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Studying the Specific (Adaptogenic and Anabolic) Activity of the Supramolecular Complex of 20-Hydroxyecdysone Triacetate with β-Cyclodextrin

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Abstract

BACKGROUND: A supramolecular complex of ecdysterone triacetate with β-cyclodextrin (β-CD) was synthesized to obtain new potentially bioactive substances and study the effect of bulky pharmacophore-functional groups on the preservation of the specific activity of 20-hydroxyecdysone. The adaptogenic and anabolic activities of the resulting water-soluble inclusion complex were studied. It was established that with the introduction of 3 acyl-pharmacophore groups in the form of encapsulated and hydrophilic supramolecular forms to experimental animals at a dose of 10 mg/kg, the studied complex has anabolic and adaptogenic effects, including increased stress resistance, increased physical endurance, as well as slightly increased weight of the heart and gonads compared to the control.

AIM: Study of the specific activity of the supramolecular complex of ecdysterone 3-acetate with β-CD.

METHODS: During the experimental study of the adaptogenic and anabolic effects of the supramolecular complex of ecdysterone 3-acetate with β-CD, the "open field" and "free swimming" tests were used. Statistical processing of the results was carried out using the Statistica 8 software package. Intergroup differences were assessed using the non-parametric Mann–Whitney U-test. For unpaired related groups, the non-parametric Wilcoxon test was used.

CONCLUSIONS: It has been established that physical activity (swimming) simultaneously has a significant stressor effect on animals and is characterized by an increased supply of glucose into the blood and the breakdown of blood proteins. It was revealed that the supramolecular complex of ecdysterone 3-acetate with β-CD at a dose of 10 mg/kg has anabolic and adaptogenic effects, in particular increased stress resistance and physical endurance of animals, as well as a slightly increased weight of the heart and gonads compared to the control.

Introduction

At present, one of the urgent problems of medicine is the problem of human adaptation to the environment, which is associated with increased environmental and social pressure, an increase in the number of stress factors affecting a person at the present stage of society development. As a result, in economically developed countries, there is a significant depression of the protective and compensatory mechanisms of a person and an increase in diseases related to the so-called "diseases of civilization" [1], [2]. One of the ways to solve the problem of increasing human resistance to adverse environmental factors is the use of pharmacological agents-adaptogens, represented by agents of natural origin [3]. Adaptogens are stress response modifiers that increase the body's non-specific resistance to stress by increasing its ability to adapt and survive. Adaptogens show polyvalent positive effects against chronic inflammation, atherosclerosis, neurodegenerative cognitive impairment, metabolic disorders, cancer, and other diseases associated with aging. The current and potential use of adaptogens is mainly related to stress fatigue and cognitive function, mental illness, and behavioral disorders [4].

The arsenal of adaptogenic agents of natural origin is very limited; the needs of healthcare in such agents are satisfied only by 20–25% [5].

Athletes and bodybuilders have recognized for decades that the use of anabolic steroids can promote muscle growth and strength. Anabolic steroids are being considered for the treatment of cachexia associated with chronic diseases and to address the problem of muscle wasting in the elderly, but, nevertheless, their

effectiveness has yet to be demonstrated in terms of improving physical functions and quality of life [6].

Anabolic steroids perform a variety of functions in the human body. They contribute to the fixation of calcium in the bones and an increase in bone mass in osteoporosis. The effect of anabolic steroids on bone tissue is a dose-dependent increase in cell proliferation and an increase in the activity of alkaline phosphatase produced by osteoblasts.

Anabolic steroids stimulate the synthesis of intracellular enzymes, especially cytochromes, as a result of which the processes of tissue respiration and oxidative phosphorylation, the formation of adenosine triphosphate and creatine phosphate, which are necessary for many biochemical processes, are enhanced. Under the influence of anabolic steroids, the rate of glycogen synthesis increases, the action of insulin increases, and the level of glycemia decreases. Noteworthy is the ability of anabolic steroids to potentiate the action of endogenous somatotropin (growth hormone).

