Steroids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Bioavailability and structural study of 20-hydroxyecdysone complexes with cyclodextrins

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ARTICLE INFO

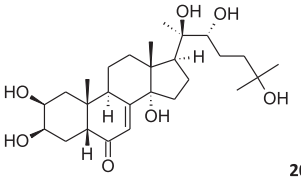
Keywords:
Ecdysterones
Cyclodextrins
Inclusion complexes
NMR spectroscopy

ABSTRACT

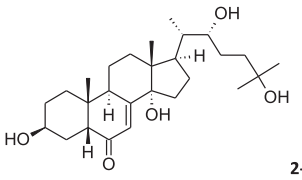
20-Hydroxyecdysterone – (2β,3β,5β,22*R*)-2,3,14,20,22,25-hexahydroxycholest-7-en-6-one was isolated in satisfactory yield using ethanol extraction from the aerial part of *Silene wolgensis* (Hornem.) Otth; sometimes *Silene wolgensis* (Willd.) Bess. ex Spreng. The complexation of the phytoecdysteroid with β-cyclodextrin was studied by NMR spectroscopy. By studying the changes in chemical shifts of protons of substrates and receptors it was found that ecdysterone interacts with cyclodextrins to form supramolecular inclusion complexes of stoichiometric composition of 1:1 or 1:2. Ecdysterone-β-cyclodextrin complexes exhibit 100 times higher solubility in water than the parent compound.

1. Introduction

In recent years, the interest in metabolic-type and natural products drugs that have the ability to activate plastic processes in various organs and tissues, improve the energy status of their cellular systems, and thereby improve the body resistance to various adverse factors, increased significantly. According to the results of the experimental and clinical studies, one of the most effective drugs in this area could be "Ecdisten" (Five Stars Laboratory, Bulgaria) developed on the basis of the natural ecdysterone (**20E**) contained in various plant sources such as *Leuzea carthamoides* alias *Rhaponticum carthamoides*, *Rhaponticum integrifolium*, *Silene brahuica*, *Ajuga turkestanica* etc. by the Institute of the Chemistry of Plant Substances (Tashkent, Republic of Uzbekistan), and the "Ekdifit" preparation developed at the International Research and Production Holding "Phytochemistry" (Karaganda, Republic of Kazakhstan), which is the sum of the extracs of *Serratula coronata* and contains **20E** as the main active component and additional amounts of ecdysteroids and flavonoids [1–4]. Generally, these compounds are considered to be non-toxic and safe to be used as a food supplements and APIs.



20E



2-DE

At present, special attention is paid to another plant-supercontentrator *Silene wolgensis* of family *Caryophyllaceae* marked by a high content of **20E** (1.76%) superior to its content in *Serratula coronata* (1.5%), which is the plant base of the domestic anabolic and adaptogenic preparation "Ekdifit" and a promising alternative industrially significant species among plants of Kazakhstan.

The main problem encountered in working with phytoecdysteroids, and the related substances and drugs, is relative difficulty of their isolation and poor water solubility. This in turn leads to a low bioavailability of the drug and, in the future, a slow and incomplete release of the drug. To eliminate the above-mentioned problems, we proposed to use industrially available cyclodextrins (CDs) in the development of substance delivery systems.

The most promising and intensively studied area is the preparation and investigation of inclusion complexes of steroid compounds with CD

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https://doi.org/10.1016/j.steroids.2018.11.007
Received 14 August 2018; Received in revised form 23 October 2018; Accepted 14 November 2018

at the present stage of development of supramolecular chemistry see e.g., [5–8]. Utilisation of CD complexes was also claimed in several patents [9–11].

In this connection, new encapsulated inclusion complexes based on **20E** (isolated from the aerial parts of *Silene wolgensis* collected in 2016 at the flowering stage in the vicinity of Balyktykol village of Nurinsky district, Karaganda region, Republic of Kazakhstan) have been investigated in the present work. It was found that the β-CD inclusion complexes have 100-times higher solubility in water.

In addition to the main component **20E**, it was possible to isolate and characterize the minor ecdysteroid, 2-deoxyecdysone, i.e., (3β,5β,22*R*)-3,14,22,25-tetrahydroxycholest-7-en-6-one (**2-DE**), previously isolated from the Kazakhstan plant *Silene cretaceae* Fisch. and characterised by its stereochemical and X-ray diffraction study [12]. Ethanol extract of the aerial part of *Silene wolgensis* was treated with a mixture of petroleum ether and ethyl acetate followed by 2-methylpropan-1-ol to remove the non-polar components. Compounds **20E** and **2-DE** were isolated from the residue by chromatography.

