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Supramolecular Complexes of 3-Epi-2-deoxyecdysone with Cyclodextrins and Their Anti-Inflammatory Activity

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Abstract—The ecdysteroid 3-epi-2-deoxyecdysone has been isolated from the aerial part of *Acanthophyllum gypsophiloides Regel*. The complexes formation of the ecdysteroid with α-, β-, γ-, and 2-hydroxypropyl-β-cyclodextrins has been studied by means of NMR spectroscopy. Anti-inflammatory activity of the obtained 3-epi-2-deoxyecdysone complexes with cyclodextrins has been investigated.

Key words: 3-epi-2-deoxyecdysone, cyclodextrins, supramolecular complexes, inclusion complexes

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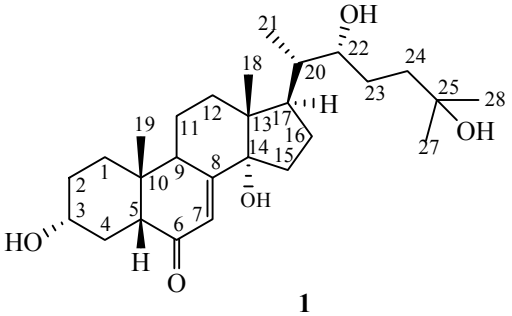
Phytoecdysteroids exhibit anabolic, tonic, adaptogenic, immunostimulatory, and hypoglycemic activity. The use of phytoecdysteroids in pharmacy is limited due to their low solubility in water. It is known that supramolecular complexation with cyclodextrins is among the most efficient approaches to increase the drug solubility in water [1]. Supramolecular self-assembly of the phytosteroids complexes with cyclodextrins improves the substance solubility in water, enhances its bioavailability and physico-chemical stability, protects from biodegradation, and reduce the toxicity [2–4]. Cyclodextrins are comparatively available compounds produced from renewable raw material – starch. The most common cyclodextrins are α-, β-, γ-cyclodextrins, containing 6, 7, and 8 glucopyranose units, respectively. The interest to cyclodextrins has emerged because of their cyclic structure and ability to form supramolecular complexes of the *host-guest type* (receptor-substrate) in water due presence of the internal hydrophobic cavity [5–7].

3-Epi-2-deoxyecdisone **1** [3α,14α,22*R*,25-tetrahydroxy-5β(*H*)-cholester-7-en-6-one, Scheme 1], isolated earlier [8] and comprehensively studied by means of mass spectrometry and NMR-spectroscopy [9], was chosen as a substrate for the investigation of supramolecular self-assembly of the complexes with α-, β-, γ-, and 2-hydroxypropyl-β-cyclodextrins and further bioscreening of their anti-inflammatory activity.

Supramolecular complexes were prepared via the interaction of equimolar amounts of ecdysone **1** and α-, β-, γ-, or 2-hydroxypropyl-β-cyclodextrin in an aqueous-ethanolic solution at 50°C during 5 h. The structure investigation of supramolecular complexes by NMR spectroscopy was based on the determination of the difference between the values of the ^{1}H chemical shifts of the substrate (**1**) and the receptors (cyclodextrins) in the free state and upon the complex formation via the intermolecular interactions. The formation of internal, external, and mixed complexes

Scheme 1.