Anabolic steroids used in an attempt to enhance athletic performance have been a widespread practice in both amateur and professional sports. The rationale for such use (or abuse) of these agents is based upon the musculature-developing effects. The anabolic steroids by athletes are various synthetic derivatives of testosterone, substances with longer durations of action and/or greater potencies than the physiological androgens [7].

According to their origin, adaptogens can be divided into two groups: Natural and synthetic. The sources of natural adaptogens are terrestrial and aquatic plants, animals, and microorganisms [8].

Plant ecdysteroids have tonic, anabolic, adaptogenic, and other types of biological activity. One of the main reasons for the limited use of phytoecdysteroids in medical practice is their low water solubility. It is known that one of the effective ways to increase the water solubility of pharmaceutical preparations is the supramolecular and clathrate complexation of phytoecdysteroids with arabinogalactan, gum arabic, and cyclodextrins (CDs) [9], [10], [11], which also have antidiabetic activity and hypolipidemic action. Supramolecular complexation of phytoecdysteroids with CDs makes it possible to increase the solubility of the substance in water and improve their physicochemical properties, and due to this, they can be used to increase their bioavailability by increasing the absorption of drugs, which ultimately reduces the doses of the drugs used [12], [13], [14].

It should also be noted that at present the development of low-dose drugs is one of the important tasks of modern pharmaceutical chemistry and pharmacology. One of the promising directions in the development of such dosage forms with increased bioavailability is an approach based on the method of supramolecular complex formation of pharmacies in low stoichiometric ratios.

In this regard, the supramolecular complex of 20-hydroxyecdysone triacetate ((22R)-14, 20, 25-trihydroxy-6-oxo-2β, 3β, 5β-cholest-7-ene-2, 3, 22-trityl triacetate with β-CD, previously synthesized by us, was identified in sufficient detail by nuclear magnetic resonance spectroscopy, and its anti-inflammatory activity was studied [15].

However, an exhaustive study of the specific (adaptogenic and anabolic) activity of the water-soluble form obtained on its basis has not been carried out.

In this regard, the supramolecular complex of 20-hydroxyecdysone triacetate with β-CD was chosen by us to study its adaptogenic and anabolic activity.

The choice of 20-hydroxyecdysone triacetate as a synthon and a substrate for supramolecular self-assembly with β-CD is also due to the fact that acyl derivatives of ecdysterone have a wound-healing effect, the effectiveness of which increases significantly when included in liposomes, and are poorly soluble in water [16].

Research objectives

1. Supramolecular complex 2, 3, 22-acetoxy-14, 20, 25-hydroxy-5, 9 (H)-cholest-7-en-6- it is synthesized on the basis of 20-hydroxyecdysone triacetate (ecdysterone) and β-CD
2. When experimentally studying "20E-3Ac+β-CD" for adaptogenic and anabolic effects, behavioral tests are used to integrally assess the functional capabilities of the whole organism.

Materials and Methods

20E-3Ac+βCD-Inclusion complex of 20-hydroxyecdysone triacetate with β-CD.

The experimental part was carried out in accordance with the "rules of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes" and in accordance with the requirements for the study of new pharmacological substances on mature male rats (12 animals), initial body weight 290–320 g.

Rats were obtained from the vivarium of the JSC "Phytochemistry" (Karaganda). The animals were kept under standard vivarium conditions on a normal diet and free access to water and food. In addition, observations were made of the general condition of the animals: Changes in body weight of animals, physical activity, appetite, and response to external

stimuli. The animals were divided into two groups;, each group consisted of six animals. The animals of the experimental group were intragastrically injected with the test compounds at a dose of 10 mg/kg for 3 weeks. Animals that received an equivalent volume of starch mucus intragastrically daily for 3 weeks served as control.

Throughout the experiment, the animals were under daily observation. The following indicators were chosen as tests characterizing the state of animals under the influence of the “20E-Ac3” sample:

1. The intensity and nature of motor activity
2. Consumption of feed and water
3. Study of physical endurance
4. The death of animals
5. Measurement of body weight of animals
6. Weighing of the heart and gonads
7. Biochemical analysis of animal blood.

