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ISOLATION OF TURKESTERONE FROM THE EPIGEAL PART OF *Ajuga turkestanica* AND ITS ANABOLIC ACTIVITY

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The technological isolation of turkesterone from the epigeal part of Ajuga turkestanica *(Rgl.) Brig. is described, a method is proposed for the quantitative determination of turkesterone, and the results of an investigation of its biological activity are given.*

The perennial plant *Ajuga turkestanica* (Rgl.) Brig. growing in Uzbekistan and Tadzhikistan is a rich source of biologically active substances [1].

B. Z. Usmanov et al. [2] have made a detailed study of the ecdysteroids of *A. turkestanica* roots. Together with known ecdysteroids, they isolated a new compound of this class — turkesterone [3-5] (with a yield of 0.052% on the weight of the raw material), possessing a pronounced capacity for activating the biosynthesis of protein in the animal organism [6].

We have investigated the isolation of turkesterone from the epigeal part of *A. turkestanica* gathered in the Surkhandar'inskaya Oblast, Uzbekistan, and have determined its biological activity. To select the best reagent we studied the extraction of the raw material with alcohol in various concentrations and with methanol:

	Extractant	Yield, % on the weight of raw material
Ethyl alcohol	60%	0.05
	70%	0.12
	80%	0.14
	90%	0.07
	95%	0.05
Methyl alcohol		0.14

The best results were obtained when turkesterone was extracted from the raw material with 80% ethanol and with methanol.

We studied the extraction of turkesterone in time (Fig. 1). Analysis was conducted by a chromatospectrophotometric method [7] in a Soxhlet apparatus [sic], and the chromatographic separation of turkesterone from accompanying substances on plates with a fixed layer of silica gel of type L5/40 μ in the solvent system chloroform–methanol–acetone (6:2:1), with determination of the optical densities of the eluates at λ 241 nm. Four hours was necessary to achieve the equilibrium concentration at first contact of the phases, while phase equilibrium was reached at second contact after 3 h and at the third and fourth contacts after 2 h (see Fig. 1). The extraction curves were typical isotherms tending to equilibrium. With a decrease in the amount of extractive substances in the raw material the relative rate of extraction rose.

In addition to turkesterone, the extract contained other ecdysteroids, and also iridoids, carbohydrates, tanning substances, and other impurities. Accompanying substances of hydrophobic nature were eliminated by treatment with chloroform. When an aqueous solution was extracted with chloroform five times, 3.4% (of the weight of the raw material) of accompanying substances of hydrophobic nature was eliminated.

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Turkesterone was extracted from the aqueous solution with butanol, the extraction dynamics being given below:

Number of extractions	Amount of turkesterone in each extract, % of the amount in the aqueous solution
1	30
2	35
3	20
4	8
5	2.5
Total:	95.5

As can be seen, five extractions with butanol are sufficient for the exhaustive extraction of turkesterone from aqueous solution. Besides turkesterone, the concentrated butanolic extract contained iridoids. The latter were eliminated by precipitation from a butanol–acetone mixture. Turkesterone was obtained from the filtrate by chromatography on a column of alumina. The best result was achieved on elution with chloroform–methanol (4:1) at a ratio of total material to sorbent of from 1:15 to 1:20.

The results of the investigation showed that turkesterone isolated both from the epigeal part and from the roots of *A. turkestanica* possessed a pronounced anabolic action on the organism of experimental animals (Tables 1 and 2). Thus, the turkesterone from the roots (below — sample I) stimulated an increase in the body weight of the animals by a factor of 1.77, while turkesterone from the epigeal part (below — sample II) stimulated an increase in body weight by a factor of 1.74 (in comparison with a control).

The changes in the weights of the organs and their total protein contents, which are characteristic for drugs with an anabolic type of action, were also fairly close in groups of animals that had received samples I and II, respectively.

Under the influence of sample I, the weights of the liver and kidneys increased by 27.8 and 31.3%, while for sample II the corresponding figures were 25.2 and 33.8%. The weight of the heart and of the tibialis anterior muscle rose by 25.2 and 30.9% in the first case and by 24.2 and 28.5% in the second case. On the administration of sample I to rats, the total protein content rose in the liver, kidneys, heart, and muscle tissue by 32.4, 42.1, 34.7, and 33.7%, and in the case of sample II by 28.2, 44.9, 30.4, and 29.8%, respectively (its percentage content in the organs and tissues of the experimental animals did not change in comparison with the control).

