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ECDYSTEROID ACETATES FROM *Ajuga reptans*

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The compounds 20-hydroxyecdysone-22-acetate and viticosterone E (20-hydroxyecdysone-25-acetate) are observed for the first time in Ajuga reptans *L. growing at the northern limit of its range in a middle taiga subzone of European northeastern Russia.*

The plant *Ajuga reptans* L. (Lamiaceae) is interesting because it is traditionally used in the folk medicine of many peoples as a tonic and stimulant owing to the presence in it of ecdysteroids [1].

It has been shown that the ecogeographic conditions under which *A. reptans* grows can affect the ecdysteroid composition [2-5]. We found that *A. reptans* growing at the northern limit of its range contains polypodin B, 20-hydroxyecdysone, 29-norcyasterone, 29-norsengosterone, sengosterone, and ajugalactone, which are characteristic of this species. Cyasterone is absent. Ajugasterone B is observed for the first time [5]. Minor ecdysteroids are known to be present in addition to the principal ones. The purified minor ecdysteroids of *A. reptans* were obtained by preparative and semi-preparative normal-phase HPLC (Table 1).

Mass spectral analysis of compounds **1** and **2** showed that the principal fragmentation patterns are cleavage of the MH^+ and MNH_4^+ ions at the C_{17}-C_{20} and C_{20}-C_{22} bonds and loss of one or several water molecules from the MH^+ and MNH_4^+ ions, side-chain fragments, or fragment ions. The fragment ions of **1** and **2** that are formed by cleavage at the C_{17}-C_{20} and C_{20}-C_{22} bonds (Table 2) are identical to those from 20-hydroxyecdysone. The side-chain fragment ions are *m/z* 203 ($C_{10}H_{19}O_4^+$) and 159 ($C_{18}H_{15}O_3^+$), which differ from the side-chain ions of 20-hydroxyecdysone by 42 mass units. The unsaturation factor (UF) of these ions is 1. Furthermore, the side-chain ions that are formed by cleavage of MH^+ and MNH_4^+ at the C_{20}-C_{22} bonds have the same UF as the side-chain fragments from cleavage at the C_{17}-C_{20} bonds. This indicates that the side chains are unsaturated after the C_{20} atoms. The collection of ions with *m/z* 271, 240, 222, and 180 of **1** and **2** results most probably from fragmentation with simultaneous cleavage of the C_{13}-C_{17} and C_8-C_{14} bonds of the steroid with subsequent loss of water molecules and H atoms from the resulting fragments.

The fragmentation of **1** and **2** is distinguished by the rather facile cleavage of species of mass 42, 43, and 60 amu from both the molecular ions and side-chain ions. Such fragmentation of **1** gives ions with *m/z* 479, 461, and 443; for **2**, *m/z* 481, 463, 445, and 427. The side-chain ions of **1** and **2** have *m/z* 160 and 143. Thus, the characteristic fragmentation of **1** and **2**, the difference in the side-chain masses of 20-hydroxyecdysone, and the UF of the side-chain fragments from cleavage at the C_{17}-C_{20} and C_{20}-C_{22} bonds confirms the presence of acetate groups after C_{20}. It is interesting that **2** loses C_2H_2O and C_2H_3O from the molecular ion and then further fragments at the C_{23}-C_{24} bond. As a result, ions with *m/z* 425, 424, 406, 389, 388, 371, and 370 form. For **1**, this fragmentation pattern does not occur. The loss of fragments with mass 42 and 43 from MH^+ is not so evident as for **2**, apparently owing to steric factors at C_{25}.

Thus, **1** and **2** from *A. reptans* are identified by chromatographic retention time using pure standards, melting point, and mass spectrometry as 20-hydroxyecdysone-22-acetate and viticosterone E (20-hydroxyecdysone-25-acetate) (Fig. 1). The content of 20-hydroxyecdysone-22- and -25-acetates is 10^{-4} g/g of dry plant.

It should be mentioned that ecdysterone acetates, for example, 20-hydroxyecdysone-2-acetate and -3-acetate were previously isolated as minor components from *A. reptans* grown in Spain [3]. We observed the 22-acetate and viticosterone E (20-hydroxyecdysone-25-acetate) in *A. reptans* growing at the northern limit of its range. The differences in the content of ecdysone acetates is explained by the differences in the ecogeographical conditions under which the plant was growing.

