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DISTRIBUTION OF PHYTOECDYSTEROIDS IN PLANTS OF UZBEKISTAN AND THE POSSIBILITY OF USING DRUGS BASED ON THEM IN NEUROLOGICAL PRACTICE

Z. Saatov,[a] D. A. Agzamkhodzhaeva,[b] and V. N. Syrov[a]

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The distribution of phytoecdysteroids in plants of the families Labiatae, Compositae, and Caryophyllaceae growing in Uzbekistan has been analyzed. It has been shown that some of them possess a capacity for lowering the levels of urea and residual nitrogen in the blood and for improving the functional state of the kidneys in various pathological states. The dependence of biological activity on the structure of the compounds is discussed. The possibility of broadening the indications for the use of the drug ékdisten, the first to have been created from compounds of this class, in complications affecting the eyes of patients suffering from chronic glomerulonephritis has been substantiated experimentally and clinically.

The isolation of phytoecdysteroids from locally grown raw material has been reported previously [1—3]. These substances have proved to be extremely interesting because of their capacity for enhancing the biosynthesis of protein in the organism of experimental animals in a similar way to the steranabols without at the same time exhibiting androgenic, thymolytic, and other side effects [4, 5]. Since there is a great demand for compounds with this type of action at the present time, we have made an analysis of the distribution and quantitative levels of phytoecdysteroids in plants of the Labiatae, Compositae, and Caryophyllaceae families growing on the territory of Uzbekistan among which the finding of ecdysteroid-containing specimens was most likely [6]. In addition to the properties of phytoecdysteroids discovered previously [5], we have studied the influence of those found most frequently on the nitrogen metabolism. The latter is closely connected with the protein metabolism, and in the case of favorable results in this direction (for example, the detection of hypoazotemic activity in phytoecdysteroids) the possibility of the practical use of these compounds would appear. This is all the more the case since the drug ékdisten based on ecdysterone has been approved by the Pharmaceutical Committee for medical use as a general tonic [5], while a total preparation based on the phytoecdysteroids of *Ajuga turkestanica* has been passed for clinical trial [7]. It is important to note that the good hypoazotemic activity of synthetic testosterone derivatives has not found wide use because of their specific hormonal action on the organism [8].

Among plants of the Labiatae family we investigated the epigeal and hypogeal organs of *Ajuga turkestanica*. According to the world literature, many representatives of the genus *Ajuga* contain compounds with the activity of the hormones of insect molting and metamorphosis [4]. From the species investigated we isolated eight substances with the chemical structure of ecdysteroids. A considerable number of ecdysteroids were also detected in plants of the genera *Rhaponticum* and *Serratula*, which belong to the Compositae family. However, we detected the largest number of ecdysteroids in representatives of the family Caryophyllaceae found in Uzbekistan. Incidentally, it was just in this family that we also detected the largest number of ecdysteroid-containing species. The ecdysteroids isolated from plants of this family are distinguished favorably by both their quantitative and their qualitative compositions. The ones found most frequently were ecdysterone, α-ecdysone, 2-deoxyecdysterone, and 2-deoxy-α-ecdysone. Also characteristic was the presence of glycosides of ecdysterone, of integristerone A, and of viticosterone E. In addition, we detected ecdysteroid esters: benzoates, acetates, and sulfates. All these results are shown in Table 1.

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a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (371) 120 64 75; b) Second Tashkent State Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 209—215, March-April, 1999. Original article submitted December 21, 1998.

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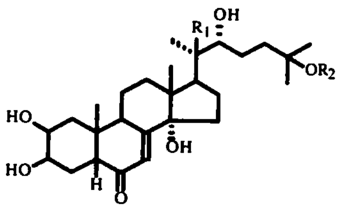

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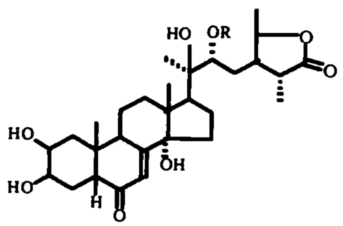

