PHYTOECDYSONES of *Ajuga turkestanica*.

III. THE STRUCTURE OF TURKESTERONE

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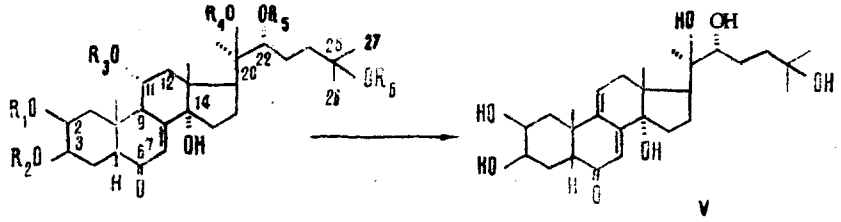
Studying plants of the genus *Ajuga*, we have isolated from *A. turkestanica* (Rgl) Briq. family Labiatae, cyasterone, ecdysterone [1], and a new phytoecdysone which we called turkesterone [2].

The mass spectrum of turkesterone lacks the peak of the molecular ion. However, the presence in it of dehydration peaks with m/e 460 ($M - 2 \cdot H_2O$), 442 ($M - 3 \cdot H_2O$), 343, and 325, which are characteristic of phytoecdysones containing four hydroxy groups in the steroid nucleus [3-6], in combination with the fragments from the decomposition of the side chain with m/e 143, 125, 99, 81, and 69 [7] permit the conclusion that turkesterone (I) has mol. wt. 496 ($C_{27}H_{44}O_8$).



V

	R_1	R_2	R_3	R_4	R_5	R_6
I.	H	H	H	H	H	H
II.	Ac	Ac	Ac	H	Ac	H
III.	Ac	Ac	Ac	H	Ac	Ac
IV.	> CMe_2		H	> CMe_2		H

In acetone solution in the presence of phosphomolybdic acid, turkesterone (I) forms a diacetonide (IV) the mass spectrum of which shows the peak of the molecular ion with m/e 576. Peaks with m/e 201, 143, 125, 102, 99, and 81, which are characteristic for decomposition of the side chain of the diacetonide of ecdysterone must also be noted. Consequently, if the side chain of turkesterone has a composition similar to that of ecdysterone, the new phytoecdysone must include seven hydroxy groups: four in the androstane part of the molecule and three in the side chain.

The acetylation of turkesterone with acetic anhydride in pyridine gave a mixture of an amorphous tetraacetate (II) and an amorphous pentaacetate (III). The characteristics of the fragmentation of the tetraacetate under the action of electron impact agree with the assumption that the steroid nucleus of turkesterone has four hydroxy groups. In actual fact, the ions *a* and c [8], and also their subsequent fragments, are shifted two units in the region of lower masses in comparison with the analogous fragment of ecdysones containing three hydroxy groups in the tetracyclic nucleus. This can obviously be explained by the fact that

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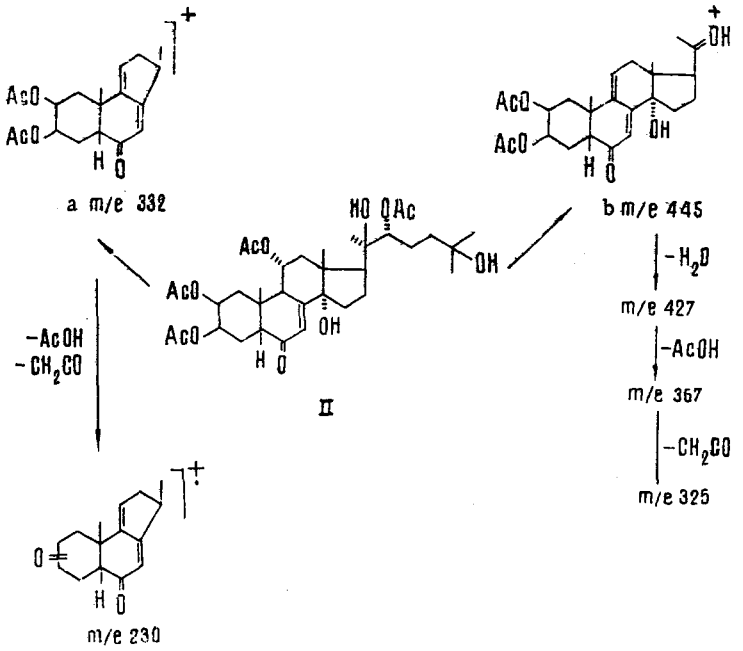