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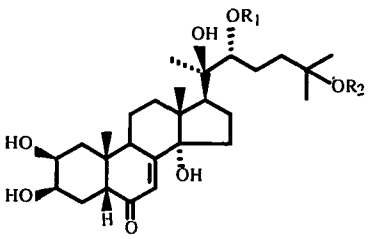
TABLE 1. Physicochemical Properties of *Ajuga reptans* Ecdysteroids

Compound	HPLC retention time, min			Molecular wt.	mp, °C
	syst. 1	syst. 2	syst. 3		
20-Hydroxyecdysone-25-acetate (viticosterone E)	14.0	10.4	5.9	522	198
20-Hydroxyecdysone-22-acetate	18.0	13.6	10.9	522	152
20-Hydroxyecdysone	25.0	19.2	16.8	480	240-242

TABLE 2. Principal Mass-Spectral Fragmentation Patterns of *A. reptans* Ecdysteroids

Compound	$\underline{MNH_4^+}$ MNH_4^+-nH_2O (*m/z*)	$\underline{MH^+}$ MH^+-nH_2O (*m/z*)	Fragmentation at bond				Other ions
			C_{17}-C_{20}		C_{20}-C_{22}		
			Parent (*m/z*) Side-chain ion (*m/z*)	Empirical formula of fragment ions	Parent (*m/z*) Side-chain ion (*m/z*)	Empirical formula of fragment ions	
1	540 522	523 505, 487, 469	320 203, 185, 167	MH^+-$C_{10}H_{19}O_4^+$-nH_2O	364, 363, 346, 345, 327 176, 159	(MH^+, MNH_4^+)-$C_8H_{15}O_3^+$-nH_2O	479, 461, 443: 160, 143, 125, 116 271, 240, 222, 180
2	540 522	523 505, 487, 469	320, 302 203, 185, 167	MH^+-$C_{10}H_{19}O_4^+$-nH_2O	364, 363, 346, 345, 328, 327, 310 176, 159	(MH^+, MNH_4^+)-$C_8H_{15}O_3^+$-nH_2O	481, 463, 445, 427, 160, 143, 116 271, 240, 222, 180, 424, 406, 388, 370, 425, 389, 371
3	498 480, 462, 444, 426	481 463, 445, 427, 409	320, 303, 285 161, 160, 143, 178	MH^+-$C_8H_{17}O_3$-nH_2O	364, 380, 346, 363, 329, 345, 327 117, 116	(MH^+, MNH_4^+)-$C_6H_{13}O_2$-nH_2O	313, 249, 187, 168, 151, 133



	R_1	R_2
20-Hydroxyecdysone-22-acetate	$COCH_3$	H
Viticosterone E		
20-Hydroxyecdysone-25-acetate	H	$COCH_3$

EXPERIMENTAL

A. reptans was collected in the Syktyvkar district of the Komi Republic and was air-dried at 60°C.

The literature method for extraction and preliminary purification was modified [3]. Dry plants (50 g) were ground and extracted with methanol (500 ml) for 30 min. Nonpolar compounds were removed with hexane. The methanol fraction was evaporated. The dry solid was dissolved in water and placed on a column filled with diasorb $C_{16}T$ (Biokhimmak plant, Russia, 40-100 μm fraction, 100 g, 45×150 mm) previously activated with methanol and washed with water. Gradient elution used methanol:water, 1:4 (500 ml) and 1:1 (500 ml). The column was washed with methanol (500 ml) after the elution. Fractions containing ecdysteroids were combined, evaporated to dryness, and placed on silica gel (30 g). Chromatography was performed on a silica-gel column (50-150 μm, 70 g, 30×260 mm) using $CHCl_3$ and $CHCl_3$:CH_3OH (6:1, 4:1, 2:1, 1:1, and 1:2, 500 ml portions). Pure compounds were isolated from the resulting fractions using preparative and semi-preparative HPLC.

Preparative normal-phase (np) HPLC (Zorbax-Sil 21.2×250 mm column, CH_2Cl_2:isopropanol:water 125:40:3 eluent, elution rate 10 ml/min, system 1) and semi-preparative np-HPLC (Zorbax-Sil 9.4×250 mm column, cyclohexane:isopropanol:water 100:40:3 eluent, elution rate 4 ml/min, system 2) were performed on DuPont Instruments (France) equipment with a pump and 8800 control system, 850-UV detector, and $\lambda = 254$.

Ecdysteroids were analyzed by np-HPLC (Zorbax-Sil 4.6×250 mm column, cyclohexane:isopropanol:water 100:40:3 eluent, elution rate 1 ml/min, system 3; and Zorbax-Sil 21.2×250 mm column, CH_2Cl_2:isopropanol:water 125:40:3, elution rate 1 ml/min, system 4).

Mass spectra were recorded by chemical ionization in ammonia and direct probe in a Riber 10-10B instrument (Nermag S. K., France).

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