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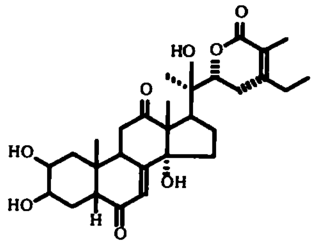

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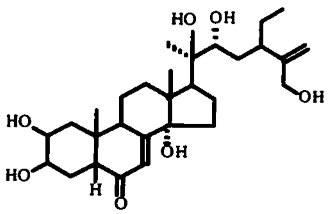

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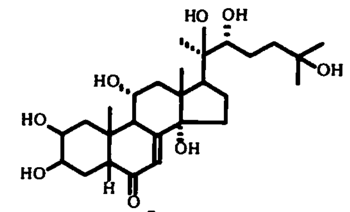

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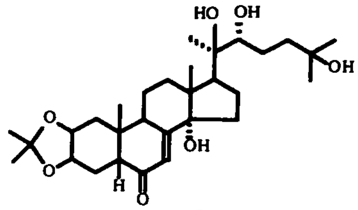

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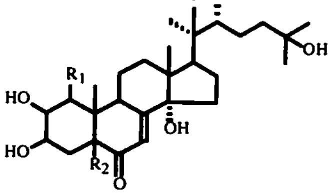

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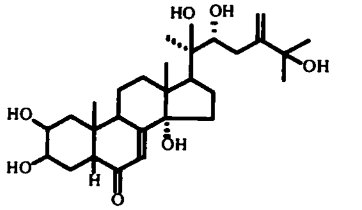

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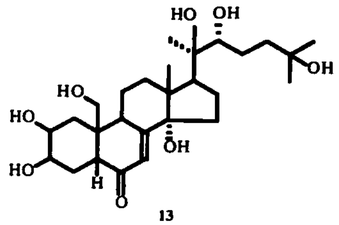

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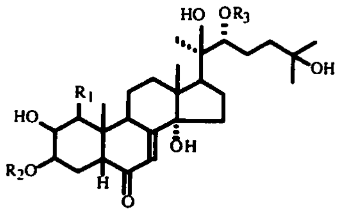

15. R_1=R_2=H; R_3=α-*D*-Gal
16. R_1=R_3=H; R_2=α-*D*-Gal
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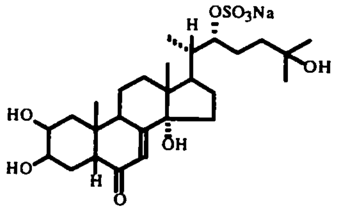
18. R_1=OH; R_2=H; R_3=α-*D*-Gal



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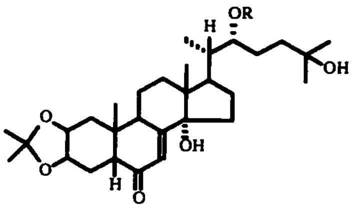

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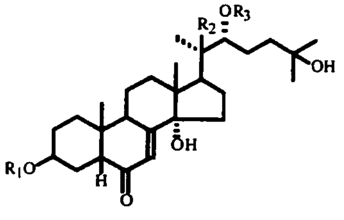
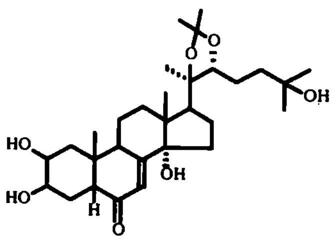


20. R_1=R_2=R_3=H
22. R_1=Ac; R_2=R_3=H;
28. R_1=R_2=H; R_3=COC_6H_5
29. R_1=R_3=H; R_2=OH



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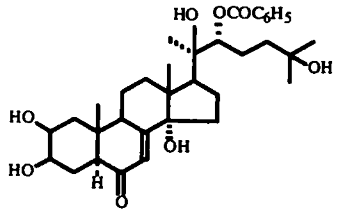

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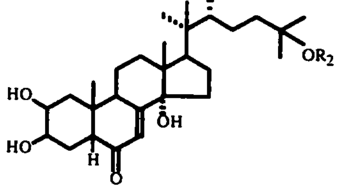
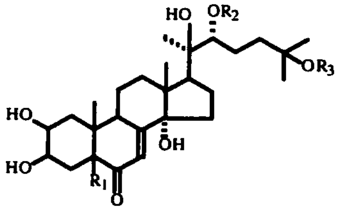