As can be seen from the figures given in Tables 1 and 2, the differences between the values of any index investigated in the experimental groups were small and statistically insignificant.

However, we may note that the turkesterone from the epigeal part of this bugle (as also from the roots) was not inferior in anabolic effect to nerobol (methandrostenolone) — a drug from the steranobol series (see Tables 1 and 2).

Consequently, turkesterone isolated from the epigeal part of *Ajuga turkestanica*, may be of considerable interest both in the economic and in the biological respect for the creation of a metabolically active drug that could be used successfully in practical medicine. An advantage of turkesterone over the steroidal anabolic drugs that are widely used in clinical medicine [8] consists in the complete absence of the specific androgenic effect that limits the use of such drugs, particularly in relation to children and women [9]. As can be seen from Table 3, in experiments on castrated sexually immature male rats turkesterone, unlike nerobol, did not exert a stimulating influence on the weight of the ventral prostate and the seminal vesicles. The administration of nerobol to the animals led to increases in the weights of these organs by factors of 1.92 and 2.1, respectively.

EXPERIMENTAL

Choice of Solvent for the Extraction of Turkesterone. Comparative extraction was conducted under identical conditions of fivefold treatment of 1-kg samples of *Ajuga turkestanica* with the solvents under investigation.

Kinetics of the Extraction Process. To establish phase equilibrium on the first contact of the phases, 1-kg batches of comminuted raw material were charged into six 10-liter extractors, and 80% ethyl alcohol was used as the extractant. In the first extractor the time of extraction was 1 h; in the second, 2 h; in the third, 3 h; in the fourth, 4 h; in the fifth, 5 h; and in the sixth, 6 h. After the passage of the given times, the extracts were decanted off and their turkesterone contents were determined quantitatively.

TABLE 1. Influence of the Substances Investigated on the Increase in Body Weight of Intact Sexually Immature Rats ($M \pm m$, n = 6-8)

Nature of the experiment	Increase in body weight, mg/g/day	P	Effect, %
Control	14.8±2.4		–
Turkesterone (sample I)	26.2±3.8	<0.05	77.0
Turkesterone (sample II)	25.8±3.2	<0.02	74.3
Nerobol (methandrostenolone)	26.8±2.8	<0.01	81.1

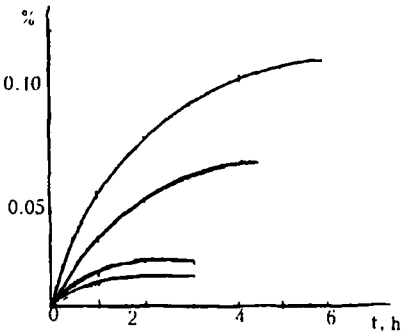

Fig. 1. Kinetics of the extraction of turkesterone.

To establish phase equilibrium at the second phase contact, 1-kg batches of raw material were extracted in four extractors for 4 h. The extracts were decanted off, and fresh portions of solvent were added. The extract was decanted off from the first extractor after 1 h, from the second after 2 h, from the third after 3 h, and from the fourth after 4 h, and the turkesterone contents were determined quantitatively. Depending on the concentration of turkesterone in the sample to be analyzed, for each case the amount of solution deposited on a 20 × 20 cm glass plate with a fixed layer of type L5/40μ silica gel was selected in such a way that the level of turkesterone was within the limits of sensitive determination.

The plate with the deposited samples was dried in the air for an hour and was placed in a chamber with the solvent mixture chloroform–methanol–acetone (6:2:1) and chromatographed by the ascending method.

Turkesterone was detected with a 1% solution of vanillin in concentrated sulfuric acid. The zones containing turkesterone were transferred quantitatively into flasks with ground-in stoppers, 10 ml of methanol was added to each, and elution was carried on with continuous shaking for 4 h. The eluates were filtered off through a pore 16 Schott funnel and their optical densities were determined at λ 241 nm in a cell with a layer thickness of 10 mm.

The optical density of a solution of GSO ékdisten was measured in parallel. The turkesterone content (X, %) was calculated from the formula

$$X = \frac{D_x \cdot C_o \cdot V_1 \cdot V_3 \cdot 100 \cdot 100}{D_o \cdot m \cdot V_2 \cdot (100 - W)},$$

where D_x is the optical density of the eluate of the experimental solution;

D_0 is the optical density of the GSO ékdisten eluate;

C_0 is the concentration of the GSO ékdisten solution;

m is the weight of the preparation, g;

V_1 is the volume of the experimental solution, ml;

V_2 is the volume of the experimental solution deposited on the chromatogram:

V_3 is the volume of the eluate of the experimental solution, ml: and

W is the loss in weight on drying.