In the experimental study of a substance for adaptogenic and anabolic effects, behavioral tests are used to integrally assess the functionality of the whole organism. At the same time, indicators of motor activity and emotional reactivity are of great importance [17].

During the study of adaptogenic and anabolic effects, an “open field” test was performed after 2 weeks.

The “open field” is a rounded area with a diameter of 100 cm, limited in circumference by boards 40 cm high. The area is divided into 16 squares. The animal was placed in the center of the field and within 2 min the number of stances (vertical motor activity) and locomotions (horizontal motor activity), as well as grooming and the number of defecations (boluses) were visually recorded [18].

The experiment was carried out twice, to record the initial data on the behavior of animals, and after using the test compound “20E-Ac3” for 2 weeks, The effect of the “20E-Ac3” sample on the physical performance of experimental animals was studied in the “free swimming” test. The effect of the drug on the working capacity of animals was judged by the amount of time the rats stayed in the water until they were completely tired, placed in an aquarium 15 × 15 × 40 cm, and filled 1/3 with water (27°C). Testing was performed twice: Swimming without load and then swimming with a load of 10% of body weight. Before the start of the experiment, the animals were trained a day before, without taking into account the time the rats spent in water until they were completely tired.

Forced swim without load: Dynamic load was assessed by endurance time in a standard test of swimming to complete fatigue (swimming “to failure”), for 10 min. The criterion for the onset of complete fatigue in the test was the complete immersion of the animal’s head under water for 5 s. At this point, the animal is quickly removed from the water and dried with a dry towel for 2 min.

Forced swimming with a load of 10% of body weight: Animals swim with a load until fatigue, as evidenced by the immersion of the animal to the bottom of the cylinder. At this point, the animal is quickly removed from the water and dried with a dry towel for 2 min.

At the end of the study, the rats were decapitated under light ether anesthesia, and biochemical parameters (total protein, glucose) were determined in the blood serum. Plasma total protein and glucose levels were determined using reagent kits manufactured by High Technology, Inc. Determination of biochemical parameters was performed on the analyzer “HTI BioChemSA.”

After decapitation of the experimental animals and blood sampling, a macroscopic examination of the heart and gonads was performed, and their mass was determined.

Statistical processing of the results was carried out using the Statistica 8 software package. The results obtained are presented as “mean ± standard error of

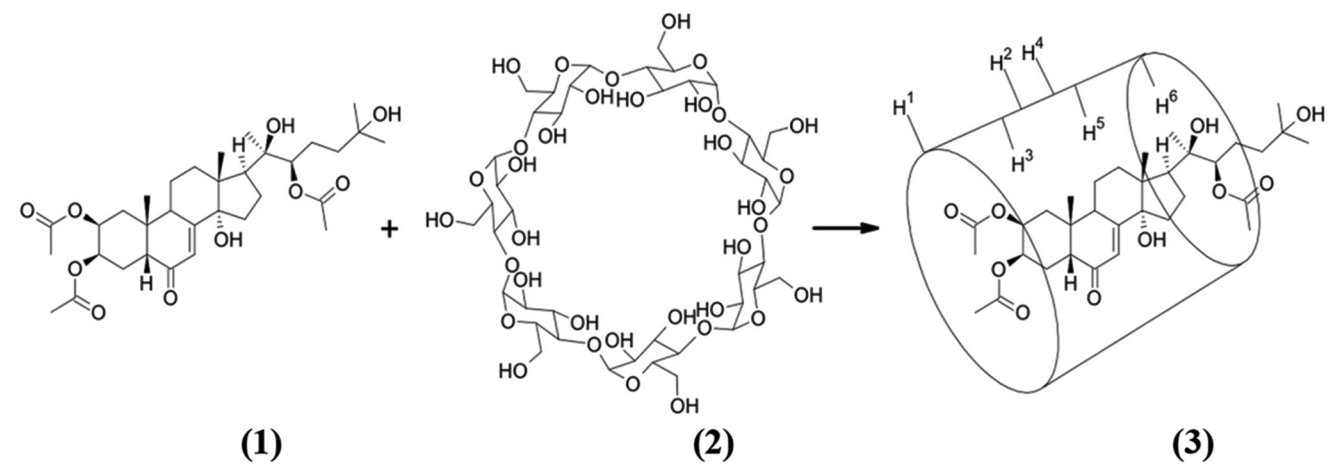

Figure 1: Scheme for the synthesis of the supramolecular complex of 20-hydroxyecdysone triacetate (3)