Supramolecular inclusion complexes of **20E** with cyclodextrins were obtained by interaction of equimolecular amounts of substrate with receptors in ethanol solutions of reacting substances at 50 °C for 5 h followed by isolation of complexes by drying. The complexes were also tested for cytotoxicity using MTS assay on a panel of seven cancer cell lines (see Supplementary information) and two non-cancerous lines [13]. No cytotoxicity was observed up to tested maximum concentration of 50 μM.

2. Results and discussion

2.1. NMR study

One of our goals was to characterize and uncover more details on the molecular geometry of **20E** complexes with β-CD. Firstly, we attempted a complete signals assignment, and to identify the intermolecular interactions (contacts) between **20E** and **CD** via the 2D ROESY NMR experiments.

The complete signals assignment of both ^{1}H and ^{13}C was done for pure **20E** in DMSO-d_6 (15.5 mg of pure **20E** in 0.5 mL of low water content DMSO-d_6), excluding the OH protons, which have two broad signals at 4.09 and 4.56 ppm, three broad overlaid signals around 4.36 ppm, and a sharp signal at 4.63 ppm, which was identified as OH at carbon C14 due to strong HMBC correlation to carbon C13, and two weak correlations to carbons C14 and C15. The broad signal of residual water at 3.30 ppm has intensity of 0.17 H. The other signals and most of their characteristics are summarized in the Table 1. The values of J_{HH}, intensity of crosspeaks in HMBC and ROESY were used to set up geometry of the steroid compartment of **20E** (the aliphatic part from C23 appears to freely rotate), and to confirm the molecular model after its optimization *via* semi empirical method PM3 (Fig. 1).

The **20E**-β-CD complex (9.5 mg dissolved in D_2O 0.5 mL) was studied via 1D and 2D NMR experiments. We observed a precipitation after a month storing of the solution.

The ^{1}H spectrum confirmed 1:1 composition of the complex, and symmetry of the β-CD compartment. Since **20E** is asymmetrical molecule, the complex **20E**-β-CD is expected to be asymmetrical as well. The observed symmetry can be explained via fast rotation of the complex components, including a decomplexation step despite of **20E** low solubility in water. All signals were assigned unequivocally, and if possible, the multiplicities of the signals were described with help of 1D TOCSY experiments (Table 1).

Analogously, the **20E**-2(β-CD) complex **20E**-2(β-CD) was studied (9.5 mg of **20E**-β-CD in 0.5 mL D_2O). The signals assignment was successful for all ^{1}H and ^{13}C signals, which is summarized in the Table 1. Similarly, to **20E**-β-CD, we observed a precipitation after a month of storage.

The ^{1}H spectrum confirmed only 1:1.8 composition of the **20E**-2(β-

Table 1
NMR signals assignment for **20E** and **CD**.