To determine the time of extraction for the third phase contact, 1-kg batches of raw material were extracted for 4 h, the extract was decanted off, fresh solvent was added, and the raw material was extracted for another 3 h, after which the time necessary to reach phase equilibrium was determined. The time of extraction for the fourth phase contact was determined similarly.

TABLE 2. Influence of the Substances Investigated on the Weights of Organs and on their Protein Contents in Intact Sexually Immature Rats ($M \pm m$, n = 6-8)

Organ	Nature of the experiment	Weight of the organ, mg	Protein content of the organ	
			%	mg
Liver	Control	4586± 268	17.2±0.38	788±46.0
	Turkesterone, sample I	5862±218*	17.8±0.40	1043±62.4*
	Turkesterone, sample II	5744±224*	17.6±0.36	1010±58.8*
	Nerobol	5698±216*	17.6±0.38	1002±42.8*
Heart	Control	388±16.0	16.2±0.40	62.8±3.8
	Turkesterone, sample I	482±12.0*	17.4±0.62	84.6±5.6*
	Turkesterone, sample II	486±12.8*	17.0±0.64	81.9±4.2*
	Nerobol	490±14.6*	17.6±0.56	86.2±5.8*
Kidneys	Control	396±13.4	15.9±0.70	62.9±3.8
	Turkesterone, sample I	520±24.8*	17.2±0.88	89.4±4.8*
	Turkesterone, sample II	530±26.0*	17.2±0.82	91.2±5.2*
	Nerobol	544±25.8*	17.4±0.86	94.6±6.0*
Tibialis anterior muscle	Control	168±8.8	17.8±0.14	29.9±1.2
	Turkesterone, sample I	220±10.2*	18.2±0.78	40.0±2.6
	Turkesterone, sample II	216±9.2*	18.0±0.80	38.8±2.8*
	Nerobol	228±10.4*	18.6±0.82	42.4±3.2*

*Significant at P 0.05.

TABLE 3. Influence of the Substances Investigated on the Weight of the Ventral Prostate and the Seminal Vesicles of Castrated Sexually Immature Rats ($M \pm m$, n = 10)

Nature of the experiment	Weight of the ventral prostate, mg	P	Weight of the seminal vesicles, mg	P
Control	8.6±0.32	-	9.6±0.48	-
Turkesterone (sample I)	9.2±0.48	<0.5	10.2±0.38	<0.5
Turkesterone (sample II)	9.0±0.38	<0.5	10.0±0.50	<0.5
Nerobol	16.5±0.95	<0.001	20.4±0.80	<0.001

Dynamics of Extraction of Turkesterone from Aqueous Solution. A dried chloroform-treated extract of 1 kg of *Ajuga turkestanica* was extracted with butanol (5 × 300 ml). The turkesterone content of each extract was determined by the method described above.

The anabolic activity of turkesterone was studied on intact sexually immature male rats weighing 60-80 g, using the procedure described by N. A. Yuldaev and B. V. Pokrovskii [1]. The drug was administered to the animals orally (through special sound into the stomach) in a dose of 5 mg/kg. For comparison we used the known anabolic agent nerobol (methandrostenolone), which was administered to the animals similarly in a dose of 10 mg/kg. Ten days after the beginning of the administration of the substances, each rat was decapitated under light ether narcosis (after it had been weighed), and the heart, liver, kidneys, and tibialis anterior muscle were dissected out and weighed. In addition, the protein contents of all the organs were determined by the method of O. Lowry et al. [9].

To investigate a possible androgenic influence of turkesterone and nerobol we used a procedure described by L. Hershberger et al. [12]. For this, turkesterone and nerobol in doses of 5 and 10 mg/kg, respectively, were administered to castrated sexually immature rats weighing 50-55 g. The substances were administered orally for 7 days. Then the animals were decapitated, and, on a torsion balance, we determined the weights of their seminal vesicles and ventral prostates, which are

the target organs for substances with an androgenic activity [13]. The numerical material obtained in the course of the experiments was treated statistically as described by M. L. Belenkii (1969).

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