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SYNTHESIS OF 20-HYDROXYECDYSONE 25-ACETATE

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A scheme has been developed for the selective conjugation of 20-hydroxyecdysone (20E) at the 25- position, with the synthesis of 20E 25-acetate as an example. The proposed scheme also permits the selective synthesis of 20E 22-esters from both lower and higher fatty acids, which opens up prospects for the creation from them of drugs with a prolonged action.

The ecdysteroids are a group of natural compounds possessing a broad spectrum of biological activity. In insects they are molting hormones [1]. In mammals and man these compounds possess a pronounced tonic action. Prospects have been shown for their use as components of drugs with an adaptogenic, cardiotonic, antiatherosclerotic, antiulcer, and wound-healing action [2].

In addition to the free ecdysteroids, conjugates of them have been detected in natural materials of both plant and animal origin. The most widespread class of ecdysteroid conjugates is formed by monoesters of α-ecdysone and 20-hydroxyecdysone (20E), namely their 2-, 3-, and 25-acetates and their 22-esters with higher carboxylic acids [3-6]. Their physiological functions have not yet been elucidated in detail. So far as concerns invertebrates, they are regarded as inactive, reserve, forms of the hormones [7]. In view of what has been said above, the synthesis of monoesters of ecdysteroids and the creation from them of a new type of ecdysteroid-containing drugs with a prolonged action is a promising direction.

The task of our investigation was the development of a scheme for the selective conjugation of 20E at the 25- position, using as an example the synthesis of 20E 25-acetate (20E25Ac).

Structurally, 20E (1) is a polyhydroxy compound. Since the capacity for the esterification of the hydroxy groups of 20E decreases in the sequence C-2 > C-22 > C-3 >> C-25 [6], the synthesis of its 25-esters is possible only if the diol groups are protected. A method is known for obtaining 20E25Ac by the preliminary protection of the diol groups in the form of a diacetonide [3]. However, hydrolysis of the product of the acylation of 20E diacetonide gave 20E25Ac with a yield of less than 0.3%. The main hydrolysis product was 20E25Ac 20,22-monoacetonide, which shows the resistance of 20E 20,22-monoacetonide to hydrolysis. Our attention was then attracted to a paper on the synthesis of an ecdysteroid with a long side-chain from cyasterone by Ourisson et al. [8], who succeeded in selectively protecting the 20,22-diol group in the form of the phenylboronate and the 2,3-diol group in the form of the acetonide.

We have used this method of protecting the diol groups in developing a scheme for the selective conjugation of 20E at C-25 for the exemplary case of the synthesis of 20E 25-acetate. The 2,3-acetonide of 20E 20,22-phenylboronate (3) proved to be stable under the conditions of esterification. The resulting 2,3-acetonide of the 25-acetate (5) was stable under the conditions for the hydrolysis of the phenylboronate (with an aqueous solution of hydrogen peroxide). The acetonide protection was readily eliminated in a weakly acid medium (under the action of a cation-exchanger in the H^+- form).

The steps and conditions for the chemical transformation of 20E (1) at the 25- position are shown in the scheme. The proposed scheme also permits the selective synthesis of 22-esters of 20E. In later studies we propose to show the use of this scheme for obtaining 22-esters of 20E with acetic acid and higher fatty acids.

