TERPENOIDS FROM *Crepis capillaris* (L.) WALLR.

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From the root extract of *Crepis capillaris* mixtures of triterpene acetates, free triterpenols, triterpene hydroxyacids, sterols and sterol glucosides were separated. Twelve compounds were identified by spectroscopic methods and GLC.

In the previous papers[1-3] the isolation and structure determination of several new sesquiterpene lactones from *Crepis capillaris* (L.) WALLR. (syn. *Crepis virens* L.), *Compositae*, were reported. In the course of chromatographic separation of sesquiterpene lactones from the root extract of the plant[3] five fractions (A – E), containing a number of triterpenoids and steroids, were collected. This paper deals with their identification on the basis of GLC, MS and IR analyses.

The first two fractions A and B were triterpene-positive by TLC-spot tests. GLC analyses revealed the presence of at least two peaks in each of the fractions. Compounds in the fraction A were shown to be triterpene acetates with molecular peak at m/z 468 and common fragment at m/z 408 (M – 60). In the IR spectrum absorption bands at 1 740 and 1 250 cm^{-1} characteristic for acetates were observed. Saponification of the fraction A with methanolic potassium hydroxide afforded fraction A_1. The original compounds were recovered again on acetylation of fraction A_1 with acetic anhydride in pyridine. Fraction B contained a mixture of free triterpenols (IR band at 3 390 cm^{-1}) of molecular weight 426 with hydroxyl group attached to rings A or B (m/z 207 and 189)[4]. Moveover, in the mass spectra of fractions A and B characteristic RDA-fragments were observed by the presence of peaks at m/z 218 and 203, a common feature of Δ^{12}-oleanenes or Δ^{12}-ursenes. Relatively intense mass fragment ions at m/z 204, 302 (in A) and 344 (in B) pointed to Δ^{14}-taraxerenes[4]. On the basis of above results and GLC analyses of the fraction A, A_1 and B (Table I) α-amyrin, β-amyrin, taraxerol and their acetates were identificed by comparison of R_t with those of authentic samples.

From fraction C crystalline mixture of phytosterols was separated. Mass spectrum of the mixture showed two molecular peaks at m/z 414 and 412. GLC confirmed the presence of two compounds and their R_t were found to correspond with those

of β-sitosterol and stigmasterol standard samples. Mass fragmentation pattern and IR spectrum were also characteristic for the two compounds.

The IR spectrum of fraction D showed hydroxyl (3 400 cm^{-1}), carboxyl (1 690 cm^{-1}) functions and unsaturation (1 640 cm^{-1}). In the mass spectrum molecular peak at m/z 456 was observed and fragmentation pattern was characteristic for triterpene hydroxy acids of α- or β-amyrin series. An intense peak at m/z 207 and prominent peaks, resulting from RDA-fragmentation of ring C, at m/z 248 and 203 indicated the presence of $C_{(3)}$ hydroxyl group, $C_{(12)}$ double bond and $C_{(1j)}$ carboxyl group[4]. Fraction D after methylation and silylation was subjjected to GLC analysis. Two peaks corresponding with those of uracilic and oleanolic acid methylsilyl derivatives were observed.

The most polar fraction E was proved to be a mixture of stigmasterol and β-sitosterol glucosides. Both acid and enzymatic hydrolysis (β-glucosidase) of the fraction afforded sterol mixture E_1 and glucose (identified by PC and TLC). The mass spectrum of fraction E revealed peaks characteristic for glucose moiety (m/z 145, 109, 73, 60, 61). The remaining ions corresponded with those of β-sitosterol and stigmasterol. The two compounds were identified in the GLC analysis of fraction E_1.

TABLE I

GLC analyses of triterpene and sterol fractions

Fraction	Identified compounds	Column I R_t, min		Column II R_t, min
A	α-amyrin acetate	11·1		—
	β-amyrin acetate	11·1		—
	taraxerol acetate	12·3		—
B[a]	α-amyrin	10·0	15·0	11·7
	β-amyrin	9·7	13·8	10·2
	taraxerol	12·6	18·0	18·0
C[b]	β-sitosterol	6·9		—
	stigmasterol	6·0		—
D	oleanolic acid[c]	12·9		—
	ursolic acid[c]	14·5		—

[a] The same data for fraction A_1; [b] the same data for fraction E^1; [c] trimethylsilyl derivative.

