd, J1 = 1.4 Hz, J2 = 4.0 Hz, H-7), 6.67 (1H, s, H-14); 13C-NMR (CDCl3) δ 18.2 (t, C-2), 19.3 (q, C-18), 21.3 (q, C-16), 21.3 (q, C-17), 27.0 (t, C-1), 27.8 (t, C-6), 27.8 (d, C-15), 32.0 (q, C-19), 34.4 (s, C-4), 40.5 (t, C-3), 44.8 (d, C-5), 48.7 (s, C-10), 76.4 (d, C-7), 129.6 (d, C-14), 135.6 (s, C-9), 151.1 (s, C-13), 152.5 (s, C-8), 173.3 (s, C-20), 176.2 (s, C-11), 179.2 (s, C-12); EIMS m/z 328 [M]+ (5), 284 (43), 215 (100), 204 (17), 165 (14), 141 (14), 55 (20).
Rosmaquinone (14). $^1$H NMR (300 MHz) δ 0.88 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.06 (3H, d, $J = 7.0$ Hz, Me-16), 1.08 (3H, d, $J = 7.0$ Hz, Me-17), 1.22 (1H, m, H-3α), 1.45 (3H, overlap, H-1α, H-2β and H-3β), 1.60 (1H, m, H-2α), 2.05 (1H, s, H-5), 2.93 (1H, hept, $J = 7.0$ Hz, H-15), 3.21 (1H, bd, $J = 10.5$ Hz, H-1β), 4.40 (1H, d, $J = 3.3$ Hz, H-7), 4.52 (1H, d, $J = 3.3$ Hz, H-6), 6.79 (1H, s, H-14); $^{13}$C NMR (75 MHz) δ 18.2 (C-2), 21.1 (C-16 and C-17), 21.7 (C-19), 25.0 (C-1), 27.3 (C-15), 30.9 (C-18), 31.2 (C-4), 37.8 (C-3), 45.7 (C-10), 49.8 (C-5), 68.0 (C-7), 76.1 (C-6), 133.5 (C-14), 138.1 (C-9), 146.7 (C-8), 150.1 (C-13), 175.5 (C-20), 179.5 (C-11), 179.9 (C-12); Elms m/z 344 [M]$^+$ (15), 316 (25), 257 (48), 230 (44), 215 (33), 203 (45), 201 (48), 187 (43), 161 (25), 128 (39), 115 (57), 105 (26), 91 (66), 55 (100).

*Methylation of Carnosol (2).* A suspension of compound (2) (0.106 mmol), anhydrous K$_2$CO$_3$ (0.78 mmol) and Me$_2$SO$_4$ (0.64 mmol) in dry acetone (6 mL) was stirred at 0°C in darkness under a nitrogen atmosphere for 5 h. The reaction mixture was diluted with water (10 mL) and extracted with AcOEt (3×10 mL). The organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The dried residue was purified over silica gel column using hexano/AcOEt (95:5) as solvents to provide 15 as the main product (55.7%).

11,12-di-O-Methylcarnosol (15) Crystalline plates (EtOH), mp: 155-157°C. $^1$H-NMR (CDCl$_3$) δ: 0.86 (3H, s, H-19), 0.90 (3H, s, H-18), 1.18 (6H, d, $J = 7.0$ Hz, H-16 and H-17), 1.33 (IH, bd, $J = 14.0$ Hz, H-3α), 1.62-1.64 (2H, m, H-2α and H-3β) 1.70 (1H, q, H-5), 1.90 (IH, td, $J_1 = 7.0$, $J_2 = 1.5$ Hz, H-6β), 2.10 (IH, dt, $J_1 = 4.0$, $J_2 = 14.0$ H-2β), $J_2$ (IH, m, H-6α) 2.43 (IH, td, $J_1 = 4.3$, $J_2 = 14.0$, H-lα), 2.80 (IH, bd, $J = 10.0$ Hz, H-1β), 3.28 (IH, sept, $J = 7.0$ Hz, H-15) 3.81 (3H, s, Ar-OCH$_3$), 3.83 (3H, s, Ar-OCH$_3$), 5.39 (IH, dd, $J_1 = 1.5$, $J_2 = 4.5$ Hz, H-7), 6.84 (IH, s, H-14); $^{13}$C-NMR (CDCl$_3$) δ: 19.5 (C-2), 20.2 (C-17), 23.8 (C-19), 24.0 (C-16), 27.2 (C-15), 28.7 (C-l), 30.0 (C-6), 32.3 (C-18) 36.1 (C-4), 41.6 (C-3), 45.9 (C-5), 49.1 (C-l0), 61.1 (Ar-OCH$_3$), 61.4 (Ar-OCH$_3$), 77.8 (C-7), 116.5 (C-14) 129.5 (C-9), 136.1 (C-8), 142.5 (C-13) 151.2 (C-12), 151.9 (C-11), 176.2 (C-20). Elms m/z (%) 358 [M]$^+$ (11), 315 (22) 314 (100), 299 (30) 271 (9) 245(10), 243 (13), 232 (231, 229 (21), 201 (191, 141 (231, 128(32), 115 (33) 69 (66), 55 (93).