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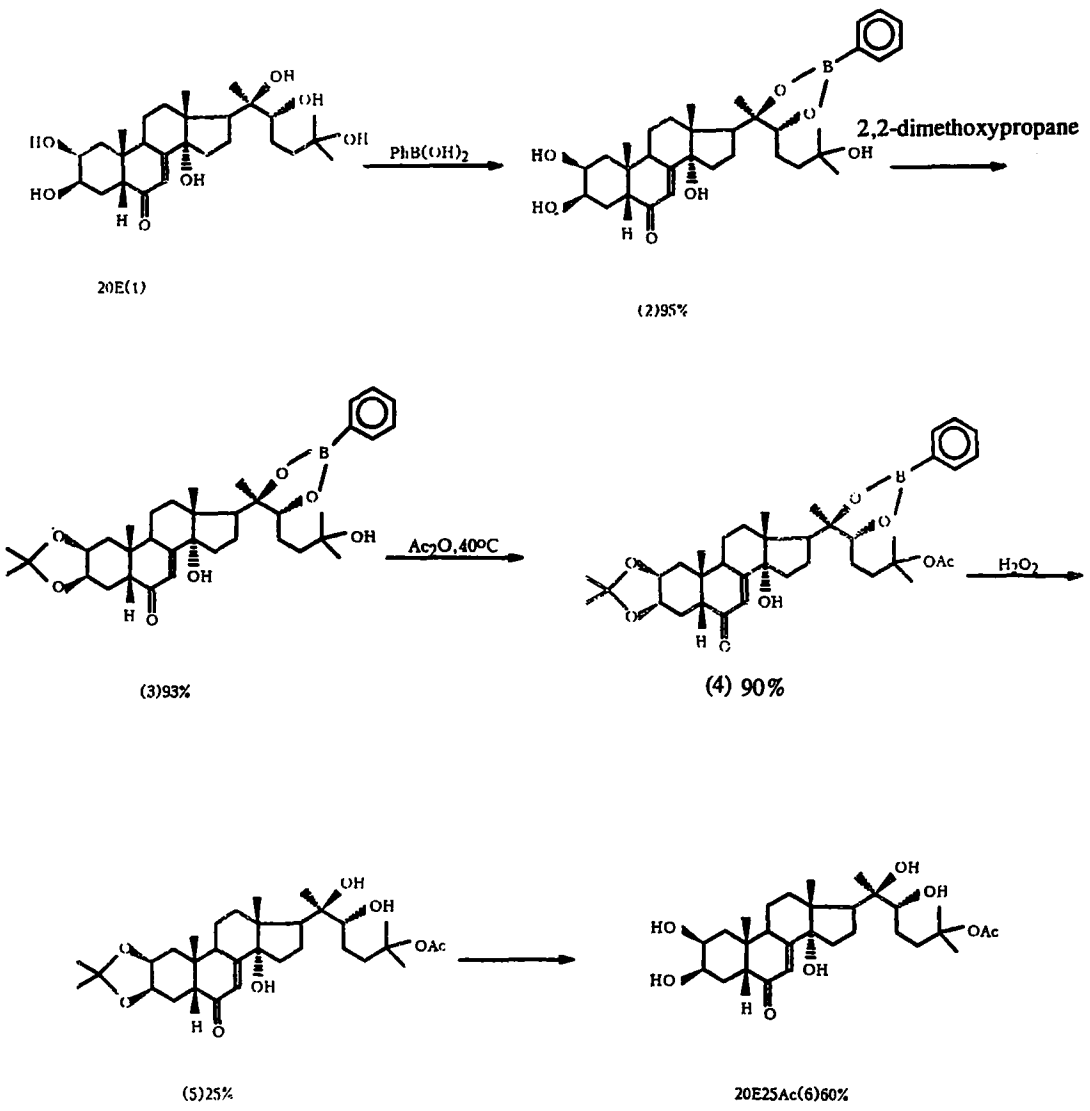
EXPERIMENTAL

The solvents — isopropanol (i-PrOH), hexane (C_6H_{14}), methanol (MeOH), acetone, pyridine, tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), ethyl acetate (AcOEt), dimethylformamide (DMFA), toluene, and butan-1-ol (BuOH-1) — were purified and rendered absolute by the methods described in [9]. In the case of mixed solvents, the proportions are given by volume.



Chemical modification of 20E (1) at the 25-position.

Phenylboronic acid ($PhB(OH)_2$ from Fluka (Switzerland) was used without purification.