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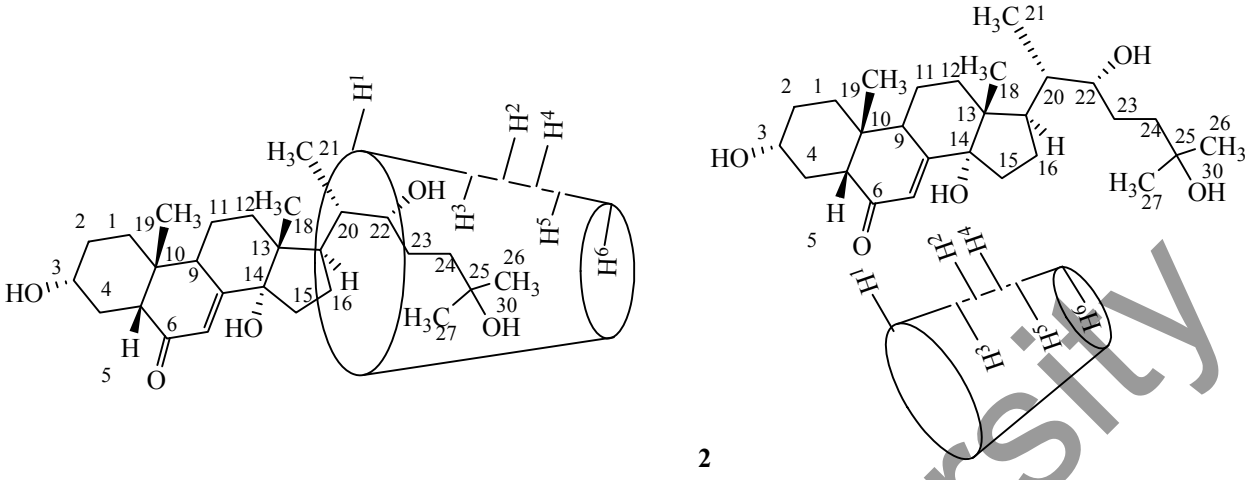
Scheme 2.



2

could be evidenced by the value of the chemical shifts of the internal and external protons of cyclodextrins, respectively. The change in the ^{1}H chemical shifts in the substrate spectrum allowed to determination the direction of inclusion of the substrate into the cyclodextrin cavity [10–12].

The structure of ecdysone **1** was elucidated basing on the ^{1}H and ^{13}C NMR data using the DMSO-d_6 as solvent (Table 1). The accuracy of the one-dimensional ^{1}H and ^{13}C NMR signals assignment was confirmed by the data of two-dimensional correlation ^{1}H–^{1}H TOCSY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C HMQC, and ^{1}H–^{13}C HMBC experiments. The ^{1}H NMR spectral data for α-, β-, γ-, and 2-hydroxypropyl-β-cyclodextrins in the free state and included in the supramolecular complexes with ecdysone **1** obtained in D_2O are given in Tables 1 and 2.

Investigation of the supramolecular complexes of α-cyclodextrin with ecdysone **1** revealed that the signals of the steroid molecule were practically absent in the NMR spectra. That fact demonstrated that there was no quantitative interaction between α-cyclodextrin and ecdysone **1**. Probably, insignificant size of the hydrophobic cavity of α-cyclodextrin (in comparison with other analogs) prevented the formation of the supramolecular complex.