No.	**20E** in $CDCl_3$	**20E** in DMSO-d_6	**20E**-β-CD in D_2O	**20E**-2(β-CD) in D_2O
*Signals of **20E***				
C1	37.96	36.610	35.449	35.473
H1α		1.263, dd, 13.3, 12.0	1.299, dd, 13.5, 12.5	1.307, dd, 13.6, 12.4
H1β		1.598, dd, 13.3, 4.3	1.788, dd, 13.5, 4.4	1.793, dd, 13.6, 4.4
C2	68.03	66.754	67.367	67.369
H2		3.604, ddd, 11.9, 4.3, 3.1	3.880	3.880, br d, 12.3
C3	68.12	66.570	67.128	67.095
H3		3.764, ~q, 2.9	3.968, ~q, 2.9	3.950, ~q, 3.0
C4	32.41	31.526	31.154	31.163
H4α		1.473, ~dt, 13.7, 3.8	1.705, br d	1.720, br d
H4β		1.593, td, 13.4, 2.5	1.627	1.629, br t
C5	51.37	50.072	50.358	50.342
H5		2.200, dd, 13.1, 4.2	2.270, dd, 12.6, 5.0	2.292, dd, 13.4, 4.2
C6	203.43	202.620	208.101	207.992
C7	121.64	120.432	121.017	121.004
H7		5.626, d, 2.6	5.891, d, 2.6	5.900, dd, 2.7, 0.8
C8	166.08	165.187	168.242	168.171
C9	34.43	33.144	33.831	33.829
H9		3.007, ddd, 11.6, 7.1, 2.7	3.025, ddd, 11.5, 7.2, 2.6	3.033, ddd, 11.5, 7.2, 2.7
C10	38.64	37.601	38.161	38.161
C11	21.11	20.061	20.133	20.151
H11α		1.520, ~qd, 12.8, 5.1	1.655	1.663
H11β		1.648	1.797	1.810
C12	31.96	30.842	31.154	31.163
H12α		1.725, ddd, 12.5, 4.9. 2.2	1.891	1.906
H12β		2.024, ~td, 13.0, 5.0	~1.923	1.962
C13	48.04	46.845	47.233	47.216
C14	84.16	82.963	84.745	84.666
C15	31.98	30.303	30.454	30.482
H15α		1.781, ~td, 11.6, 5.5	1.979, ~td, 10.4, 6.2	1.978
H15β		1.507, ~q, 9.6	1.631, ~dt	1.634
C16	21.47	20.251	20.493	20.565
H16α		1.871	1.859, ~q, 11.1	1.874
H16β		1.556	1.759, ~dt, 14.1, 3.4	1.733
C17	50.08	48.676	49.216	49.228
H17		2.259, ~t, 9.0	2.250, dd, 10.0, 8.8	2.266, dd, 9.8, 8.6
C18	17.87	17.114	17.268	17.302
H18		0.763, s	0.796, s	0.819, s
C19	24.46	23.841	23.352	23.405
H19		0.836, s	0.925, s	0.943, s
C20	76.82	75.682	77.599	77.513
C21	21.68	20.959	20.095	20.195
H21		1.062, s	1.175, s	1.204, s
C22	77.52	76.182	77.010	76.952
H22		3.115, dd, 10.5, 1.7	3.341, dd, 10.6, 1.9	3.337, dd, 10.6, 1.9
C23	27.45	26.073	26.018	26.023
H23		1.475, ~tdd, 12.4, 3.9, 1.8	1.535	1.535, dddd, 13.5, 10.4, 5.0, 1.9
H23′		1.111, dddd, 13.6, 11.6, 10.6, 4.5	1.237	1.248
C24	42.62	41.377	40.818	40.874
H24		1.645, ~td, 12.8, 5.0	1.651, ddd, 13.2, 10.6, 5.3	1.634
H24′		1.253, ddd, 13.1, 11.6, 4.3	1.417, ddd, 13.2, 11.1, 4.3	1.428, ddd, 13.3, 10.0, 5.0
C25	69.52	68.673	71.265	71.167

(*continued on next page*)

Table 1 (*continued*)

No.	**20E** in $CDCl_3$	**20E** in DMSO-d_6	**20E**-β-CD in D_2O	**20E**-2(β-CD) in D_2O
C26	29.99	29.972	28.052	28.178
H26		1.077, s	1.138, s	1.135, s
C27	30.09	28.990	27.949	27.910
H27		1.052, s	1.135, s	1.129, s
Signals of β-CD				
C1	–	–	102.238	102.135
H1			4.991, d, 3.8	4.992, d, 3.9
C2	–	–	72.161	72.138
H2			3.575, dd, 9.9, 3.7	3.577, dd, 9.9, 3.7
C3	–	–	73.248	73.203
H3			3.885, dd, 9.9, 9.0	3.886, dd, 9.9, 9.0
C4	–	–	81.466	81.379
H4			3.512, ~t, 9.3	3.515, ~t, 9.3
C5	–	–	71.929	71.899
H5			3.781	3.787
C6	–	–	60.282	60.286
H6 (2H)			~3.79	~3.79

CD) complex, which proves incomplete formation, i.e., a lower stability, of 1:2 complex. Similarly, to **20E**-β-CD complex, the symmetry of the β-CD compartment was observed. Since the observation of two different β-CD parts would be expected as was recently reported for 1:2 complex of cholesterol with permethylated β-CD at temperature under 20 °C [13], the ^{1}H spectra were recorded at temperature 27, 21, 16, 11, 6, and 1 °C. In all cases, only one set of signals was observed for β-CD, although, the broadening and tiny changes in chemical shifts on most of signals were observed. The signal of H1 proton of β-CD was chosen as an inner reference, since this proton is out of the β-CD cavity and thus it should be least affected by a complexation (Fig. 2). The changes ($\delta^{27°C} - \delta^{1°C}$) on H1, H2, H3, H4, and H5 signals of CD part were 0, +4,