24. R_1=COC_6H_5; R_2=H
25. R_1=R_2=COC_6H_5
27. R_1=COC_6H_5; R_2=Ac



30. R_1=OH; R_2=Ac; R_3=H
31. R_1=R_3=H; R_2=Ac
32. R_1=H; R_2=α-*D*-Gal; R_3=Ac

TABLE 1. Distribution of Ecdysteroids in Plants of the Families Labiatae, Compositae, and Caryophyllaceae

Family, species, and ecdysteroids	Plant organ*	Content, % on the weight of the dry raw material
Labiatae		
Ajuga turkestanica Regel. [4]		
Ecdysterone (**1**)	e.p.	0.020
Ecdysterone	r.	0.045
Cyasterone (**2**)	e.p.	0.025
Cyasterone	r.	0.010
Ajugalactone (**4**)	e.p.	0.001
Ajugalactone	r.	0.001
Ajugasterone B (**5**)	e.p.	0.002
Ajugasterone B	r.	0.003
22-Acetylcyasterone (**3**)	e.p.	0.050
22-Acetylcyasterone	e.p.	0.12
α-Ecdysone (**6**)	e.p.	0.04
Ecdysterone 2,3-monoacetonide (**8**)	e.p.	0.08
Turkesterone (**7**)	e.p.	0.17
Turkesterone	r.	0.043
Compositae		
Rhaponticum integrifolium C. Winkl. [6]		
Ecdysterone	i.	0.23
Integristerone A (**9**)	i.	0.013
Integristerone B (**10**)	i.	0.0003
24(28)-Dehydromakisterone A (**11**)	i.	0.0002
Rhaponticum nanum Lipsky		
Ecdysterone	e.p.	0.007
Integristerone A	e.p.	0.0025
Serratula sogdiana Bunge		
Ecdysterone	i.	0.520
Ecdysterone	l.	0.170
Viticosterone E (**12**)	i.	0.0014
Viticosterone E	l.	0.027
Sogdysterone (**13**)	i.	0.003
Serratula algida Iljin		
Ecdysterone	i.	TLC
Caryophyllaceae		
Silene brahuica Boiss. [9]		
Viticosterone E	e.p.	0.012
Polypodine B (**14**)	e.p.	0.0020
Ecdysterone	e.p.	0.034
Ecdysterone	r.	0.094
Integristerone A	e.p.	0.002
Integristerone A	r.	0.004
Sileneoside A (**15**)	r.	0.020
Sileneoside D (**16**)	r.	0.0013
Sileneoside B (**17**)	r.	0.0045
Sileneoside C (**18**)	r.	0.0032
α-Ecdysone 22-sulfate (**19**)	r.	0.001
Silene scabrifolia Kom.		
Ecdysterone	e.p.	0.046
2-Deoxy-α-ecdysone (**20**)	e.p.	0.20
Ecdysterone 22-O-benzoate 2,3-monoacetonide (**21**)	e.p.	0.003
2-Deoxy-α-ecdysone 3-acetate (**22**)	e.p.	0.0011
Ecdysterone 2,3-monoacetonide (**8**)	e.p.	0.0013
Ecdysterone 20,22-monoacetonide (**23**)	e.p.	0.0026
Ecdysterone 22-O-benzoate (**24**)	e.p.	0.016
Ecdysterone 22,25-di-O-benzoate (**25**)	e.p.	0.0008

TABLE 1. (Continued)