*Acetylation of Carnosol (2).* To a solution of carnosol (2) (50 mg) in pyridine (1.5 ml), acetic anhydride (3.0 ml) was added and the mixture was allowed to stand at room
temperature overnight. The reaction mixture was worked up in the usual manner, to afford carnosol diacetate (92.5 %).

11,12-diacetyl-carnosol (16). Isolated as an amorphous solid, mp: 158-159°C. $^1$H-NMR (300 MHz, CDCl$_3$) δ 0.86 (3H, s, Me-19), 0.91 (3H, s, Me-18), 1.19 and 1.20 (each 3H, d, $J = 7.0$ Hz, Me-16 and Me-17), 2.29 (3H, s, Ar-OAc), 2.30 (3H, s, Ar-OAc), 2.67 (IH, bd, $J = 10.0$ Hz, H-1β), 2.90 (1H, hept, $J = 7.0$ Hz, H-15), 5.50 (1H, dd, $J_1 = 1.4$ Hz, $J_2 = 4.0$ Hz, H-7), 7.10 (1H, s, H-14); EIMS m/z (%) 414 [M]+ (10), 354 (50), 327 (10), 286 (100), 228 (19), 202 (8), 69 (55) 55 (100).

2. IN VITRO SQUALENE SYNTHASE ACTIVITY ASSAY

Squalene Synthase activity was evaluated by determining the amount of $[^3$H] FPP converted to squalene as previously described by Amin et al. (1992). The assay was performed in 1 mL of 50 mM phosphate buffer, pH 7.4, containing rat liver microsomes (18 μg protein/mL), 0.5 mM NADPH, 10 mM MgCl$_2$, various concentrations of the phytochemicals dissolved in EtOH, and $[^3$H] FPP (0.5 μM, 0.27 Ci/mmol) in a glass screw-cap tube. All components except $[^3$H] FPP were preincubated for 10 min at 37 °C. 10 minutes after the addition of $[^3$H] FPP, at the same temperature, was added 1 mL 15% KOH in EtOH. The mixture was incubated at 65 °C for 30 min, extracted with 2.5 mL n-hexane, which was subsequently washed with 1 mL distilled water. Then 1.5 mL of the upper organic phase was counted with 2.5 mL of scintillation liquid using a Beckman scintillation counter. Each assay was performed in duplicate, and IC$_{50}$ values represent the mean concentration of compounds that inhibits the activity of the enzyme by 50%.
Table S1. SQS inhibitory activity showed by abietane diterpenes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration* (mg/100 g DW)</th>
<th>SQS inhibitory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Carnosol 2</td>
<td>220.0</td>
<td>43</td>
</tr>
<tr>
<td>Rosmanol 3</td>
<td>64.9</td>
<td>18</td>
</tr>
<tr>
<td>7α-Methoxyrosmanol 4</td>
<td>22.0</td>
<td>2</td>
</tr>
<tr>
<td>7α-Ethoxyrosmanol 5</td>
<td>12.7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Galdosol 6</td>
<td>24.5</td>
<td>21</td>
</tr>
<tr>
<td>Rosmadial 7</td>
<td>6.8</td>
<td>15</td>
</tr>
<tr>
<td>Epirosmanol 8</td>
<td>1.5</td>
<td>----</td>
</tr>
<tr>
<td>Isorosmanol 9</td>
<td>9.4</td>
<td>16</td>
</tr>
<tr>
<td>Isogaldosol 10</td>
<td>1.7</td>
<td>----</td>
</tr>
<tr>
<td>Demetilsalvicanol 11</td>
<td>1.8</td>
<td>----</td>
</tr>
<tr>
<td>7β-Methoxyrosmanol 12</td>
<td>1.6</td>
<td>----</td>
</tr>
<tr>
<td>Carnosol quinone 13</td>
<td>----</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Rosmaquinone 14</td>
<td>----</td>
<td>54</td>
</tr>
<tr>
<td>11,12-di-O-Methylcarnosol 15</td>
<td>----</td>
<td>38</td>
</tr>
<tr>
<td>11,12-Diacetyl-carnosol 16</td>
<td>----</td>
<td>3</td>
</tr>
</tbody>
</table>