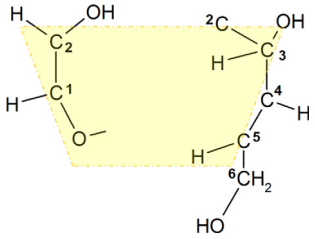

Fig. 2. A view on the cross-section of β-CD cavity.

−12, −7, and −8 ppb, respectively, i.e., the large change was observed on the inner H3 of β-CD. The analogous changes were observed on H1α (−2), H2 (+38), H3 (+18), H5 (−13), H7 (−8), H9 (+4), H17 (+3), H18 (−9), H19 (−19), H21 (−6), H22 (−3), H23 (−8), H24′ (0), H26 (−2), H27 (−4) signals of the **20E** part; the other signals are not reported due to overlaying. The largest changes on signals of the **20E** ring A means the largest changes in chemical environment upon the change of temperature, which could be attributed to stabilisation of the 1:2 complex, and changes in ring A configuration (*vide infra*).

A comparison of ^{1}H and ^{13}C chemical shifts ($\delta_{1:1} - \delta_{1:2}$) revealed various changes on **20E** part from −39 ppb on H12β to +26 ppb on H16β, and −126 ppb on C26 to +109 ppb on C6. The changes on the β-CD part were less than 6 ppb in ^{1}H chemical shifts, and +103, +23, +45, +87, +30, and −4 ppb on C1, C2, C3, C4, C5, and C6, respectively. As changes in chemical shifts are result of various contributions, an unambiguous conclusion is mostly unfeasible on those changes only. On the other hand, the observed changes are significant, which means that the second β-CD molecule affects a molecular composition, i.e., there is a formation of a 1:2 complex or complexes. All differences are given in supplementary materials in the xls file.

In general, the unambiguous conclusion on geometry of a complex can be done via interpretation of intermolecular interactions in ROESY spectra. However, since only one set of signals for β-CD was observed in

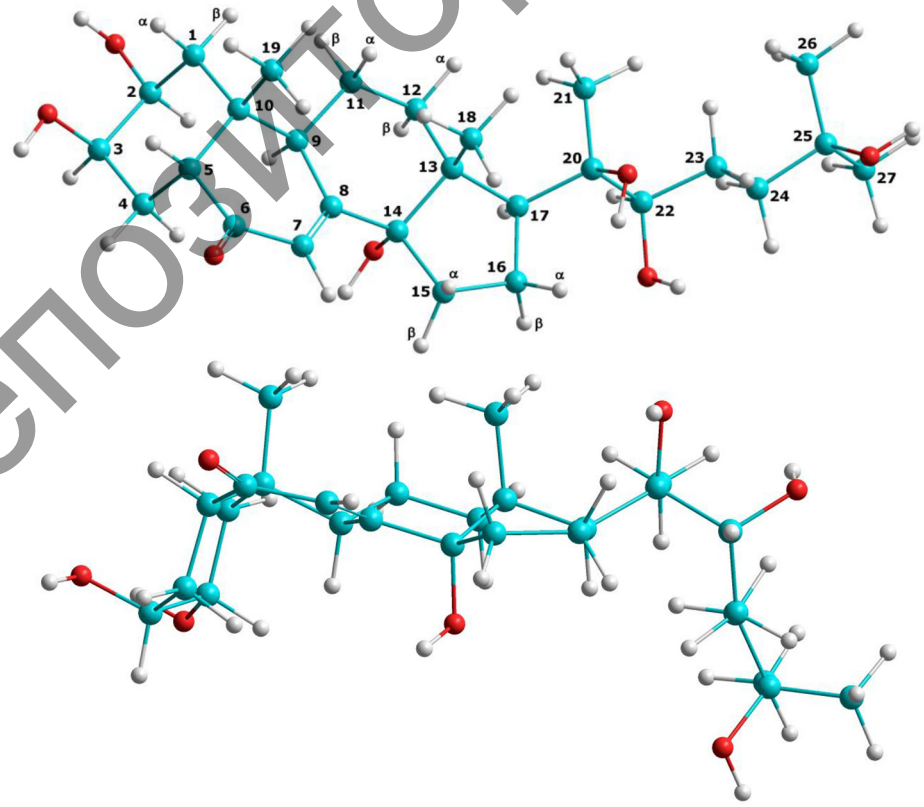

Fig. 1. Top and side views on a molecular model of **20E** as derived from NMR data and PM3 optimization.