2,2-Dimethoxypropane was synthesized as described in [10] and was purified by vacuum distillation.

p-Toluenesulfonic acid (*p*-TSA) was synthesized and purified by the procedure described in [11] and was dried over P_2O_5.

Acetic anhydride (Ac_2O) was purified by vacuum distillation. The purities of the solvents, the Ac_2O, and the 2,2-dimethoxypropane were evaluated from their refractive indices.

The cation-exchanger (KU-2) in the H^+ form was dewatered by azeotropic distillation in toluene and was then dried in vacuum.

Thin-layer chromatography (TLC) was conducted on Silufol-UV 254 plates (Czechoslovakia). The solvent systems are described in the text. Detection was achieved in UV light.

Low-pressure column chromatography was performed on alumina (Brockmann activity grade II) and silica gel L 100/250 μm (Czechoslovakia). The solvent systems are described in the text. Preparative high-performance liquid chromatography (HPLC) was conducted in the isocratic regime on 250 × 25 mm columns with Diasorb C_{16}/T (10 μm). The mobile phase used was H_2O–MeOH–BuOH-1 (100:5:5). Rate of elution 20 ml/min. Detection was effected with a RIDK-101 refractometer (Czechoslovakia). Analytical HPLC was conducted in the isocratic regime on a 150 × 4.6 mm column with Separon SGX (5 μm). The eluent used was C_6H_{14}–i-PrOH–H_2O (100:40:3). Rate of elution 0.5 ml/min. Monitoring by means of a UV detector at a wavelength of 254 nm.

Extraction of the Raw Material and Isolation of 20E (1). The whole epigeal part of *Serrulata coronata* L. (500 g) was extracted in a Soxhlet apparatus with 2 liters of MeOH for 8 h. The extract was evaporated to a volume of 0.3 liter, diluted with water to 2 liters, filtered through a Schott filter, and evaporated to 0.5 liter. The ecdysteroids were extracted from the purified aqueous solution with water-saturated BuOH-1 (3 × 1 liter). The combined aqueous-butanolic extracts were evaporated to a syrupy mass which was then diluted with 0.5 liter of MeOH and deposited on a column of alumina (200 g, 250 × 60 mm). Elution was carried out with $CHCl_3$–MeOH (4:1) and was monitored by TLC (eluent $CHCl_3$–MeOH (4:1)). The total ecdysteroid fraction obtained in this way was evaporated and recrystallized (from AcOEt-MeOH), giving 5.4 g of ecdysteroids. 20E was obtained from the total ecdysteroid fraction by reversed-phase preparative HPLC. As a result we obtained 5 g of 20E with a purity of 98% by weight.

20E 20,22-Phenylboronate (2). The synthesis was carried out by the procedure described in [8]. A solution of 0.48 g (1.0 mmole) of substance (1) in 2 ml of absolute methanol (or acetone) was treated with 0.18 g (1.1 mmole) of $PhB(OH)_2$, and the mixture was stirred until dissolution was complete. After the resulting solution had been left at room temperature for 20 min, TLC monitoring (eluent: CH_2Cl_2–MeOH (85:15); R_f(PBA) 0.76; R_f(1) 0.18; R_f(2) 0.49) showed completion of the reaction. The solvent was distilled off in vacuum at 30°C. The solid residue was dissolved in the minimum amount of EtOAc and was chromatographed on a column (80 × 20 mm) with 10 g of SiO_2 in EtOAc. Elution was performed with MeOH in EtOAc (0-5%), giving 0.40 g of substance (2). Yield 90%.