*Expressed as miligrams of compound per 100 grams of dried weight sample.
3. DOCKING ANALYSIS

For the analysis was selected the crystallographic structure of a human SQS available from Protein Data Bank (PDB ID: 3V66) and AutoDock Vina as the docking algorithm. A docking grid was generated in the 3V66 receptor, in the active site region defined by the co-crystallized receptor ligand, a pyrrolobenzoxazepine derivative. The docking method was validated in order to assure effectiveness of the analysis. This was made re-docking the co-crystallized ligand, employing the same parameters intended to use for our compounds. Comparing the best result docking pose for the ligand with the co-crystallized one, a RMSD of 0.566 was obtained. As this value is lower than 1.5-2 Å, it is considered a successful analysis and a docking protocol suitable for predicting ligand poses (Henever et al. 2009).

Table S2. Binding free energy derived from the docking studies on SQS for compounds 2, 14 and 15.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta G_b$ (Kcal/mol)</th>
<th>Principal Residues Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnosol 2</td>
<td>-8.7</td>
<td>Hydrophobic cavity residues Ala176, Met207, Gly208</td>
</tr>
<tr>
<td>Rosmaquinone 14</td>
<td>-8.8</td>
<td>Hydrophobic cavity residues Leu76</td>
</tr>
<tr>
<td>11,12-di-O-Methylcarnosol 15</td>
<td>-8.1</td>
<td>Hydrophobic cavity residues</td>
</tr>
</tbody>
</table>

Figure S2. Docked compounds with Squalene synthase (Carnosol: lila; 11,12-di-O-methylcarnosol: brown; Rosmaquinone: golden). The color of the surface indicates hydrophilicity (blue) passing by white to red (hydrophobicity), framing with a circle the receptor hydrophobic pocket.
**Figure S3.** Surface of the hydrophobic pocket with a right cavity colored in green, and left cavity in blue. Docked compounds are carnosol (lila), 11,12-di-O-methylcarnosol (brown) and rosmaquinone (golden).

**Figure S4.** Residue interactions with the docked compounds (Carnosol: lila; 11,12-di-O-methylcarnosol: brown; Rosmaquinone: golden).

**References**


**1H-NMR SPECTRA OF ALL COMPOUNDS**

**Figure S5.** 1H-NMR spectra of Carnosol 2

**Figure S6.** 1H-NMR spectra of Rosmanol 3
Figure S7. 1H-NMR spectra of 7α-Methoxyrosmanol 4

Figure S8. 1H-NMR spectra of 7α-Ethoxyrosmanol 5
Figure S9. $^1$H-NMR spectra of Galdosol 6

Figure S10. $^1$H-NMR spectra of Rosmadial 7
Figure S11. $^1$H-NMR spectra of Epirosmanol 8

Figure S12. $^1$H-NMR spectra of Isorosmanol 9
Figure S13. $^1$H-NMR spectra of Isogaldosol 10

Figure S14. $^1$H-NMR spectra of Demetilsalvicanol 11
Figure S15. $^1$H-NMR spectra of $7\beta$-Methoxyrosmanol 12

Figure S16. $^1$H-NMR spectra of Carnosol quinone 13
Figure S17. $^1$H-NMR spectra of Rosmaquinone 14

Figure S18. $^1$H-NMR spectra of 11,12-di-O-Methylcarnosol 15
Figure S19. $^1$H-NMR spectra of 11,12-Diacetyl-carnosol 16