all cases, the studied systems are under fast chemical exchanges. Therefore, the observed ROE has to be considered as related to an average structure of all conformers and all species. That is also reason the most of NMR characteristics of **20E**-β-CD and **20E**-2(β-CD) samples are similar, thus, we evaluated them jointly (see supplementary information Fig. S1). Similar complications were reported on complexation of cholesterol *via* various CD derivatives [15].

As expected, no conclusive ROE between **20E** and H1, H2, or H4 signals (outer hydrogens; see Fig. 2) of β-CD was observed on all samples. That suggests that **20E** is complexed inside the β-CD cavity exclusively, or the concentration of other complexes is undetectable at studied conditions. That attests that all observed intermolecular ROEs are due to the fact that **20E** is more or less inside the β-CD cavity.

In spite of a number of signal overlays, it was possible to rule out whether the aliphatic or steroid part of **20E** are placed within β-CD at the O6 (ROE to H5/H6) or O3 (ROE to H3) rim, based on unambiguous presence/absence of ROE on recognizable signals. The H5 and both H6 signals of β-CD are overlaid; however, as they are in proximity within the β-CD molecule, it is not a severe problem. The H3 signal of β-CD is overlaid by the H2 signals of **20E**, thus only the ROE crosspeaks, which are not observed in pure **20E** can be assigned to H3 of β-CD. Fortunately, these signals are separated at 1 °C, which enables more unambiguous interpretation (see supplementary information Fig. S2).

We identified a strong ROE of H5/H6 of β-CD to **20E** signals H23, H23′, H24′, H26/H27, as well as ROE of H3 of β-CD to **20E** signals H17, H18, H19, and H22. That suggests the aliphatic part of **20E** is inserted deeply into β-CD through the O3 rim. Surprisingly, there is also weak ROE of H5/H6 of β-CD to **20E** signals H18, H21 and H22, and H3 of β-CD to **20E** signals H26 and H27. That can be explained by simultaneous complexation through the O6 rim, or by complexation through the O3 rim, however, with β-CD having at least one sugar unit turned over.

The ROEs of H3 of β-CD to **20E** signals H1β, H4α, H4β, H5, and H19, suggests complexation of the A ring, however, there is no ROE to H1α. That can be understood as a hindering of H1α from a ROE constant by the nearby methyl H19 and the OH group(s).

The comparison of ROESY spectra of **20E**-β-CD and **20E**-2(β-CD) revealed that a small ROE of H5/H6 of β-CD to H7 of **20E** is observed only in the case of **20E**-2(β-CD) sample both at 27 and 1 °C. Surprisingly, there is no ROE to H9 of **20E**, which could be expected. That made it impossible to build ~~up~~ a reasonable molecular model of a 1:2 complex. We conclude that the oddness can origin from a change of the A ring conformation upon ~~a~~ complexation. That is supported by observation that pure **20E** has a strong ROE between H9 and H2, and no ROE between H9 and H3, which is consistent with a molecular model where distance H9-H2 is 0.18 nm, while H9-H3 distance is 0.38 nm. However, both **20E**-β-CD and **20E**-2(β-CD) have also a significant ROE between H9-H3 (see Supplementary information Fig. S3). A change of the ring A conformation can be supported also by the largest changes in chemical shifts on that ring upon the change of temperature (*vide supra*). Unfortunately, we were unable to identify significant changes in HMBC to uncover more details on the geometry changes.