20E 2,3-Acetonide 20,22-Phenylboronate (3). The synthesis was conducted by the method described in [8]. A solution of 0.25 g (0.44 mmole) of substance (2) in 5 ml of absolute acetone was treated with 0.35 ml (2.5 mmole) of 2,2-dimethoxypropane, and then with 3 mg (0.17 mmole) of *p*-TSA, and the mixture was stirred until the solid matter had dissolved. After the resulting solution had been left at room temperature for three hours, TLC monitoring (eluent: CH_2Cl_2–MeOH (9:1); R_f(*p*-TSA) 0.17; R_f(2) 0.44; R_f(3) 0.53) showed that the reaction was complete. The acetone and 2,2-dimethoxypropane were distilled off in vacuum at 30°C. The solid residue was dissolved in the minimum amount of EtOAc and was chromatographed on a column (200 × 20 mm) with 25 g of SiO_2 in EtOAc. Elution by MeOH in EtOAc (0-15%), giving 0.25 g of substance (3). Yield 93%.

20E 2,3-Acetonide 20,22-Phenylboronate 25-Acetate (4). The acylation reaction was conducted by the procedure described in [8]. In solution in 1.5 ml of absolute pyridine, 0.06 g (0.1 mmole) of substance (3) was acylated with 1 ml of Ac_2O at 40°C for 45 h. The solvent and the excess of Ac_2O were distilled off in vacuum at 30°C, and the solid residue was dissolved in the minimum amount of EtOAc. TLC analysis (eluent: CH_2Cl_2–MeOH (9:1); R_f(3) 0.51; R_f(4) 0.65) of the solution showed the presence of substances (3) and (4). It was chromatographed on a column (50 × 10 mm) with 5 g of SiO_2 in EtOAc with elution by EtOAc, giving 0.06 g of substance (4). Yield 90%.

20E 20,22-Phenylboronate 25-Acetate (5). The synthesis was conducted by the procedure described in [8]. A solution of 0.06 g (0.09 mmole) of substance (4) in 0.6 ml of aqueous THF (THF–H_2O (9:1)) was treated with 0.82 ml (7 mmole) of a 30% aqueous solution of H_2O_2. After the reaction mixture had been left at room temperature for 3 h, TLC monitoring (eluent: CH_2Cl_2–MeOH (9:1); R_f(4) 0.63; R_f(5) 0.55; R_f($PhB(OH)_2$) 0.70) showed completion of the reaction. Then 0.6 ml of distilled water was added, and the THF was driven off in vacuum at 30°C. The residue was repeatedly extracted with EtOAC (not less than 5 × 5 ml). The organic phase was washed twice with 1 N NH_4Cl, 1 N $Na_2S_2O_3$, 0.1 N HCl, and saturated NaCl solution, and was dried over anhydrous Na_2SO_4. Then the solvent was distilled off in vacuum at 30°C, and the solid residue was dissolved in the minimum amount of EtOAc and chromatographed on a column (80 × 20 mm) with 10 g of SiO_2 in CH_2Cl_2. Elution by MeOH in CH_2Cl_2 (0-10%), giving 0.012 g of compound (5). Yield 25%.

20E 25-Acetate (6). The synthesis was conducted by the procedure described in [8]. A solution of 0.012 g (0.02 mmole) of substance (6) in 0.4 ml of CH_2Cl_2–MeOH (1:1) was treated with 0.2 g of cation-exchanger (KU-2) in the H^+-form. The mixture was stirred at room temperature for three days and the liquid was decanted off. The cation-exchanger was washed repeatedly with MeOH. The solutions were combined, and the solvent was distilled off in vacuum at 30°C. The solid

residue was dissolved in CH_2Cl_2–MeOH (1:1), and TLC analysis of the solution (eluent: CH_2Cl_2–MeOH (85:15); R_f(**5**) 0.53; R_f(**6**) 0.47; R_f(**1**) 0.18) showed that the product was the individual substance (**6**). The yield of (**6**) was 0.006 g (60%).

The yield of the final product (**6**), referred to (**1**), was 15%.

The purity of the initial substance 20E (**1**) and of the reaction products at each stage of the synthesis was confirmed by normal-phase HPLC.

The initial 20E and the reaction products at each stage of the synthesis were identified by IR, UV, and ^{13}C and ^{1}H NMR spectroscopies and mass spectrometry.

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