the mean." Intergroup differences were assessed by the non-parametric Mann–Whitney U-test. For pairwise related groups, the non-parametric Wilcoxon test was used.

Results and Discussions

Chemical research

β-CD was used as a clathrate to obtain a composite based on triacetate 20E and for its further study due to its universal properties. Supramolecular encapsulated complex (3) based on compound (1) with β-CD (2) was obtained by the interaction of equimolecular amounts of triacetate 20E (1) with CD in water-ethanol solution at 50°C for 8 h (Figure 1).

The fine structure of compound (1) was determined by ^{1}H and ^{13}C NMR spectroscopy in a DMSO-d_6 solution (Table 1). The assignment of signals in 1D ^{1}H and ^{13}C NMR spectra was confirmed by the data of 2D correlations ^{1}H-^{1}H COSY, ^{1}H-^{1}H ROESY, ^{1}H-^{13}C HMQC, and ^{1}H-^{13}C HMBC (Table 1). As can be seen from the data in Table 1, the greatest change in the chemical shifts of the protons of the glucopyranose unit is experienced by the internal protons H-3 and H-5, which are included in the inner part of the truncated CD cone. This allows us to conclude that the substrate molecule (1) enters the internal cavity of β-CD with the formation of an inclusion complex (3). The ratio of the integral intensities of the proton signals of the substrate (1) and β-CD in the complexes showed that there is one molecule of β-CD per one molecule of the substrate (1).

The synthesized supramolecular complex (3) has sufficient solubility in water and has an anti-inflammatory effect [15].

The results of bioscreening on the study of the specific activity of the supramolecular complex of 3-acetate 20E with β-CD (3) showed that the complex at a dose of 10 mg/kg has anabolic and adaptogenic effects.

Results of biological screening for specific activity in vivo

The effect of the influence of the sample "20E-Ac3+βCD" on the weight gain of the animals is presented in Table 2. After 3 weeks of administration of the studied samples, the dynamics of the growth of the body weight of the animals were recorded.

As can be seen from Table 2, the studied sample "20E-Ac3+βCD" showed significant activity in the direction of increasing the body weight of rats. The increase in body weight of animals at the end of the experiment, receiving the test substance, was significant.

Table 1: Chemical shifts of the 1H and 13C nuclei of substrate 1 and β-cyclodextrin in the free state (δ0) and in the composition of complex 3 (δ)