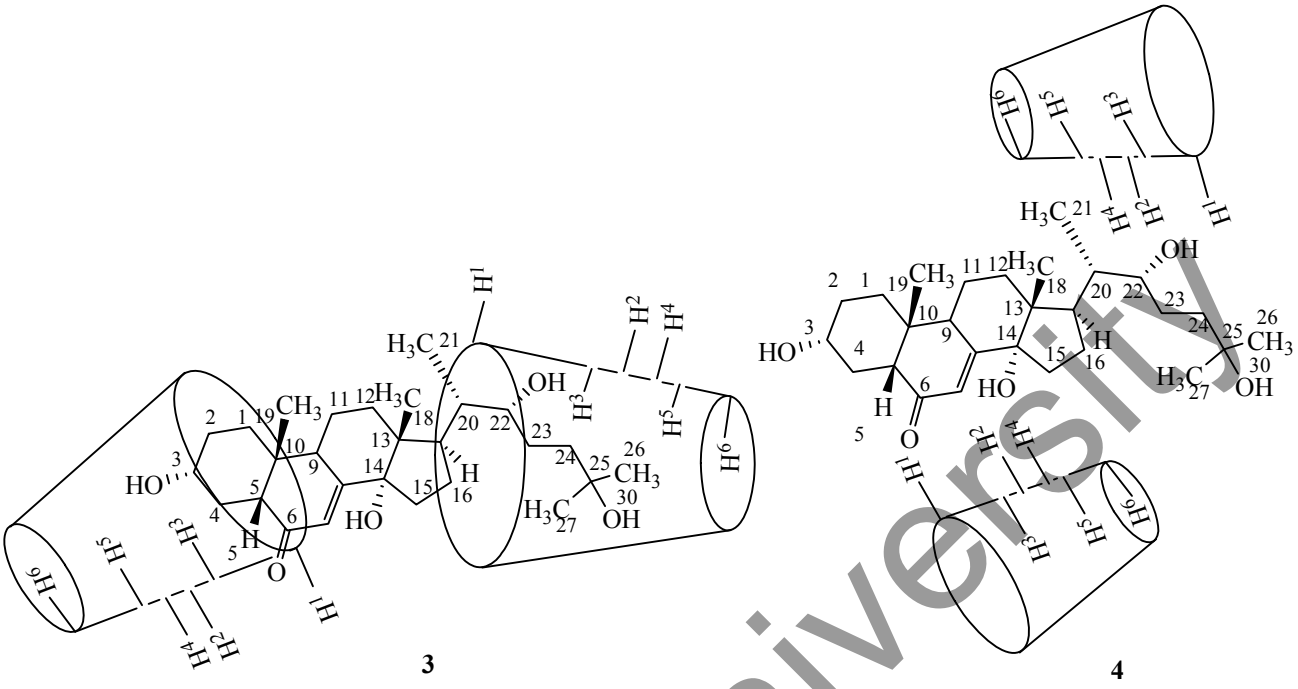
The comparison of integral intensities of the signals in the ^{1}H NMR spectra of ecdysone molecule **1** with β-, γ-, and 2-hydroxypropyl-β-cyclodextrins in the complexes showed that ecdysone **1** formed complexes **2** of the 1 : 1 composition with β-cyclodextrin, while γ- and 2-hydroxypropyl-β-cyclodextrins afforded complexes **3** and **4** of the 1 : 1 composition. The formation of supramolecular complex **2** was accompanied by the change in the chemical shifts of the cyclodextrin protons $\Delta\delta_1$ was similar for internal hydrophobic protons (H^3, H^5, and H^6) as well as for external hydrophilic protons (H^1, H^2, and H^4) (atoms numbering is given in Scheme 2). The most prominent change in the NMR spectrum of ecdysone **1** molecule were observed for the protons of steroid rings (H^7, H^{15}, and H^{16}) and the methine (H^{22}) and methylene (H^{21}, H^{26}, and H^{27}) protons. Those results showed the supramolecular self-assembly gave rise to the formation of *host-guest* complexes and the conventional ones [12, 13]. Water-soluble aggregates formed upon that process could solubilize lipophilic molecules of substrates via non-inclusive complexation [14].

The most prominent change in the ^{1}H NMR chemical shifts upon the formation of complex **3** was observed for the hydrophobic internal proton H^3 of cyclodextrin (atoms numbering is given in Scheme 3). The changes in the chemical shifts were observed for the protons of both internal and external spheres of γ-cyclodextrin. The changes in the chemical shifts of the steroid molecule protons were most strong for the cyclic protons (H^3, H^{12}, and H^{17}) and also for the non-cyclic proton H^{22}. Since in that complex one steroid molecule corresponded to two molecules of γ-cyclodextrin and the strongest change in the chemical shifts of the substrate molecule **1** were observed for both cyclic and acyclic protons, we could suggest the formation of the *host-guest* complex **4** with the steroid molecule accommodated in the cyclodextrin cavity via different parts of the molecule. The changes in the chemical shifts of the external protons of γ-cyclodextrin were possible due to the interaction of

Table 1. ^{1}H and ^{13}C chemical shifts of ecdysone **1** in the free state (δ_0) and in the complexes **2** (δ_1), **3** (δ_2), and **4** (δ_3)