Family, species, and ecdysteroids	Plant organ*	Content, % on the weight of the dry raw material
5α-Ecdysterone 22-O-benzoate (**26**)	e.p.	-
Silene wallichiana Klotzch		
Viticosterone E 22-O-benzoate (**27**)	e.p.	0.0012
2-Deoxy-α-ecdysone 22-O-benzoate (**28**)	e.p.	0.0010
Viticosterone E	e.p.	0.006
2-Deoxy-α-ecdysone	e.p.	0.071
2-Deoxyecdysterone (**29**)	e.p.	0.040
Ecdysterone 22-O-benzoate	e.p.	0.033
Ecdysterone	e.p.	0.046
Meandrium turkestanicum (Rgl). Vve d [10, 11]		
Polypodin B 22-acetate (**30**)	e.p.	0.0004
Ecdysterone 22-acetate (**31**)	e.p.	0.00071
Polypodine B	e.p.	0.00017
Ecdysterone	e.p.	0.085
Integristerone A	e.p.	0.005
Melandrioside A (**32**)	e.p.	0.00071
Sileneoside A	e.p.	0.0028
Sileneoside D	e.p.	0.0017
Dianthus hoeltreri Winkl. [12]		
Viticosterone E	e.p.	0.035
Polypodine B	e.p.	0.052
Ecdysterone	e.p.	0.3

* e.p. — epigeal part; r. — root; i. — inflorescence; l. — leaves.

TABLE 2. Results of an Investigation of the Hypoazotemic Activity of Phytoecdysteroids

Experimental conditions	Urea	Residual nitrogen	Experimental conditions	Urea	Residual nitrogen
	change, % on the initial level			change, % on the initial level	
Ecdysterone	-22*	-26*	2-Deoxy-α-ecdysone 3-acetate	-4	-6
Viticosterone E	-18*	-20*	Sileneoside A	-14*	-28*
Ecdysterone 2,3-monoacetonide	-5	-6	Sileneoside C	-13	-15
Ecdysterone 22-O-benzoate	-10	-12	Cyasterone	-26*	-30*
Ecdysterone 22-O-benzoate 2,3-monoacetonide	-7	-9	22-Acetylcyasterone	-22*	-25*
2-Deoxyecdysterone	-14	-17	Turkesterone	-30*	-35*
α-Ecdysone	-8	-11	Polypodin B	-21*	-24*
2-Deoxy-α-ecdysone	-6	-7	Integristerone A	-11	-13

*Significant changes at $P < 0.05$. There were ten animals in each group.

An experimental study of the influence of a number of the phytoecdysteroids on the level in rat blood of the final products of the nitrogen metabolism showed the existence of appreciable hypoazotemic activity (Table 2). The least pronounced lowering of the levels of urea and residual nitrogen in the blood of the animals (with respect to the initial levels) 2 h after administration was observed under the action of ecdysterone 2,3-monoacetonide, ecdysterone 22-O-benzoate 2,3-monoacetonide, α-ecdysone, and 2-deoxy-α-ecdysone and its acetate (the effect amounted to only 4—11% at $P > 0.05$).

The most pronounced hypoazotemic effect was shown by cyasterone and turkesterone. Under their influence the level of urea in the blood fell by 20—30% and that of residual nitrogen by 30—35% ($P < 0.05$). The prolonged introduction of the phytoecdysteroids into the animal organism gave a clearer and constant hypoazotemic effect throughout the experiment. However, in both cases, as also in the study of anabolic activity [4, 13], a dependence of biological activity on the chemical structure of the compounds was traced fairly clearly. Thus, on comparing the figures of Table 1 and the structural formulas of the phytoecdysteroids given in the scheme, we can see that, with retention of the overall stereochemistry of the steroid nucleus their hypoazotemic activity depends mainly on the number and positions of the hydroxy groups in the molecule. Their conversion into acyl or isopropylidene groups is accompanied by a fall in the degree of expression of the corresponding effect. The glycosides of certain plants that were considered exhibited a somewhat higher activity than their aglycons.

The ability of phytoecdysteroids to lower the levels of urea and residual nitrogen in rat blood serum has served as a basis for studying their curative effect in some forms of renal pathology. Our investigations have shown that under the conditions of experimental nephritis evoked by a mixture consisting of equal amounts of a 0.1% solution of uranyl acetate and glycerol, these compounds not only considerably reduced the degree of expression of uremic intoxication but even improved a whole series of indices characterizing the functional state of the kidneys [14]. We completely confirmed these results in clinical studies using one of the compounds of this class (which subsequently acquired the drug name ékdisten). With nephrological patients, in addition their state of health being improved, anemia and proteinuria were decreased, the levels of urea and creatinine in the blood serum were lowered, and its protein composition was restored [15].