№ atom	Group	δ_0, ppm		δ, ppm		$\Delta\delta = \delta-\delta_0$	
		^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Compound 1							
$1_{ax}1_{eq}$	CH_2	1.65–1.70 m	37.19	1.65–1.70 m		0.00	
		2.11–2.22 m		2.11–2.20 m			
2	CH	4.95d, ^{3}J = 12.0Hz	68.75	4.94 d		−0.01	
3	CH	5.17 br.s	67.34	5.16		−0.01	
$4_{ax}4_{eq}$	CH_2	1.65–1.70 m	31.41	1.65–1.70 m		0.00	
		1.90–1.93 m		1.90–1.92 m		−0.01	
5	CH	2.11–2.22 m	51.10	2.11–2.20 m		0.02	
6	>C = O	-	201.32	-			
7	CH	5.65 s	121.07	5.66		0.01	
8	>C<	-	165.83	-			
9	CH	3.02–3.08 m	34.09	3.02–3.08 m		0.00	
10	>C<	-	38.24				
$11_{ax}11_{eq}$	CH_2	1.65–1.70 m	21.37	1.65–1.70 m		0.00	
		1.78–1.81 m		1.78–1.82 m		0.01	
$12_{ax}12_{eq}$	CH_2	1.90–1.93 m	30.81	1.90–1.92 m		–0.01	
		2.44–2.48 m		2.44–2.48 m		0.00	
13	>C<	-	47.34	-			
14	>C<	-	83.51	-			
$15_{ax}15_{eq}$	CH_2	1.91 s	33.52	1.90 s		−0.01	
		2.11–2.22		2.11–2.20		−0.02	
$16_{ax}16_{eq}$	CH_2	2.11–2.22 m	21.70	2.11–2.20		−0.02	
		2.44–2.48		2.44–2.48		0.00	
17	CH	3.02–3.08	49.58	3.02–3.08		0.00	
18	$-CH_3$	0.88 s	23.90	0.88 s		0.00	
19	$-CH_3$	0.73 s	17.68	0.72 s		−0.01	
20	>C<	-	79.11	-			
21	$-CH_3$	1.13 s	23.91	1.12 s		−0.01	
22	CH	4.63d, ^{3}J = 10.0Hz	81.86	4.63 d		0.00	
$23_{ax}23_{eq}$	CH_2	1.78–1.81 m	26.54	1.76–1.82 m		0.01	
		2.11–2.22 m		2.11–2.20 m		−0.02	
$24_{ax}24_{eq}$	CH_2	1.78–1.81 m	40.63	1.76–1.82 m		0.01	
		2.11–2.22 m		2.11–2.20 m		−0.02	
25	>C<	-	75.24	-			
26	$-CH_3$	1.28 s	26.29	1.27 s		−0.01	
27	$-CH_3$	1.33 s	29.09	1.32 s		−0.01	
2	$-OOC-CH_3$	1.88 s	170.31	1.87 s		−0.01	
3	$-OOC-CH_3$	1.98 s	170.18	1.97 s		−0.01	
22	$-OOC-CH_3$	2.05 s	172.92	2.04 s		−0.01	
β-cyclodextrin							
1	CH	4.77d, ^{3}J = 4.0Hz	102.40	4.78 d	102.45	0.01	0.05
2	CH	3.26d, ^{3}J = 12.1Hz	72.83	3.26 d	72.92	0.00	0.09
3	CH	3.58 t, ^{3}J = 8.3Hz	73.54	3.60 t	73.56	0.02	0.02
4	CH	3.28t, ^{3}J = 10.0Hz	81.98	3.28 t	82.03	0.00	0.05
5	CH	3.50 s	72.50	3.53 s	72.55	0.03	0.05
6	CH_2	3.58 s	60.42	3.59 s	60.42	0.01	0.00

As a result of the study, the following indicators were recorded in the open field test: Freezing episodes, an average level of mobility, avoidance of the central part of the arena, a high level of defecation, and long grooming. After a 2-week period of introduction of the "20E-Ac3+βCD" sample, a second "open field" test was performed.

As a result of the test, it was revealed that the animals of the experimental group showed a higher level of orienting and exploratory activity in the "open field" test. It turned out that in experimental animals the level of anxiety was lower; the number of urinations and defecations was lower than in the control group, and the latency of leaving the center of the "open field" was higher. However, when assessing the difference between the final and baseline indicators, no significant changes are observed (Table 3).

Table 2: The index of the dynamics of body weight growth in rats with daily intragastric administration of the "20E-3Ac + βCD" sample

Group	Number of animals	Initial mass	1 week	2 weeks	3 weeks	The difference before exp. and in 3 weeks
Control	♂6	298.7 ± 25.9	309.7 ± 26.7	314.3 ± 28.9	316.5 ± 32.3	17.8
20E-3Ac + βCD	♂6	315.7 ± 15.7	299.7 ± 52.5	333.2 ± 18.5	337.7 ± 15.7	22

Table 3: The effect of the "20E-3Ac + βCD" sample on the behavior of rats in the "open field" test before and after the administration of the substance after 2 weeks

