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LABDANE-TYPE DITERPENES FROM *Cistus creticus*

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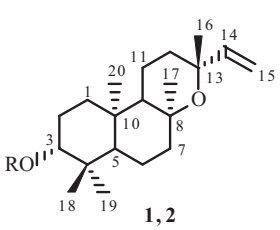
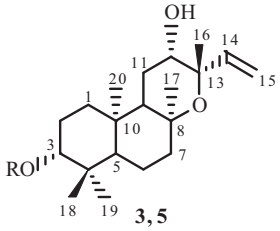
The genus *Cistus* (Cistaceae) is represented by five species in Turkish flora [1]. The *Cistus* genus has widespread utilization in Turkish folk medicine such as against rheumatism, for hemorrhoids, to cure sterility, kidney, and urinary inflammations, as a hemostatic, antipyretic, expectorant, and sedative, and for peptic ulcer as well as diabetes mellitus [2–5]. Anti-inflammatory, anti-helicobacter pylori, antihypertensive, anti-microbial, and cytotoxic effects of *Cistus* species have also been reported [6–10]. Diterpenes [11–14], proanthocyanidins [15], lignan glycosides [16], and flavonoids [13, 15, 16] have been reported previously from *Cistus* species. This paper describes the isolation and structural elucidation of labdane-type diterpenes from *Cistus creticus*.

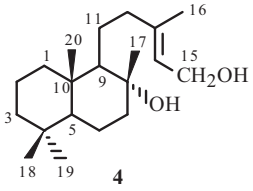
Cistus creticus was collected from Kusadasi in July 2005. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 02016). Open air-dried and powdered aerial parts of the plant (650 g) were extracted with *n*-hexane. After filtration, the combined extracts were evaporated under vacuum to dryness. The *n*-hexane extract was fractionated by silica gel column chromatography, followed by open CC on silica gel and Sephadex LH-20 to yield compounds **1–5**. The purified compounds were identified by EI-MS and NMR spectrum using a Finnigan MAT 95 spectrometer and Varian Mercury plus spectrometer at 400 MHz for proton and 100 MHz for carbon.



1, 2



3, 5



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1, 3: R = Ac

2, 5: R = H

The spectral data of the isolated compounds are reported in below.

***ent*-3β-Acetoxy-13-*epi*-manoyl Oxide (Ribenol Acetate) (1)**. EI-MS *m/z* 333 $[M - CH_3]^+$ (calcd for $C_{22}H_{36}O_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.00 (1H, dd, J = 11.3, 17.9, H-14), 4.95 (1H, dd, J = 1.0, 17.9, H-15b), 4.90 (1H, dd, J = 1.0, 11.0, H-15a), 4.47 (1H, dd, J = 4.76, 11.3, H-3), 2.21 (1H, dd, J = 3.2, 9.3, H-12b), 2.04 (3H, s, CO<u>Me</u>), 1.21 (3H, s, H-17), 1.13 (3H, s, H-16), 0.85 (3H, s, H-18), 0.82 (3H, s, H-19), 0.75 (3H, s, H-20). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 171.0 (<u>C</u>OMe), 147.8 (C-14), 109.8 (C-15), 81.0 (C-3), 75.9 (C-8), 73.6 (C-13), 58.4 (C-9), 55.5 (C-5), 43.1 (C-7), 38.0 (C-4), 37.5 (C-1), 36.7 (C-10), 34.9 (C-12), 32.8 (C-16), 28.2 (C-18), 24.0 (C-17), 23.8 (C-2), 21.5 (CO<u>Me</u>), 19.6 (C-6), 16.5 (C-20), 16.3 (C-11), 16.1 (C-19) [17].

***ent*-3β-Hydroxy-13-*epi*-manoyl Oxide (Ribenol) (2)**. EI-MS *m/z* 291 $[M - CH_3]^+$ (calcd for $C_{20}H_{34}O_2$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.10 (1H, dd, J = 11.0, 18.0, H-14), 4.97 (1H, dd, J = 1.0, 18.0, H-15b), 4.92 (1H, dd, J = 1.0, 11.0, H-15a), 3.22 (1H, ddd, J = 5.5, 5.5, 11.0, H-3), 2.22 (1H, dd, J = 3.1, 10.0, H-12b), 1.78 (1H, dd, J = 3.1, 9.0, H-7a), 1.22 (3H, s, H-17), 1.13 (3H, s, H-16), 0.98 (3H, s, H-18), 0.75 (3H, s, H-19), 0.73 (3H, s, H-20). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 147.8 (C-14), 109.8 (C-15), 79.1 (C-3), 76.0 (C-8), 73.6 (C-13), 58.5 (C-9), 55.5 (C-5), 43.2 (C-7), 39.1 (C-4), 37.8 (C-1), 36.7 (C-10), 35.0 (C-12), 32.9 (C-16), 28.2 (C-18), 27.5 (C-2), 24.0 (C-17), 19.8 (C-6), 16.2 (C-11), 16.1 (C-20), 15.4 (C-19) [17, 18].