Atom number	Group	δ_C, ppm	δ_H, ppm						
		δ_0	δ_0	δ_1	$\Delta\delta_1 = \delta_1 - \delta_0$	δ_2	$\Delta\delta_2 = \delta_2 - \delta_0$	δ_3	$\Delta\delta_3 = \delta_3 - \delta_0$
1_{ax}	CH_2	29.62	1.41			1.40	–0.01	1.41	0
1_{eq}			1.46	1.41	–0.05	1.46	0	1.44	–0.02
2_{ax}	CH_2	28.73	1.54			1.56	0.02	1.48	–0.06
2_{eq}			1.56	1.53	–0.03	1.59	0.03	1.52	–0.04
3	CH	63.22	3.40	3.44	0.04	3.59	0.19	3.37	–0.03
	OH		4.45						
4_{ax}	CH_2	32.55	1.54						
4_{eq}			1.56	1.53	–0.03	1.59	0.03	1.52	–0.04
5	CH	47.65	2.23	2.27	0.04	2.25	0.02	2.30	0.07
6	CO	203.23	–	–	–	–	–	–	–
7	CH	120.76	5.60	5.84	0.24	5.75	0.15	5.83	0.23
8	C	165.91	–	–	–	–	–	–	–
9	CH	36.58	3.01	3.04	0.03	3.02	0.01	3.05	0.04
10	C	36.58	–	–	–	–	–	–	–
10	C	36.58	–	–	–	–	–	–	–
11_{ax}	CH_2	20.60	1.46			1.46	0	1.44	–0.02
11_{eq}			1.56	1.53	–0.03	1.59	0.03	1.52	–0.04
12_{ax}	CH_2	31.00	1.56	1.53	–0.03	1.59	0.03	1.52	–0.04
12_{eq}			1.95	1.92	–0.03	1.80	–0.15	1.92	–0.03
13	C	47.37	–	–	–	–	–	–	–
14	C	83.39	–	–	–	–	–	–	–
	OH								
15_{ax}	CH_2	29.62	1.54	1.53	–0.01	1.56	0.02	1.48	–0.06
15_{eq}			1.85	1.76	–0.09	1.79	–0.06	1.84	–0.01
16_{ax}	CH_2	21.58	1.56						
16_{eq}			1.83	1.76	–0.07	1.79	–0.04	1.81	–0.02
17	CH	47.37	1.95	1.92	–0.03	1.80	–0.15	1.92	–0.03
18	CH_3	15.85	0.55	0.63	0.08	0.58	0.03	0.63	0.08
19	CH_3	24.36	0.81	0.87	0.06	0.84	0.03	0.83	0.02
20	CH	42.35	1.56	1.53	–0.03	1.59	–0.03	1.52	–0.04
21	CH_3	13.42	0.79	0.87	0.08	0.79	0	0.83	0.04
22	CH	72.87	3.34	3.44	0.10	3.57	0.23	3.37	0.03
	OH		4.17						
23_{ax}	CH_2	24.58	1.18	1.23	–0.05	1.24	0.06	1.16	–0.02
23_{eq}			1.31	1.27	–0.04	1.31	0	1.35	0.04
24_{ax}	CH_2	41.79	1.29	1.27	–0.02	1.31	0.02	1.27	–0.02
24_{eq}			1.76	1.75	–0.01	1.76	0	1.76	0
	OH		4.47						
25	C	69.33	–	–	–	–	–	–	–
	OH								
26	CH_3	30.53	1.01	1.09	0.08	1.08	0.07	1.08	0.07
27	CH_3	26.27	1.03	1.09	0.06	1.08	0.05	1.08	0.05

Scheme 3.



3 **4**

ecdysone **1** with hydroxyl groups the and the protons located out of the cyclodextrin cavity.

When 2-hydroxypropyl-β-cyclodextrin, more hydrophilic than the other cyclodextrins used in this study (due to presence of additional 2-hydroxypropyl fragments) was utilized as the component of supramolecular self-assembly system, there was almost no change in the chemical shifts of both internal and external protons the matrix upon formation of the complex **4**. The most prominent changes in the chemical shifts of ecdysone molecule **1** were observed for protons H^7, H^{18}, and H^{26}. In that case, probably, supramolecular self-assembly led to the formation of complexes without inclusions **4** due to the intermolecular interactions of the hydroxyl groups of the steroid molecule and 2-hydroxypropyl-β-cyclodextrin.

