316. PMR: 8.12 (1H, br.s, OH), 5.62 (1H, s, H-19), 4.4 (2H, m, CH_2-11), 2.46 (2H, dd, J = 14 Hz, CH_2-15), 1.05 (3H, s, CH_3-12), 0.98 (3H, d, J = 6 Hz, CH_3-13), 0.84 (3H, s, CH_3-14), 0.79 (1H, dd, J = 11.5 Hz, J = 2 Hz, H-10), 2.35-0.85 (11H, m). The ^{13}C NMR spectrum is given in Table 1.

Synthesis of Sesquiterpenylbutylaminohydroxyquinone (VIII). A solution of 63 mg of (V) in 100 ml of 50% aqueous ethanol was treated with 0.1 ml of n-butylamine. After 24 h the alcohol was evaporated off in vacuum, the aqueous residue was extracted with $CHCl_3$, and the solvent was evaporated off. Yield 90%. After crystallization from $CHCl_3$, red crystals were obtained with mp 141-143°C. Mass spectrum, m/z (%): 399 (M^+, 5), 209 (100), 191 (5), 180 (25), 166 (12), 152 (20). UV spectrum (λ_{max}^{MeOH}, nm): 210, 322, 500. PMR: 8.45 (1H, br.s, OH), 6.43 (1H, m, NH), 5.39 (1H, s, H-19), 4.45 (2H, m, CH_2-11), 3.16 (2H, m, CH_2-22), 2.45 (2H, dd, J = 14 Hz, CH_2-15), 1.05 (3H, s, CH_3-12), 0.96 (3H, d, J = 6 Hz, CH_3-13), 0.83 (3H, s, CH_3-14), 0.79 (1H, dd, J = 11.5 Hz, J = 2 Hz, H-10), 0.85 (3H, t, CH_3-25), 2.4-0.9 (11H, m).

Synthesis of 17-Hydroxy-20-methylaminoavarone (IX). A solution of 20 mg of (I) in 40 ml of 50% aqueous ethanol was treated with 100 mg of $CH_3NH_2 \cdot HCl$ and 0.5 ml of pyridine. After 20 h, the alcohol was driven off in vacuum and the aqueous residue was extracted with $CHCl_3$. The solvent was eliminated and the dry residue was chromatographed on LH-20 in $CHCl_3$. This gave 15 mg of (IX). Crystallization from aqueous methanol gave red crystals with mp 215-217°C. Mass spectrum, m/z (%): 357 (M^+, 5), 191 (20), 167 (100). UV spectrum (λ_{max}^{MeOH}, nm): 221, 294, 322, 500. PMR: 8.4 (1H, br.s, OH), 6.45 (1H, m, NH), 5.38 (1H, s, H-19), 5.14 (1H, br.s, H-3), 2.92 (3H, d, J = 5 Hz, N—CH_3), 2.56 (2H, dd, J = 14 Hz, CH_2-15), 1.54 (3H, d, J = 2 Hz, CH_3-11), 1.00 (3H, s, CH_3-14), 0.97 (3H, d, J = 6.1 Hz, CH_3-13), 0.83 (3H, s, CH_3-12), 2.1-0.95 (9H, m).

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ECDYSTEROIDS OF A CULTURE OF TISSUES AND CELLS OF *Ajuga turkestanica*

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The possibility has been studied of obtaining ecdysteroids — ecdysterone and turkesterone — with the aid of a culture of the tissues and cells of the plant Ajuga turkestanica. *Conditions have been selected under which the yield of ecdysteroids in the culture of tissues and cells is comparable with amounts of the same substances in the organs of the whole plant.*

It is known that ecdysterone has been isolated from the leaves from *Ajuga turkestanica* (Rgl.) Brig (family Labiatae) while in the roots, in addition to the ecdysteroid mentioned, turkesterone (11α-hydroxyecdysterone) has been detected [1].

We have considered the possibility of producing ecdysteroids with the aid of a culture of the tissues and cells of this plant. To induces callusogenesis and the growth of the cell culture we investigated several modifications of nutrient media. The variants that proved to be the best are given in the Experimental section.

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TABLE 1. Comparative Amounts of Ecdysterone and Turkesterone in the Plant Raw Material, Callus Tissue, and a Cell Culture of *A. turkestanica*

Raw material	Ecdysterone	Turkesterone
	% on the weight of the air-dry raw material	
Leaves [1]	0,020	—
Roots [1]	0,045	0,052
Callus tissue:		
experiment 1	0,100	0 032
experiment 2	0,110	0,033
Cell culture:		
experiment 1	0,120	0,035
experiment 2	0,120	0,036

In the course of the growth cycle (35-40 days) the callus tissue of *A. turkestanica* increased its crude weight 5-7 times. The growth cycle of the suspension culture amounted to 14 days. In this case, the weight of the culture increased 10-12 times. Analysis of extracts from the biomass of the callus and cell cultures carried out by the TLC method showed the presence of two ecdysteroids which, after isolation, were identified from their constants as ecdysterone and turkesterone (Table 1). It can be seen from Table 1 that several times more ecdysterone is synthesized in the callus tissue and in a culture of the cells of *A. turkestanica* than in the epigeal part and roots of the plant. The amounts of turkesterone in the roots of a plant growing under natural conditions, in the callus tissue, and in the cell culture were fully comparable.

EXPERIMENTAL

Alumina (activity grade IV) was used for column chromatography.

Conditions for the Cultivation of *A. turkestanica*. Callus tissue was grown from the ovary of a mature plant. The tissue was cultivated on Murashige–Skoog medium [2] with the addition of α-naphthylacetic acid (NAA) (1 mg/liter) and thidiazuron (0.002 mg/liter).

A suspension culture of cells was obtained by transferring callus tissue to a liquid nutrient medium: half the amount of mineral salts for Murashige–Skoog medium with the addition of NAA (1 mg/liter) and thidiazuron (0.0002 mg/liter). The culture was grown on a shaking machine with an intensity of 100 cycles/min. Callus formation and the multiplication of the cells was carried out at a temperature of 26 ± 2°C with an illumination of 5000-7000 lux during a 16-h photoperiod, while the cell culture was grown in the dark. The callus tissue was transplanted every 4 weeks, and the suspension culture every 14 days. To perform chemical analysis, samples were taken on the 40th-45th day and 17th-20th day, respectively. The experiments were repeated.

Isolation of the Ecdysteroids. The comminuted dry biomass of the callus tissue (40 g) was extracted at 70°C with 500 ml of methanol for 4 h. The extraction was repeated five times. After concentration, the extract was diluted twofold with water and the precipitate that had deposited, which contained no ecdysteroids, was removed. The ethanol was evaporated and the aqueous residue was extracted several times with hexane. The ecdysteroids were extracted exhaustively with butanol from the purified aqueous solution. The solvent was distilled off and the total ecdysteroids obtained were chromatographed on a column of alumina. The column was eluted with chloroform–methanol (9:1). This gave 43 mg of a compound with mp 242-244°C (from acetone–methanol), which was identified from its R_f value and its spectral characteristics as ecdysterone. The further washing of the column with the same solvent system led to the isolation of 13 mg of turkesterone, shown to be identical with an authentic sample by the magnitude of its migration in TLC [chloroform–methanol (4:1) system] and its spectral indices.

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