We have obtained similar results in the present investigation. At the same time, another important feature of the action of ékdisten under appropriate conditions was found. Thus, the administration of ékdisten to patients with chronic glomerulonephritis led to the optimization of the morphometric indices of the microcirculation of the bulboconjunctiva in the majority of patients examined. The frequency of vascular, intravascular and perivascular changes in the bulbar conjunctiva decreased. The frequency of finding twisting, irregularity, aneurism, and reticulation of the vessels, phenomena of stagnation of the venous networks and of zones of degeneration fell substantially. In addition to this, a distinct tendency to a normalization of the diameter of the arterioles, capillaries, and venules appeared. The most pronounced effect was observed in patients with the nephrotic form of the disease (Table 3).

Thus, the wide distribution of phytoecdysteroids among medicinal plants growing on the territory of Uzbekistan, the appreciable hypoazotemic activity of some of them, their ability to alleviate the course of chronic glomerulonephritis and to prevent the occurrence of ocular complications in this pathology show the necessity for further chemical and pharmacological investigations in this direction.

TABLE 3. Influence of Ékdisten on the State of the Microcirculation of the Bulboconjunctiva in Patients with Chronic Glomerulonephritis (CGN)

Experimental conditions (CGN)	Diameter of the vessels: arteriole	capillary	venule	Arteriolovenular coefficient
Mixed form	12.6±0.9 / 12.6±0.8	6.8±0.3 / 7.1±0.5	28.8±1.5 / 26.7±1.2	0.41±0.04 / 0.48±0.02
Nephrotic form	13.5±0.6 / 15.7±0.4*	6.9±0.4 / 8.3±0.3*	31.5±2.2 / 24.2±1.8*	0.46±0.02 / 0.64±0.05*
Hypertonic form	11.6±0.8 / 11.3±0.6	6.1±0.4 / 6.7±0.4	30.2±1.8 / 26.2±2.3	0.39±0.03 / 0.43±0.04
Control	16.3±0.6	9.1±0.3	23.6±1.6	0.7±0.09

*Numerator — before treatment with ékdisten; denominator — after treatment. Asterisk — significant at $P < 0.05$.

EXPERIMENTAL

General Procedure. To obtain the total ecdysteroids the plants were extracted with alcohol. The extract was concentrated and diluted with water. The hydrophobic impurities that deposited were removed. The alcohol was evaporated off in vacuum, and the aqueous residue was treated with hexane or chloroform. The ecdysteroids were extracted from the purified aqueous fraction first with ethyl acetate and then with butanol. After the ethyl acetate and butanol had been distilled off, the dry residue was chromatographed on a column of alumina or silica gel [1—3].

For the experimental pharmacological investigations we used male rats weighing 120—150 g. The phytoecdysteroids were administered through a special sound into the stomach in the form of an aqueous emulsion with apricot gum in a dose of 5 mg/kg. The efficacy of the preparations as hypoazotemic agents was judged from their ability to lower the levels of urea [16] and residual nitrogen [17] in the animals' blood serum both on single dose (after a lapse of 2 h) and on repeated (for 10 days) administration.

The experimental clinical investigations were conducted in the nephrological division of clinic No. 2 of Tashkent State Medical Institute. 20 women and 15 men were examined, their average age being 35.3±7.1 years. Depending on the form of the chronic glomerulonephritis, the patients were divided into the following groups: 14 with the mixed form, 10 with the nephrotic form, and 11 with the hypertonic form. A control group of 17 patients received the traditional treatment and an experimental group of 18 patients were given, in addition, 0.005-g ékdisten tablets three times a day for 10 days. Together with the usual clinical laboratory investigation of the patients that permits monitoring of the course of the pathological process (dynamics of the general state, determination of arterial pressure, and of hemoglobin, erythrocytes, protein, urea, and creatinine in the blood serum and the urine), our main attention was devoted to a study of the influence of ékdisten on the morphometric indices of the microcirculation of the capillaries of the bulbar conjunctiva, for which we used a TM-1 capillaroscope specially adapted for these purposes. The results of the investigation were recorded on a video tape recorder. All the numerical values obtained during the experiments were subjected to statistical treatment [18].

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