The obtained complexes **2**–**4** were sufficiently soluble in water.

The results of screening study of anti-inflammatory activity of complexes **2**–**4** showed that the complex **2** in the dose of 25 mg/kg exhibited high anti-inflammatory activity (the model of acute exudative reaction), exceeding that of the reference drug Sodium Diclofenac by 1.6 times, while complex **3** exhibited

Table 2. ^{1}H chemical shifts of β-, γ-, and 2-hydroxypropyl-β-cyclodextrins in the free state (δ_0) and in the complexes **2** (δ_1), **3** (δ_2), and **4** (δ_3)

Atom number	β-Cyclodextrin			γ-Cyclodextrin			2-Hydroxypropyl-β–cyclodextrin		
	δ_0, ppm	δ_1, ppm	$\Delta\delta_1 = \delta_1 - \delta_0$	δ_0, ppm	δ_2, ppm	$\Delta\delta_2 = \delta_2 - \delta_0$	δ_0, ppm	δ_3, ppm	$\Delta\delta_3 = \delta_3 - \delta_0$
1	4.87	4.92	0.05	4.96	4.92	–0.04	4.92	4.92	0.00
2	3.45	3.49	0.04	3.51	3.51	0.00	3.48	3.48	0.00
3	3.77	3.81	0.04	3.78	3.70	–0.08	3.87	3.86	–0.01
4	3.39	3.44	0.05	3.44	3.43	–0.01	3.44	3.44	0.00
5	3.68	3.72	0.04	3.72	3.68	–0.04	3.72	3.72	0.00
6	3.68	3.72	0.04	3.72	3.68	–0.04	3.72	3.72	0.00

low activity, and complex **4** did not show any anti-inflammatory properties.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra of the solutions of ecdysone **1** in DMSO-d_6 and host-guest complexes **2**–**4** in D_2O were registered using a Jeol JNM-ECA 400 spectrometer (399.78 and 100.53 MHz, respectively) at room temperature.

β-, γ- and 2-hydroxypropyl-β-cyclodextrins (99% purity) were purchased from Fluka.

Synthesis of supramolecular complexes 2–4. 0.124 g (0.11 mmol) of β-cyclodextrin [or 0.142 g (0.11 mmol) of γ-cyclodextrin, or 0.182 g (0.11 mmol) of 2-hydroxypropyl-β-cyclodextrin] dissolved in 4 mL of distilled water were added to 0.05 g (0.11 mmol) of ecdysone **1** dissolved in 3 mL of anhydrous ethanol. The mixture was stirred for 8 h at 50°C. The precipitate was filtered off, washed with ethanol, and dried in a vacuum oven at 40°C. The complexes were white powders.

Complex 2. Boiling point 293–296°C (ethyl acetate–methanol). IR spectrum (KBr), ν, cm^{-1}: 1030, 1165, 1647 (C=O), 2357, 2932, 3445 (OH).

Complex 3. Boiling point 314–317°C (ethyl acetate–methanol). IR spectrum (KBr), ν, cm^{-1}: 578, 1030, 1157, 1377, 1655 (C=O), 2928, 3414 (OH).

Complex 4. Boiling point 350°C (decomp.) (ethyl acetate–methanol). IR spectrum (KBr), ν, cm^{-1}: 1022, 1061, 1377, 1651 (C=O), 2365, 2943, 3433 (OH).

Investigation of anti-inflammable activity was performed as described elsewhere [15]. The chosen objects were studied at oral dose of 25 mg/kg dose of a starch slime. The reference drug diclofenac sodium was studied at a dose of 25 mg/kg as well. Statistical data processing was performed using Statistica 6.0 software package. The intergroup differences were estimated by the nonparametric Mann–Whitney *U*-criterion. The difference was considered significant at the confidence level $p < 0.05$.

This study was conducted according to the applicable international, national, and institutional guiding principals on the use and treatment of animal subjects.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors.

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