TABLE 1. Chemical Shifts of the Methyl Groups*, ppm

Compound	C_{18}	C_{19}	C_{21}	$C_{26}/_{27}$
Turkesterone (I)	1,23	1,29	1,56	1,35
Turkesterone tetraacetate (II)	0 90	1,10	1,26	1,23; 1,18
Ajugasterone C [4]	1,21	1,27	1,51	0,82 (d)
Ajugasterone C tetraacetate [4]	0.89	1,10	1,23	0,88 (d)
Ecdysterone [10]	1,19	1,06	1,56	1,37
Ecdysterone triacetate [10]	0,85	1,03	1,26	1,22; 1,19

*The values of the CSs of compounds (I) and (II) are given with the correction for HMDS taken into account.

the process of ionization decomposition is preceded by the elimination of a molecule of acetic acid. On periodate oxidation, turkesterone consumed 2 moles of periodate. This excludes the C_1 and C_4 positions for the additional hydroxy groups.

Under the action of a solution of potassium bicarbonate on the tetraacetate (II), in addition to the saponification of the acetyl groups partial dehydration took place. The anhydroturkesterone (V) obtained (M^+ 478) had $\lambda_{max}^{C_2H_5OH}$ 301 nm (logε 3.94). For the conjugated unsaturated $\Delta^{7,9(11)}$-dien-6-one system, the absorption maximum calcualted by the Woodward–Fieser rule [9] is 303 nm. Consequently, the additional hydroxy group in the steroid nucleus for turkesterone must be located at C_{11}. The results of a comparison of the NMR spectra of turkesterone (I) and its anhydro derivative (V) lead to the same conclusion. In the NMR spectrum of turkesterone, the signal of the proton at C_{11} appears in the form of a broad multiplet at 4.45 ppm, and the proton at C_9 resonates in the 3.75-ppm region. In the NMR spectrum of the $\Delta^{7,9(11)}$-dien-6-one (V), these signals are absent, while the protons of the unsaturated ketone grouping appear in the form of two weak-field signals at 6.03 and 6.25 ppm.

The good agreement of the values of the chemical shifts (CSs) of the methyl groups at C_{19} and C_{18} of turkesterone and ajugasterone C [4] shows quite definitely that the hydroxy groups in the tetracyclic part of the new ecdysone are located in the 2β, 3β, 11α, and 14α positions (Table 1). This is confirmed by the results of a comparison of the CSs of the angular methyl groups of the tetraacetates of the phytoecdysones mentioned. From the positions of the CSs of the C_{26} and C_{27} methyls the side chain corresponds to the 25-hydroxyecdysones.

Thus, the facts given above lead to the conclusion that turkesterone is 11α,20R-dihydroxyecdysone.

EXPERIMENTAL METHOD

The IR spectra were obtained on a UR-20 spectrophotometer (KBr) and the NMR spectra on a J M-4H-100 MHz instrument with HMDS as internal standard (δ-scale). Abbreviations: s) singlet; d) doublet; m) multiplet; br. s) broadened singlet. The mass spectra were taken on an MKh-1303 instrument fitted with a system for the direct introduction of the substance into the ion source at an ionizing voltage of 40 V and a temperature of 140-170°C.

For chromatography we used type KSK silica gel treated in the usual way and alumina (activity grade IV). The phytoecdysones were revealed with vanillin/sulfuric acid.

Isolation of Turkesterone (I). The comminuted air-dry roots of *A. turkestanica* (6.0 kg) were extracted with methanol, the extract was concentrated to a volume of 1.0 liter and diluted with a double amount of water, and the hydrophobic compounds were extracted with petroleum ether. The aqueous methanolic fraction was repeatedly extracted with ethyl acetate. From the ethyl-acetate extract by chromatography on alumina and rechromatography on silica gels [eluents: chloroform–methanol (9:1) and (4:1)], in addition to cyasterone (595 mg) and ecdysterone (2.9 g), we isolated 3.12 g (0.052%) of amorphous turkesterone (I), $C_{27}H_{44}O_8$, $[\alpha]_D^{20}$ +52,0° (c 1.46; methanol). $\lambda_{max}^{C_2H_5OH}$ 244 nm lgε (3,95), ν_{max}^{KBr} 3300-3500 (OH), 1660 cm^{-1} (Δ^7-6-keto grouping). Mass spectrum: 460, 442, 424, 409, 379, 361, 343, 325, 300, 143, 125, 99, 81, 69. NMR spectrum (C_5D_5N): 1.12 (3H at C_{18}, s); 1.18 (3H at C_{19}, s); 1.24 (6H at C_{26} and C_{27}, s); 1,45 (3H at C_{21}, s); 6.12 (H at C_7, br. s); 4.45 (2H at C_2 and C_{11}, m); 4.06 (H at C_3, m); 3.75 (2H at C_9 and C_{22}, m).