***ent*-3β-Acetoxy-12β-hydroxy-13-*epi*-manoyl Oxide (3)**. EI-MS *m/z* 349 $[M - CH_3]^+$ (calcd for $C_{22}H_{36}O_4$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.29 (1H, dd, J = 11.2, 17.8, H-14), 5.43 (1H, dd, J = 1.4, 17.9, H-15b), 5.24 (1H, dd, J = 1.4, 11.3,

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H-15a), 4.47 (1H, dd, J = 4.5, 11.5, H-3), 3.51 (1H, dd, J = 4.7, 11.3, H-12), 2.04 (3H, s, COMe), 1.35 (3H, s, H-16), 1.24 (3H, s, H-17), 0.85 (3H, s, H-18), 0.83 (3H, s, H-19), 0.80 (3H, s, H-20). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 171.2 (COMe), 140.5 (C-14), 117.3 (C-15), 80.8 (C-3), 77.7 (C-12), 76.6 (C-13), 75.8 (C-8), 57.7 (C-9), 55.3 (C-5), 42.4 (C-7), 37.9 (C-4), 37.4 (C-1), 36.7 (C-10), 28.2 (C-16), 28.1 (C-18), 25.5 (C-17), 25.4 (C-2), 23.7 (C-11), 21.4 (COMe), 19.6 (C-6), 16.3 (C-20), 16.2 (C-19) [18, 19].

Labd-13(*E*)-ene,8α,15-diol (4). EI-MS *m/z*: 308 $[M]^+$, 290 $[M - H_2O]^+$ (calcd for $C_{20}H_{36}O_2$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.40 (1H, t, J = 8.0, H-14), 4.15 (2H, d, J = 8.0, H-15), 2.10 (2H, t, J = 8.0, H-12), 1.70 (3H, br.s, H-16), 1.14 (3H, s, H-17), 0.90 (3H, s, H-18), 0.82 (6H, br.s, H-19, H-20). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 141.2 (C-13), 123.4 (C-14), 74.4 (C-8), 61.4 (C-9), 59.5 (C-15), 56.5 (C-5), 44.7 (C-12), 43.0 (C-7), 42.3 (C-3), 39.9 (C-1), 39.5 (C-10), 33.6 (C-19), 33.4 (C-4), 24.2 (C-17), 23.8 (C-11), 21.8 (C-18), 20.6 (C-2), 18.5 (C-6), 16.7 (C-16), 15.7 (C-20) [20].

***ent*-3β,12β-Dihydroxy-13-*epi*-manoyl Oxide (5)**. EI-MS *m/z* 339 $[M - CH_3]^+$ (calcd for $C_{20}H_{34}O_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.09 (1H, dd, J = 11.3, 17.9, H-14), 5.19 (1H, dd, J = 1.4, 17.9, H-15b), 4.93 (1H, dd, J = 1.4, 11.3, H-15a), 3.22 (1H, dd, J = 4.4, 11.3, H-12), 2.92 (1H, dd, J = 4.4, 11.1, H-3), 1.09 (3H, s, H-16), 0.97 (3H, s, H-17), 0.73 (3H, s, H-18), 0.53 (3H, s, H-19), 0.51 (3H, s, H-20). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 141.5 (C-14), 115.9 (C-15), 78.2 (C-3), 77.2 (C-12), 76.1 (C-8), 75.6 (C-13), 57.7 (C-9), 55.1 (C-5), 42.4 (C-7), 38.8 (C-4), 37.7 (C-1), 36.5 (C-10), 28.6 (C-16), 28.2 (C-18), 27.3 (C-2), 25.4 (C-17), 25.2 (C-11), 19.6 (C-6), 16.1 (C-20), 15.4 (C-19) [18].

To our knowledge, *ent*-3β-acetoxy-13-*epi*-manoyl oxide [14, 21, 22], *ent*-3β-hydroxy-13-*epi*-manoyl oxide [14, 21, 22], and labd-13(*E*)-ene,8α,15-diol [15, 16, 21] have been reported from different *Cistus* species. This study is the first report on the isolation and structure elucidation of *ent*-3β-acetoxy-12β-hydroxy-13-*epi*-manoyl oxide and *ent*-3β,12β-dihydroxy-13-*epi*-manoyl oxide from *Cistus* species. Diterpenes are the most common substance in the genus *Cistus*. They play an important role in chemotaxonomic classification, and the genus can be evaluated as anticancer plants after satisfactory investigation of their biological activity.

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