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CHEMICAL CONSTITUENTS OF CHLOROFORM AND PETROLEUM EXTRACTS FROM *Cirsium palustre* FLOWER HEADS

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Cirsium palustre (L.) Scop. marsh thistle (Asteraceae) is a herbaceous biennial plant. It grows widely in Poland and other countries of Europe, inhabiting the humid meadows, marshes, and road sides [1]. Their chemical composition is still weakly recognized. In the volatile fraction of the plant a polyine compound, tridecaen(1)-pentaine(3,5,7,9,11), was identified [2]. From leaves and inflorescences, flavonoid aglycones and glycosides were isolated [3, 4]. In the MeOH extract and $CHCl_3$, Et_2O, EtOAc, and *n*-BuOH fractions of flower heads and leaves obtained from it, the antioxidant activity and total phenolic content were determined [5]. Lipophilic compounds, apart from polyine, in *C. palustre* have not been examined to date.

The crude chloroform extract of *C. palustre* inflorescences was subjected to column chromatography using hexane and mixtures of hexane–ethyl acetate with increasing content of ethyl acetate. Multiple rechromatography of chosen fractions over silica gel columns using hexane and hexane–chloroform with increasing content of chloroform gave hexadecanoic acid (**7**) and its methyl ester (**1**), and six pentacyclic triterpenes: α-amyrin acetate (**2**), β-amyrin acetate (**3**), β-amyrin (**4**), lupeol acetate (**5**), faradiol (**6**), and the phytosterol glycoside β-sitosterol 3-*O*-β-D-glucoside (**8**).

The compounds were identified by comparison of their mass spectra with mass spectra in the NIST MS Library and their ^1H and ^{13}C NMR spectral data with those of the corresponding compounds reported in the literature [6–12].

The petroleum extract was also chromatographed on a silica gel column with petroleum ether–ethyl acetate and then a C_6H_{14}–$CHCl_3$ step gradient. After separation, mixtures of compounds were obtained: in mixture A, compound **4** and lupeol (23.92% and 70.07%, respectively); in mixture B, cholesterol, campesterol, stigmasterol, and β-sitosterol (0.5, 11.2, 43.8 and 41.6%, respectively); in mixture C, compound **2** and compound **3** (24.22% and 49.59%, respectively); and in mixture D, 67.89% of compound **5**. The constituents of the mixtures were identified by analysis of their mass spectra.

All the compounds were isolated in *C. palustre* for the first time. The flower heads of marsh thistle can be a valuable source of triterpene compounds.

General. Silica gel 60 (Merck Co., 0.2–0.5 mm and 0.063–0.2 mm) was used for open column chromatography, and silica gel plates (Merck Co., Kieselgel 60 F_{254}) were used for TLC. ^1H and ^{13}C NMR analysis was performed on a Bruker Avance II 400 spectrophotometer at 400 and 100 MHz respectively (CD_3OD, Py-d_5) with TMS as internal standard. GC and GC-MS analysis was carried out using a Perkin–Elmer AutoSystem XL equipped with a Perkin–Elmer TurboMass detector and a Perkin–Elmer Elite 5MS column, 30m × 250 μm I.D., 1 μm film thickness. The oven temperature was programmed from 200°C to 330°C (13.0 min), temperature rise 6°C min^{-1}, temperature of injector 340°C, temperature of detector 330°C. The flow rate of the carrier gas (He) was 1.5 mL/min, split ratio 1:10. Mass spectra were acquired over the mass range 39–600 Da, ionization voltage 70 eV; ion source temperature 200°C, transfer line temperature 330°C.

Plant Material. The inflorescences of *C. palustre* were collected in July and August 2007 in the vicinity of Bialystok (Poland). A voucher specimen No. CP 06014 has been deposited at the Herbarium of Department of Pharmacognosy, Medical University of Bialystok.

Extraction and Isolation. The dried, ground inflorescences (1200 g) of *C. palustre* were exhaustively extracted in a Soxhlet apparatus first with petroleum ether (bp 60–80°C) and then with $CHCl_3$. The chloroform extract (49 g) was subjected to column chromatography on silica gel, eluting with hexane and mixtures of hexane–ethyl acetate (30:1, 20:1, 10:1, 5:1, 1:1, 1:2 and 1:5 v/v) and ethyl acetate. In sum, 519 fractions were obtained. The fractions were collected and pooled according to

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their TLC patterns to give several major fractions. Fractions 39–58 (10.74 g of yellowish, viscous residue) from C_6H_{14}–EtOAc (30:1 and 20:1) were repeatedly separated on several silica gel columns, eluting with C_6H_{14} and C_6H_{14}–$CHCl_3$ mixtures with increasing content of $CHCl_3$. After separation, 1520 mg of compound **1**, compound **2** (47 mg), compound **3** (124 mg), compound **4** (10 mg), compound **5** (32 mg), and compound **6** (15 mg) were obtained. Fractions 127–173 eluted with C_6H_{14}–EtOAc (10:1) were rechromatographed on a silica gel with C_6H_{14}–$CHCl_3$, mixtures with increasing content of $CHCl_3$ giving 60 mg of compound **7**. From fractions 469–519 eluted with C_6H_{14}–EtOAc (1:5) and EtOAc, after purification on Sephadex LH-20 with $CHCl_3$–MeOH (1:1) and crystallization from EtOH, 211 mg of compound **8** was obtained. The petroleum extract (75 g) was chromatographed on a silica gel column with petroleum ether–EtOAc (100:0–0:100) as eluent. Fractions 28–36 eluted with the mentioned solvents in the proportion 20:1 after crystallization from ethanol gave 327 mg of mixture A. From fractions 37–51 eluted with petroleum ether–EtOAc (15:1 and 10:1), 64 mg of mixture B was obtained. Fractions 11–18 eluted with petroleum ether–EtOAc (30:1) gave 34.79 g of a yellowish solid substance, which was rechromatographed on a silica gel column with a C_6H_{14}–$CHCl_3$ step gradient. From fractions 45–65 eluted with C_6H_{14}–$CHCl_3$ (30:1), 3.9 g of mixture C was obtained, and from fractions 66–126 [C_6H_{14}–$CHCl_3$ (30:1 and 20:1)], 8.32 g of mixture D was obtained.

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