Turkesterone 2,3,11,22-Tetraacetate (II) and 2,3,11,22,25-Pentaacetate (III). The acetylation of 526 mg of turkesterone (I) was performed in 12 ml of pyridine with 10 ml of acetic anhydride at room temperature for 3 days. After the solvent had been eliminated in vacuum, the residue was chromatographed on a columm of alumina. Elution with chloroform yielded with 108 mg of the amorphous pentaacetate (III), $C_{37}H_{54}O_{13}$, ν_{max}^{KBr} 3450-3500 (OH), 1740, 1250 (ester), 1670 cm^{-1} (Δ^7-6-keto grouping). NMR spectrum ($CDCl_3$): 0.84 (3H at C_{18}, s); 1.07 (3H at C_{19}, s); 1.22 (3H at C_{21}, s); 1.38 (6H at C_{26}, C_{27}, s); 3.40 (H at C_9, m); 4.75 (H at C_{22}, m); 5.08-5.40 (3H at C_2, C_3, C_{11}, m); 5.86 (H at C_7, br. s). Mass spectrum: 610, 586, 568, 550, 508, 493, 490, 445, 427, 388, 373, 345, 332, 230, 81, 69 m/e.

Further elution of the columm with chloroform–ethanol (19:1) yielded 387 mg of the amorphous tetraacetate (II) $C_{35}H_{52}O_{12}$, $[\alpha]_D^{20}$ +81,7° (c 1,86; methanol); ν_{max}^{KBr} 3400–3500 (OH), 1740, 1250 (ester), 1670 cm^{-1} (Δ^7-6-keto grouping). NMR spectrum ($CDCl_3$): 0.85 (3H at C_{18}, s); 105 (3H at C_{19}, s); 1.13 and 1.18 (6H at C_{26},C_{27}, s); 1.21 (3H at C_{21}, s); 3.35 (H at C_9, m); 4.76 (H at C_{22}, m); 5.05-545 (3H at C_2, C_3, and C_{11}, m); 5.85 (H at C_7, br. s). Mass spectrum: 628, 586, 568, 550, 526, 508, 493, 490, 445, 427, 388, 385, 373, 367, 345, 343, 332, 325, 307, 309, 230, 152, 99, 81, 69 m/e.

Turkesterone 2,3:20,22-Diacetonide (IV). To a supension of 500 mg of turkesterone (I) in 125 ml of dry acetone was added 17 mg of phosphomolybdic acid, and the reaction mixture was stirred at room temperature until the turkesterone had dissolved completely. After evaporation in vacuum to small volume, the residue was diluted with water and was neutralized with sodium bicarbonate. The neutral solution was extracted with ether, and the extract was chromotographed on a column of silica gel. Elution with a mixture of chloroform and methanol (19:1) gave 314 mg of amorphous turkesterone diacetonide (IV), $C_{33}H_{52}O_8$ ν_{max}^{KBr}: 3350–3550 (OH), 1670 cm^{-1} (Δ^7-6-keto grouping). Mass spectrum: M^+ 576, 561, 558, 543, 525, 500, 482, 467, 464, 442, 424, 419, 401, 384, 383, 366, 340, 339, 201, 143, 125, 102, 99, 81 m/e.

Anhydroturkesterone (V). The tetraacetate (II) (415 mg) was hydrolyzed with a solution of potassium bicarbonate in an atmosphere of nitrogen at room temperature for 3 h. Then the reaction mixture was diluted with water to 60 ml and was exhaustively extracted with butanol. After the butanol had been distilled off, the residue was chromatographed on a column of silica gel. Elution with a mixture of chloroform and methanol (4:1) gave 253 mg of anhydroturkesterone with mp 256-257°C (pyridine), $[\alpha]_D^{20}$ + 36.0° c 1.70 (methanol)

$\lambda_{max}^{C_2H_5OH}$ 301 nm (lgε 3.94); ν_{max}^{KBr} 3300–3500 (OH), 1655, 1615 cm^{-1} ($\Delta^{7,9(11)}$-6-keto grouping).

NMR spetrum (C_5D_5N): 1.10 (3H at C_{18}, s) ; 1.24 (6H at C_{26} and C_{27}, s); 1.45 (3H at C_{21}, s); 3.75 (H at C_{22}, m), 4.04 (2H at C_2 and C_3, m); 6.03 (H at C_7, br. s.); 6.25 (h at C_{11},m).

Mass spectrum: M^+ 478, 460, 442, 424, 409, 361, 343, 325, 300, 143, 125, 99, 81, 69 m/e.

SUMMARY

From the roots of *Ajuga turkestanica* (Rgl.) Briq. family Labiatae a new phytoecdysone — turkesterone — has been isolated. It has been shown that it is 11α20R-dihydroxyecdysone.

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