2.2. Chemistry

Cyclodextrins with purity of 99% were purchased from Fluka company. The optical rotation angles were determined on the Atago Polax 2L polarimeter, IR spectra (KBr) were obtained on the Avatar 360 ESP device, UV spectra were recorded on an Agilent Cary 60 spectrophotometer. Routine NMR spectra were recorded on a JNM-ECA Jeol 400 MHz instrument. Correlation NMR experiments were recorded on a 500 MHz instrument ECZ 500R (JEOL, Japan) at 27 °C. The chemical shifts (δ) are presented in ppm followed by integral intensity, multiplicity and corresponding homonuclear coupling constants (*J*) in Hz, and by the signal assignment, which is based on analysis of 2D COSY, HSQC, HMBC and ROESY correlation spectra. Both ^{1}H and ^{13}C chemical shifts are referenced to TMS (using the solvent signals as the internal reference), unless stated otherwise. The melting point were measured on the Boëtius (Hund Wetzlar, Germany) micro melting point instrument. Ultrasound bath was HO-230.00 ПС (Volgoda, Russia). Evaporation was done with Rotavapor R-3 (Büchi, Switzerland). We used electronic balances Practum 313-1 ORU (Sartorius Instruments, Germany).

For solubility prediction the Advanced Chemistry Development, Toronto, ACD/Percepta 14.2.0 (Build 2977) program was used.

Column chromatography was carried out on neutral alumina (Brockmann activity degrees used are given in the text). Sorbfil plates (IMID Krasnodar, RU) were used for TLC. HPLC analysis was performed on a Hewlett Packard Agilent 1100 Series instrument (150 × 4.6 mm analytical column, Zorbax SB-C18, mobile phase is 10% isopropyl alcohol, UV detection at 254 nm, column temperature 20 °C, 0.75 mL/min, the volume of the introduced sample is 20 μL).

PM3 optimizations were done with Hypercube HyperChem 8.0.6.

2.3. Plant material

The aerial parts of the plant *Silene wolgensis* were collected in 2016 during the flowering phase in the vicinity of Balyktykol village of the Karaganda region of the Republic of Kazakhstan. The raw material was dried in air and ground. The reference sample is deposited with International Research and Production Holding “Phytochemistry”, 470000 Karaganda, Republic of Kazakhstan.

2.4. *20E* and *2-DE* extraction and isolation

At the first stage, the aerial parts of the plant *Silene wolgensis* were extracted with 70% aqueous ethanol. Further, the content of ecdysterone was determined by the reversed-phase HPLC method.

Extraction of the aerial part (leaves, buds, stalks) of 1.0 kg of the ground air-dry raw material of *Silene wolgensis* was carried out with 10 L of 96% ethanol by heating in a water bath at the boiling point of the solvent for 1–1.5 h four times. The extract was cooled, drained and evaporated on a rotary evaporator at a temperature of no higher than 50 °C. 0.2 L of ethanol was added to the resulting thick brown syrup-like mass. Further, the resulting ethanol extract was treated with a 2:1 mixture of petroleum ether and AcOEt (0.6 L) to remove non-polar components, the remaining water-soluble part was extracted with 2-methylpropan-1-ol (0.6 L) resulting in a thick extract. The 2-methylpropan-1-ol extracts were combined and distilled to dryness in vacuum. We obtained 86.5 g of ecdysteroids with concomitant substances in the form of a thick green syrupy mass. The presence of ecdysterone was determined by thin layer chromatography (TLC) and qualitative analysis. Repeated column chromatography on alumina (activity of 1 as expressed in Brockmann degrees, sorbent mass 1.6 kg) and elution of the column with a chloroform-ethanol mixture (30:1) allowed separation of fractions 1–22, and elution with chloroform-ethanol (5:1) provided fractions 23–50. Fractions 1–22 containing **2-DE** were recrystallized from AcOEt; 0.5 g of **2-DE**, $C_{27}H_{44}O_6$, m.p. 207 °C (AcOEt-MeOH). $[\alpha]_D^{20}$ 66.0 (1.0, MeOH). IR spectrum (KBr), ν, cm^{-1}: 3320, 2950, 1650 (C═O, conjugated), 1450, 1200, 1080, 880. UV spectrum (EtOH), λ_{max}, nm: 244 (log ε 4.10). Experimental data are in accord with those from literature [12,17].

Compound isolated from the fractions 23–50 (1.0 g) was characterized as ecdysterone (**20E**), $C_{27}H_{44}O_7$, m.p. 236–238 °C (AcOEt-MeOH). $[\alpha]_D^{20}$ + 66.0 (1.0, MeOH). IR (KBr), ν,cm^{-1}: 3450, 2950, 1652, 1450, 1390, 1060, 880. UV spectrum (EtOH), λ_{max}, nm: 243 (log ε 4.10). ^{1}H and ^{13}C NMR (see Table 1).

