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STEROIDAL COMPOUNDS FROM *Helleborus caucasicus* LEAVES

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Interest in the genus *Helleborus* has heightened recently because it is a known source of cardiac glycosides of the bufadienolide group. Several new steroidal glycosides, ecdysteroids, representatives of a new class of natural compounds thionines, macrocyclic suboxides, etc. have been isolated from various *Helleborus* species in addition to bufadienolides. Total preparations of *Helleborus* have been used in medicine as analgesic, antirheumatic, and other agents. Mainly the subterrean organs of the plant are studied and used. The leaves have been neglected.

Of a total of 25 *Helleborus* species, 2 grow in Georgia, *H. caucasicus* A. Br. and *H. abchasicus* A. Br. The former is endemic to the Caucases; the latter, to Georgia. Roots and rhizomes of *H. caucasicus* and *H. abchasicus* accumulate an extraordinarily high amount (14-18%) of neutral lipids for subterrean organs. These differ in their chemical composition from typical plant lipids and exhibit a pronounced specific anticancer effect [1].

Analysis (LC/MS) of the butanol extract of the MeOH extract of roots and rhizomes of *H. caucasicus* (HC) detected 20 steroidal compounds. A total of 12 were isolated and characterized, of these 4 were new glycosides called "caucasicosides" [2, 3]. Total steroidal compounds of HC exhibited at very low concentrations (2×10^{-9} g/μL) cytotoxic activity against several malignant tumor cells [4].

HC (like other *Helleborus* species) is a perennial herbaceous rhizomous evergreen plant with large finger-like leaves. The leaf mass is 23–25% of the total weight of the whole plant. They contain a significant quantity of steroids, which prompted us to study the chemical composition of the HC organs that are reproduced yearly, the leaves.

Air-dried leaves of HC were extracted with MeOH (80%). The MeOH was distilled off. The aqueous phase was worked up with $CHCl_3$ and extracted with *n*-BuOH to afford total glycosides (8–10% of raw material). LC/MS spectral analysis of these detected over 30 steroids (Table 1). Therefore, the qualitative composition of the leaves was richer than that of the subterrean organs of HC.

The BuOH fraction (3 g) was fractionated over a column of silica gel (100 × 2.5 cm, 100/160 μm, Merck) with a mobile phase of $CHCl_3$:CH_3OH:H_2O (26:14:3) under isocratic conditions. This isolated a nonpolar fraction (630 mg), slightly polar (580), and polar (370).

The nonpolar fraction was separated by HPLC (515 HPLC, Waters R 590) over an RP XTerra C_{18} column (7.8 × 300 mm, LiChroprep RP18, 10 μm, XTerra) using MeOH of various concentrations. This isolated five pure compounds.

Compound 1, amorphous white powder, treatment with H_2SO_4 gave a blue color, fluoresced blue. The PMR spectrum showed resonances characteristic of methyls with δ 0.92 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.23 (3H, s, Me-21), 1.22 (3H, s, Me-26), and 1.22 (3H, s, Me-27); one olefinic resonance with δ 5.84 (1H, d, H-6); three methine protons with δ 3.87 (1H, m, H-2), 3.98 (1H, m, H-3), and 3.36 (1H, m, H-21), which confirmed that a secondary alcohol was present. The position of the ketone was proved by correlation of the broad spectrum between the C resonance with δ 206.9 (C-6) and the proton resonances with δ 2.41 (1H, m, H-5) and (1H, d, H-7).

Comparison of the PMR and ^{13}C NMR spectra with the literature indicated that **1** was 20-hydroxyecdysone, which was isolated in 2001 from subterrean organs of *H. torquatus* [5] and also by us from HC [2].

Compound 2, amorphous white powder, soluble in alcohol, gives color with Ehrlich reagent characteristic of furostanes. Acid hydrolysis of the compound (10 mg) produced aglycon (6.2 mg) that was identified as spirostan-5(6),25(27),20(22)-en-1β,3β,11α-triol. The sugar part of the hydrolysate contained glucose. The FAB–MS spectrum gave an ion-peak with *m*/*z* 607 $[M + H]^+$ and a MS/MS fragment with *m*/*z* 445 $[M + Na - 162]^+$ with molecular formula $C_{33}H_{50}O_{10}$. The PMR spectrum showed three resonances for quaternary methyls with δ 0.74 (3H, s, Me-18), 1.17 (3H, s, Me-19), and 1.66 (3H, s, Me-21).

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TABLE 1. Retention Time and Molecular Weights of Compounds from *Helleborus caucasicus* Leaves According to LC/MS

t, min	M^+	t, min	M^+
2.44	436	13.95	1064
3.21	364	14.44	924
4.23	942	15.01	1048
5.55	618	15.82	1122
6.11	780	16.81	1086
6.80	486	17.10	772
7.32	472	18.09	1070
7.94	436	19.04	436
8.26	630	19.35	918
9.26	610	19.84	1162
9.89	526	22.02	298
10.46	1048	25.25	436
11.18	900	28.38	326
11.43	902	29.65	540
12.17	1080	30.12	410
12.64	886	31.81	468
13.31	772	32.93	568

This confirmed the presence of a double bond for C-20–C-22. The double bond was also consistent with a correlation between chemical shifts with δ 1.66 (Me-21), 104.9 (C-20), and 152.6 (C-22) and the HMBC experiment in which a correlation peak was observed between an anomeric proton with δ 4.30 (1H, d, Glc H-1′) and the aglycon C atom with δ 72.4 (C-26).

Based on the results, **2** was characterized as 1β,3β,11α-trihydroxyfurost-5(6),25(27),20(22)-en-26-*O*-β-D-glucopyranoside. This furostane was a new organic compound that was first obtained by us from subterrean organs of HC and described in 2008 as caucasicoside A [2].

Structural studies of the other three compounds from HC leaves are continuing.

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