Indicator	Group	Control	TT-1 + βCD
Horizontal activity	Ref (1 week)	16.2 ± 5.1	15.7 ± 5.8
	End (2 weeks)	12.0 ± 7.2	11.0 ± 7.8
	Difference	−4.2	−4.7
Vertical activity	Ref (1 week)	8.2 ± 4.8	9.8 ± 6.1
	End (2 weeks)	3.6 ± 3.6	3.6 ± 2.1
	Difference	−4.6	−6.2
Grooming	Ref (1 week)	2.3 ± 1.2	0.2 ± 0.4*
	End (2 weeks)	1.0 ± 1.1	1.7 ± 3.6
	Difference	−1.2	−1.5
Defecation	Ref (1 week)	2.3 ± 2.7	2.8 ± 1.2
	End (2 weeks)	4.2 ± 0.9	1.5 ± 1.5*
	Difference	1.9	−1.3
Urination	Ref (1 week)	1.7 ± 1.4	1.2 ± 0.9
	End (2 weeks)	1.2 ± 0.9	0.0 ± 0,0*
	Difference	−0.5	−1.2

(Ref.) – the initial level of the indicator; (end) – level at the end of the experiment; (difference) – the difference between the final and initial level of the indicator.* -r.< 0.05 compared to control.

According to the results of the "forced swimming" test, the animals of the experimental troupe showed higher rates of physical endurance: Without load, the difference was 219.8 s for "20E-Ac3+βCD," with the load the difference was 177 s for "20E-Ac3+βCD," 3 s. compared to control (Table 4).

Table 4: The time of immobilization of rats in the "free swimming" test with the introduction of the "20E-3Ac + βCD" sample with and without load

Group	Time of rats immobilization, sec	
	Without	Load 10%
Control	85.5 ± 57.9	85.5 ± 57.9
20E-3Ac + βCD	305.3 ± 45.7*	305.3 ± 45.7*
Difference (Control-20E-3Ac + βCD)	219.8	219.8

(Difference) – the difference between the final and initial level of the indicator.

Results on biochemical parameters (total protein, glucose) and macroscopic examination of the heart and gonads, determination of their mass (Tables 5 and 6).

As can be seen from Table 5, there was no increased mass of the heart and gonads in animals treated with "20E-Ac3+βCD" compared with the control group.

As can be seen from the presented results, the content of total protein in the blood serum of animals receiving the sample "20E-Ac3+βCD" at a dose of 10 mg/kg did not increase compared to the control data. The content of glucose in the blood serum of animals receiving the sample "20E-Ac3+βCD" at a dose of 10 mg/kg increased compared to the control data.

Table 5: Indices of the heart mass of rats with daily intragastric administration of the sample "20E-3Ac + βCD"

Dose, mg/kg	Heart mass, g	Gonad mass, g	
Control	1.1 ± 0.1	2.0 ± 0.2	1.9 ± 0.3
20E-3Ac + βCD	0.9 ± 0.2*	1.8 ± 0.3	1.8 ± 0.5
Difference (control-20E-3Ac + βCD)	−0.2	−0.2	−0.1

Table 6: The effect of the compound "20E-3Ac + βCD" with daily intragastric administration on biochemical parameters in the blood serum of rats

Dose, mg/kg	Total protein, g/L	Glucose, mmoL/L
Control	77.7 ± 9.5	8.5 ± 3.9
20E-3Ac + βCD	56.4 ± 14.1*	13.3 ± 2.9*
Difference (control-20E-3Ac + βCD)	−21.3	4.8

Conclusions

1. The described nature of biochemical changes indicates that physical activity (swimming) simultaneously has a significant stress effect on animals and is characterized by an increased intake of glucose into the blood and the breakdown of blood proteins.
2. Thus, as a result of the experimental behavioral tests, it was found that the sample "20E-3Ac+βCD" at a dose of 10 mg/kg has anabolic and adaptogenic effects, in particular, increased stress resistance of animals, increased physical endurance of animals, as well as a slight increase in weight. Heart and gonads compared with control.
3. The work was performed in compliance with all applicable international, national, and institutional guidelines for the care and use of animals.

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