EXPERIMENTAL

The IR spectra were recorded on a Specord spectrometer in KBr discs. The mass spectra were measured on a LKB 9000 S instrumnet at 15 eV. For column chromatography Silica gel Merck, 70–230 mesh, was used. GLC analyses were carried out on MERA-ELWRO N-503 chromatograph equipped with FI detector using glass columns. column I: 160 × 0·3 cm size, packed with 0·5% OV-1 on Chromosorb W AW DMCS, 60–80 mesh, at 270°C, carrier gas N_2, flow rate 67 ml/min; column II: 120 × 0·3 cm size, filled with 1·5% OV-17 on Chromosorb W AW DMCS, 60–80 mesh, at 270°C, carrier gas N_2, flow rate 50 ml/min. All substances were dissolved in chloroform before injection into the GLC columns.

Extraction and Separation of fractions A, B, C, D and E

The air-dried roots (421 g) of *C. capillaris* were exhaustively extracted with ethanol at room temperature. The combined extracts were evaporated and the residue (68 g) was chromatographed on a silica gel (500 g) column packed in benzene. The following fractions (200 ml each) were collected and monitored by TLC: 1–37 (benzene), 38–72 (benzene–ethyl acetate, 9 : 1), 98–121 (benzene–ethyl acetate, 8 : 2), 122–149 (benzene–ethyl acetate, 1 : 1), 150–160 (chloroform), 161–164 (chloroform–methanol, 95 : 5), 165–207 (chloroform–methanol, 9 : 1). The combined fractions: 4–7, A (893 mg); 19–21, B (359 mg), 32–37, C (90 mg), 64–72, D (257 mg) and 170–171, E (72 mg) contained mixtures of triterpenoid and steroid compounds.

Fraction A: non-crystalline, IR spectrum (cm^{-1}): 1 740, 1 660, 1 460, 1 370, 1 250, 820; mass spectrum (*m/z*, ri. %): 468 (M, 27), 453 (10), 408 (6), 344 (4), 249 (9), 218 (100), 203 (13), 204 (22), 205 (18), 189 (27). Fraction A (50 mg) dissolved in dioxane (5 ml) was saponified with 10% methanolic KOH (5 ml) under reflux for 4 h. The hydrolysate after removal most of the solvent was diluted with water and extracted with ethyl ether. The extract was washed, dried (Na_2SO_4) and evaporated to afford fraction A_1 (29 mg).

Fraction B: non-crystalline, IR spectrum (cm^{-1}/: 3 390, 1 650, 1 460, 1 380, 830; mass spectrum (*m/z*, r.i.%): 426 (M, 34), 411 (9), 408 (3), 302 (4), 218 (100), 207 (35), 205 (13), 204 (21), 203 (15), 189 (27).

Fraction C: m.p. 150–164°C; IR spectrum (cm^{-1}): 3 400, 1 650, 1 375, 1 060, 1 030, 1 020, 970, 825; mass spectrum (*m/z*, r.i.%): 414 (66), 412 (100), 396 (23), 394 (9), 369 (23), 351 (32), 300 (50), 273 (43), 271 (52), 255 (68).

Fraction D: m.p. 260–275°C; IR spectrum (cm^{-1}): 3 400, 1 690, 1 640, 1 460, 1 375, 830, 820; mass spectrum (*m/z*, r.i.%): 456 (M, 4), 438 (4), 248 (100), 207 (31), 203 (33), 190 (25), 189 (11), 133 (4). Fraction D (2 mg) dissolved in ethanol (1 ml) was methylated with 1·5% CH_2N_2 in ether (5 ml) and silylated with hexamethyldisilazane (0·2 ml) and trimethylchlorosilane (0·2 ml), using Sweeley and coworkers method[5].

Fraction E: non-crystalline IR spectrum (cm^{-1}): 3 450, 1 650, 1 450, 1 380, 1 050, 1 035, 1 025; mass spectrum (*m/z*, r.i.%): 576 (1), 574 (4), 414 (25), 412 (21), 397 (42), 396 (85), 395 (100), 394 (13), 255 (36), 145 (12), 109 (12), 73 (18), 60 (14). Fraction E (50 mg) was hydrolysed in refluxing 7% ethanolic H_2SO_4 (5 ml) for 3 h. The mixture was diluted with water and extracted with ether. The washed and dried (Na_2SO_4) extract was evaporated to give fraction E_1 (26 mg). The acid layer was spotted on the paper Whatman No 1 and cellulose TLC plates with a number of representative hexones and developed with different solvent systems. The spot of the hydrolysis mixture corresponded with that of glucose. Fraction E was also hydrolysed with β-glucosidase in aqueous acetate buffer solution (pH 5) at 37°C and glucose was identified as the only sugar present in the hydrolysate.

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