Experimental data are in accord with those from literature [16–19].

2.5. Preparation of inclusion complexes.

The inclusion complexes of **20E** with β-cyclodextrin were obtained by the interaction of equimolecular amounts of solutions of **20E** and β-

Table 2
Solubilities of 20E and its β-CD complexes.

Sample	Solubility mg/mL	Solubility enhancement in comparison with **20E**
20E	0.084	–
20E-β-CD	8.87	105
20E-2(β-CD)	9.31	110

Table 3
20E water solubility ACD/Labs software prediction [20].

Thresholds	Probability	Reliability
> 10 mg/mL	0.03	Borderline (0.32)
> 1 mg/mL	0.21	Borderline (0.45)
> 0.1 mg/mL	0.61	Borderline (0.36)
> 0.01 mg/mL	0.91	Borderline (0.41)

cyclodextrin. 113 mg of β-CD (0.1 mmol), dissolved in 4 mL of distilled water were added to 50 mg (0.1 mmol) of **20E** dissolved in 3 mL of absolute ethanol. The solution was stirred on a magnetic stirrer at 50 °C for 8 h. The precipitate formed was filtered off, washed with ethanol and dried in an oven at 40 °C. The inclusion complexes of **20E**-β-CD were obtained as white powders. Similarly, we prepared the 1:2 inclusion compound. 226 mg of β-CD (0.2 mmol) was dissolved in 4 mL of distilled water and added to 0.05 g (0.1 mmol) of **20E** dissolved in 3 mL of absolute ethanol.

2.6. Solubility experiments

Experiments on solubility determination were done at 22 °C, air humidity 63%, and pH of distilled water used 6.55.

20E (100 mg) in a 2 L flask was mixed with 1 L of distilled water. The heterogeneous mixture was continuously mixed using ultrasound bath for 1 h. Then the solution was filtered through a sintered glass filter (class 2, 40–100 μm, diameter 0.5 cm). The clean solution was evaporated on rotary evaporator. Dry evaporate was weighed. Experiment was repeated in triplicate, in Table 2 are given mean values. Table 3 gives predicted values.

Similarly, both the supramolecular complexes **20E**-β-CD, and **20E**-2(β-CD) (aa 100 mg) were, in a 50 mL flask, mixed with 10 mL of water. The heterogeneous mixture was continuously mixed using an ultrasound bath for 1 h. Then the solution was filtered through a sintered glass filter (class 2, 40–100 μm, diameter 5 cm). The clean filtrate was evaporated on a rotary evaporator. The dry evaporate was weighed. The experiment was repeated in triplicate, in Table 2 are given mean values.

3. Conclusions

We prepared complexes of **20E** with α-, β-, γ-, 2-hydroxypropyl-β-, and 2-hydroxypropyl-γ-cyclodextrins. As the experience with all types of CD complexes was similar, the exact measurements and deep NMR study was done with β-CD only. On complexes of **20E** with β-CD we performed NMR studies targeting details of their molecular structures. We conclude that **20E** forms a stable 1:1 complex and weaker 1:2 complexes with β-CD. The **20E** molecule is deep inside the cavity of β-CD, entering through the O3 rim by aliphatic part to O6 rim. Since similar results we observed on complexation of cholesterol [14] we expect the geometry can be general for complexes of steroids with cyclodextrins. A more accurate view on the geometry of complexes will require support of computational studies as well as additional NMR experiments, and other analytical methods.

The results show 100 times enhanced solubility of **20E** when complexed with β-CD.

It should be noted that the water solubility of **20E** predicted by ACD/Percepta software was comparable to the experimental one. Prediction of solubility of the complexes was not possible using this software [19].

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (17-02836S), Ministry of Education and Sports of the Czech Republic (LO1304, LM2015063). This work was fulfilled under the grant project of Scientific Committee of the Ministry of Education and Science of the Republic of Kazakhstan №AP05133718.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2018.11.007.

