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CHEMICAL CONSTITUENTS OF *Centaurea cadmea*

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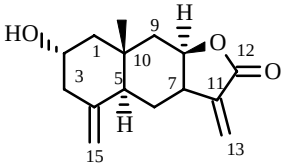
Centaurea cadmea Boiss. (Asteraceae), belonging to section *Phalolepis* (Cass.) DC., is an endemic species distributed in N, W, and SW of Turkey [1]. No previous phytochemical study has been performed on *C. cadmea*.

Our ongoing studies resulted in the isolation of a sesquiterpene lactone, 2α-hydroxy-5αH-eudesma-4(15),11(13)-dien-12,8β-olide (ivalin) (**1**) [2] known as a potent cytotoxic compound on several tumor cell lines [3], from the aerial parts of *C. cadmea*. The structure of ivalin (**1**) was determined primarily from 1D-, 2D-NMR (COSY, NOESY, HMQC, and HMBC), and LC-MS. Two polymethoxyflavones, 5,3′-dihydroxy-6,7,4′-trimethoxy-flavone (eupatorin) (**2**) [4] and 5-hydroxy-3′,4′,6,7-tetra-methoxyflavone (5-desmethylsinensetin) (**3**) [4], and two usual phytosterols, β-sitosterol (**4**) [5] and β-sitosterol-3-*O*-β-D-glucopyranoside (**5**) [6], were also isolated from the plant and identified by comparison of their ^{1}H and ^{13}C NMR spectral data with those in the literature. Compounds **2** and **3** have been reported previously from another species of *Centaurea* [7–10]. As far as could be ascertained, this is the first report of ivalin (**1**) from the genus *Centaurea*.



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General. The NMR spectra were recorded on a Varian Oxford AS400 NMR spectrometer. IR spectra were obtained on an ATI Mattson Genesis series FT-IR spectrometer. MS were determined on an Agilent series 1100 SL equipped with an ESI source. Column chromatography was carried out on Silica gel (JT Baker, 40 μm for flash chromatography), Sephadex LH-20 (Amersham Biosciences, 17-0090-02), and RP C-18 (Merck, 40 μm). TLC analyses were carried out on Silica gel 60 F_{254} plates (Merck, Germany) using $CHCl_3$–MeOH (90 : 10) and *n*-hexane–EtOAc–MeOH (10 : 10 : 1) mixture as a solvent system. Compounds were detected by UV and 1% vanillin/H_2SO_4 spraying reagent followed by heating at 105°C for 1–2 min.

Plant Material. *Centaurea cadmea* Boiss. was collected from Denizli, Evrantepe, 1512 m above sea level, in June 2004 (37° 41′ 18.6″N; 29°00′ 07″E) and identified by Prof. Dr. Ozcan Secmen from the Section of Botany, Department of Biology, Faculty of Science, Ege University, Izmir, Turkey. A voucher specimen was deposited in the Herbarium of Ege University, Faculty of Pharmacy, Izmir, Turkey (IZEF 5670).

Extraction and Isolation. The $CHCl_3$ extract (3 g) obtained from 100 g aerial parts was chromatographed on silica gel column using solvent mixtures (*n*-hexane–EtOAc–$CHCl_3$–MeOH) with increasing polarities. Twenty-one fractions were collected and monitored by TLC. Fraction 8 (1.01 g) was chromatographed over a silica gel column using $CHCl_3$–MeOH mixtures (100 : 0, 98 : 2, 96 : 4) to afford 108 fractions. Fractions 2–12 were combined (58.9 mg) and rechromatographed over a silica gel column using *n*-hexane–EtOAc–MeOH mixtures (50 : 50 : 0.5, 50 : 50 : 1, 50 : 50 : 1.5) to afford 145 fractions. Fractions 50–54 were combined to yield eupatorin (**2**) (3 mg), and fractions 68–72 were combined to yield ivalin (**1**) (5.5 mg).

The MeOH extract (11.1 g) obtained from 100 g aerial parts was chromatographed on a silica gel column, using solvent mixtures (*n*-hexane–EtOAc–$CHCl_3$–MeOH) with increasing polarities. Sixty-two fractions were collected and monitored by TLC

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(Frs. 1–62). Fractions A (Fr. 30–33, 891 mg), B (Fr. 36–37, 367 mg), and C (Fr. 50–53, 1.14 g) were selected for further purification. Fraction A was chromatographed over a silica gel column using *n*-hexane–$CHCl_3$ mixtures (55 : 45, 50 : 50) and *n*-hexane–$CHCl_3$–MeOH mixtures (10 : 10:0.25, 10 : 10 : 1) to afford 227 fractions. Fractions 32–60 were combined (145 mg) and rechromatographed over an RP C-18 column using 100% MeOH. Fractions 9–15 were combined to yield 5-desmethylsinensetin (**3**) (10.3 mg). Fraction B 154–166 (124.4 mg) was rechromatographed over a Sephadex LH-20 column using 100% MeOH. Fractions 21–24 were combined to give *β*-sitosterol (**4**) (2.9 mg). Fraction C was chromatographed over a silica gel column using $CHCl_3$–MeOH mixture (98:2) to give 197 fractions. Fractions 130–162 were combined (127.9 mg) for further purification and rechromatographed over a Sephadex LH-20 column using MeOH (100%). Fractions 23–54 were combined to yield *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) (8.6 mg).

2*α*-Hydroxy-5*α*H-eudesma-4(15),11(13)-dien-12,8*β*-olide (ivalin) (1): amorphous white powder; IR (KBr, ν_{max}, cm^{-1}): 3440, 2924, 1754, 1647, 1457, 1347, 1264, 1137; LC-MS *m/z*: 271.1294 $[M+Na]^+$ (calcd. for $C_{15}H_{20}O_3Na$: 271.1310), 519.2698 $[2M+Na]^+$ (calcd. for $C_{30}H_{40}O_6Na$: 519.2722).

^1H NMR (400 MHz, C_5D_5N, δ, ppm, J/Hz): 1.37, 2.02 (2H, m, H-1), 4.0 (1H, ddd, J = 4.8, 11.2, 15.6, H-2), 2.25, 2.87 (2H, t, J = 11.6, m, H-3), 1.74 (1H, d, J = 12.8, H-5), 1.22, 1.62 (2H, m, ddd, J = 2.8, 7.8, 13.6, H-6), 2.89 (1H, m, H-7), 4.40 (1H, dd, J = 4.8, 4.4, H-8), 1.99, 1.37 (2H, m, H-9), 5.56, 6.16 (2H, br. s, H-13), 0.77 (1H, s, H-14), 4.82, 4.44 (2H, br. s, H-15).

^{13}C NMR (100 MHz, C_5D_5N, δ, ppm): 41.2 (t, C-1), 66.3 (d, C-2), 47.5 (t, C-3), 147.3 (s, C-4), 45.7 (d, C-5), 27.6 (t, C-6), 40.6 (d, C-7), 76.8 (d, C-8), 51.9 (t, C-9), 34.1 (s, C-10), 142.8 (s, C-11), 170.0 (s, C-12), 119.5 (t, C-13), 19.1 (q, C-14), 108.2 (t, C-15).

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