References

[1] V.N. Syrov, Z.A. Khushbaktova, V.L. Yun, M.A. Dzhakhangirova, Ecdysteroid-containing drugs – representatives of a new generation of actoprotective agents, "TINBO" Bull. Tashkent 2 (2008) 92–101.
[2] B.I. Tuleuov, in: Steroid Compounds of Plants and Medicinal Preparations on their Basis. Search, Chemical Modification and Practical Aspects of Application, Glasier Karaganda KZ 2009, p. 208.
[3] N.Sh. Ramazonov, I.D. Bobayev, V.N. Syrov, Sh.Sh. Sagdullayev, A.U. Mamatkhanov, in: Chemistry, Biology and Technology for Obtaining Phytoecdysteroids: Fan va texnologiya 2016, p. 260.
[4] B.I. Tuleuov, Technology of Phytosteroid Preparations, Glasier Karaganda KZ, 2017, p. 11.
[5] L. Rinaldi, A. Binello, A. Stolle, M. Curini, G. Cravotto, Efficient mechanochemical complexation of various steroid compounds with α- β- and γ-cyclodextrin, Steroids 98 (2015) 58–62.
[6] J.-Y. Moon, H.-J. Hjung, M.Y. Moon, B.C. Chung, M.H. Choi, Inclusion complex-based solid-phase extraction of steroidal compounds with entrapped β-cyclodextrin polymer, Steroids 73 (2008) 1090–1097.
[7] P. Forgo, I. Vincze, K.E. Kover, Inclusion complexes of ketosteroids with β-cyclodextrin, Steroids 68 (2003) 321–327.
[8] A. Yasuda, M. Ikeda, Y. Naya, Analysis of hemolymph ecdysteroids in the crayfish procambarus-clarkii by high-performance capillary electrophoresis, Nippon Suisan Gakkaishi 59 (1993) 1793–1799.
[9] Y. Qin, T. Chen, Ecdysterone Cyclodextrin Inclusion Compound, Preparation, and Manufacture Thereof, 2014, CN 104225605 A.
[10] A. Meybeck, F. Bonte, G. Redziniak, Use of an Ecdysteroid in a Cosmetic or Dermatological Composition or Keratinocyte Culture Medium, 1994, WO 9404132 A2.
[11] R. Berghaus, M. Bratz, R. Dyllick-Brenzinger, Cyclodextrin-containing Suspension Concentrates and their Production Method and Use, 2012, US 20120021914 A1.
[12] B.I. Tuleuov, K.M. Turdybekov, G. Khhabdolda, S.M. Adekenov, O.A. Nurkenov, B.K. Tuleuova, A.M. Kozhanova, A.M. Almagambetov, Structure and stereochemistry of phytoecdysone from *Silene cretaceae* Fisch, Russ. J. Gen. Chem. 84 (2014) 625–628.
[13] M. Jurášek, P. Džubák, S. Rimpelová, D. Sedlák, P. Konečný, I. Frydrych, S. Gurská, M. Hajdúch, K. Bogdanová, M. Kolář, T. Müller, E. Kmoníčková, T. Ruml, J. Harmatha, P.B. Drašar, Trilobolide-steroid hybrids: synthesis, cytotoxic and antimycobacterial activity, Steroids 117 (2017) 97–104.
[14] K. Kučáková, B. Dolenský, Program and Abstract Book of the 19th International Cyclodextrin Symposium, Sophia University, Tokyo, Japan, 2018, p. 95.
[15] D. Castagne, G. Dive, B. Evrard, M. Frédérich, G. Piel, J. Pharm. Pharmaceut. Sci. 13 (2010) 362–377.
[16] http://ecdybase.org/index.php?&action=browse&row=98 (downloaded August 3, 2018).
[17] http://www.chemspider.com/Chemical-Structure.4573597.html?rid=e57812e5-cc1f-4cc2-a5ea-4dcd7a1c3c43 (downloaded July 27, 2018).
[18] I. Kubo, A. Matsumoto, F.J. Hanke, The ^{1}H-NMR assignment of 20-hydroxyecdysone, Agric. Biol. Chem. 49 (1985) 243–244.
[19] J. Huang, X. Xu, Z. Xie, Z. Xie, M. Yang, D. Yang, Isolation and purification of 20-hydroxyecdysone from radix serratulae chinensis by high-speed counter-current chromatography, J. Liquid Chromatogr. Rel. Technol 37 (2014) 1909–1916.
[20] Advanced Chemistry Development, Toronto, ACD/Percepta 14.2.0 (Build 2977).