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# SYNTHESIS OF 20-HYDROXYECDYSONE CARBOXYLATE ESTERS

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*Acylation of 20-hydroxy-2,3-*O*-isopropylideneecdysone (**2**) by carboxylic-acid anhydrides produced ecdysteroid derivatives containing various organic acids (**5**–**12**).*

**Keywords:** phytoecdysteroids; 20-hydroxyecdysone; 20-hydroxy-2,3-*O*-isopropylideneecdysone; acetylation; acetic, succinic, propionic, and butyric anhydrides; transformation.

Phytoecdysteroids constitute an important class of biologically active compounds (BACs) with broad spectra of biological and pharmacological activities. The distribution of phytoecdysteroids among plants of Uzbekistan and their biological activities and pharmacological uses were reported [1, 2]. Ecdysteroids diminish the hemolytic effects of anabolic steroids by stimulating *in vitro* and *in vivo* thymocyte proliferation [3], limit the loss of thymus mass [4], increase the protective activity of blood lymphocytes and neutrophils [5], and strengthen phagocytosis [6]. Ecdysteroids were confirmed to activate the T-cell portion of the immune system and phagocyte functioning [7]. A dose-dependent limitation of anti-IGE-induced histamine release from cloud cells of experimental animals was found. The antihistamine effect was associated with inhibition of intracellular $Ca^{2+}$ release [8].

Natural metabolites are transformed, in particular, acylated, to enhance the biological activity of BACs and to discover new types of activity. Acetylated ecdysteroid derivatives possess pronounced wound-healing and antimicrobial properties [9]. 20-Hydroxyecdysone (**1**) derivatives can activate DNA synthesis in lymphocytes if stimulated by polyclonal mitogens [10].

Studies of the chemical transformations of **1** found that the hydroxyls were reactive toward conjugation and placed them in the following order of decreasing reactivity: $C_2 > C_{22} > C_3 > C_{25} > C_{20} >> C_{14}$ [9, 11]. Therefore, tri- and tetraesters were rather easily prepared by direct esterification of **1** by a large excess of the organic acid chloride or anhydride [11].

20-Hydroxy-2,3-*O*-isopropylideneecdysone (**2**), 20-hydroxy-20,22-*O*-isopropylideneecdysone (**3**), and 20-hydroxy-2,3,20,22-di-*O*-isopropylideneecdysone (**4**) were prepared by the known method [12–14].

The mixture of **2**–**4** was separated by column chromatography. Their structures were confirmed using PMR and $^{13}C$ NMR spectra.

A method for chemical transformation of **2** was developed for further synthesis of conjugates **5**–**12**.

The reaction of **2** with acetic anhydride afforded the derivatives 22,25-di-*O*-acetyl-20-hydroxy-2,3-*O*-isopropylideneecdysone (**5**) and 22,25-di-*O*-acetyl-20-hydroxyecdysone (**6**) (Scheme 1).

Acid hydrolysis of **5** removed the acetonide protecting group and produced **6** with C-22 and C-25 acetates. This was confirmed by PMR and $^{13}C$ NMR spectra (Table 1). The PMR spectrum showed a weak-field shift by 1.56 ppm of the H-22 resonance (δ 5.32) as compared with that in **1**. The acetate methyls resonated as strong-field singlets at 1.83 and 1.95 ppm (Table 1). Resonances for C-22 and C-25 in the $^{13}C$ NMR spectrum were shifted to weaker field (80.56 and 82.35 ppm, respectively). Furthermore, the aliphatic portion of the spectrum also exhibited resonances for two C atoms at 26.52 and 26.66 ppm that were attributed to methyl C atoms. Resonances for the two acetate carbonyls appeared at weaker field at 170.74 and 171.76 ppm.

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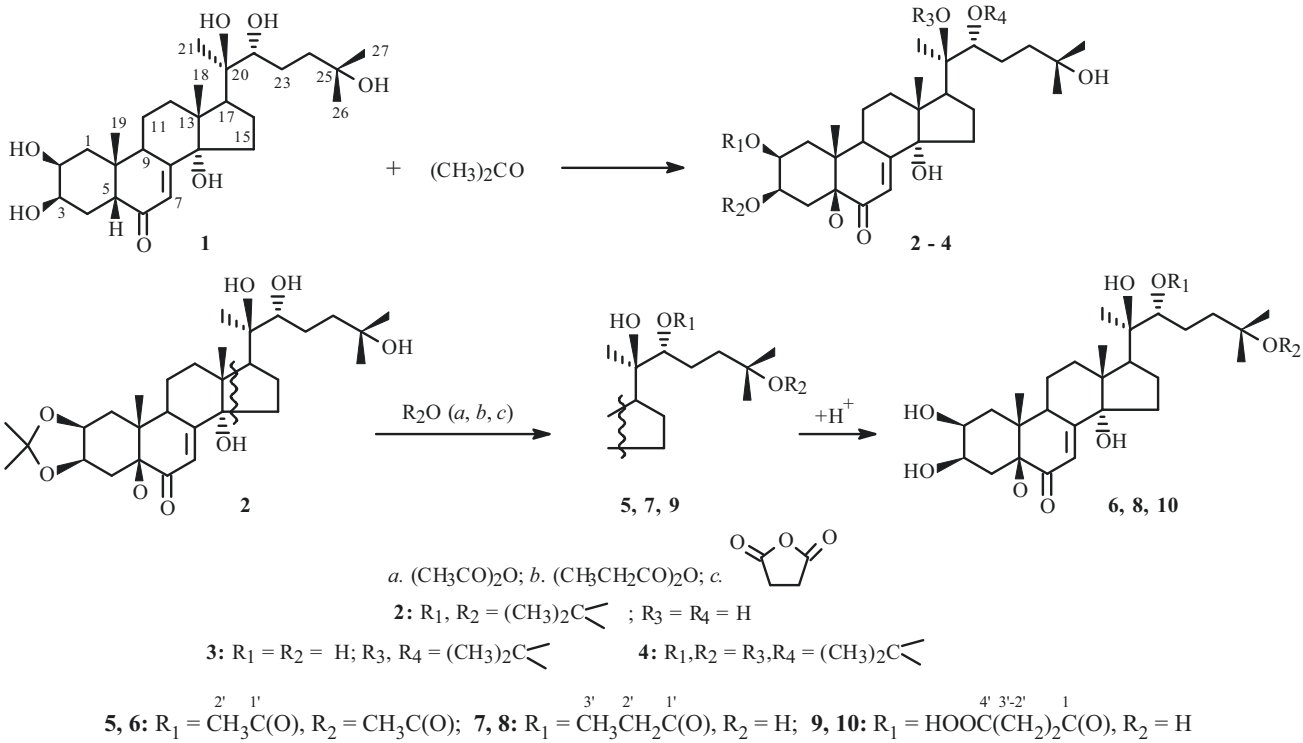



Scheme 1

The reaction of 20-hydroxy-2,3-*O*-isopropylideneecdysone (**2**) with propionic anhydride gave the new derivative 20-hydroxy-2,3-*O*-isopropylidene-22-*O*-propionylecdysone (**7**), which was converted by acid hydrolysis into 22-*O*-propionyl-20-hydroxyecdysone (**8**).

The PMR spectrum of **8** had resonances for methine protons H-2 and H-3 at 4.08 (br.d, J = 10.9 Hz) and 4.12 ppm (br.s), respectively, that did not change as compared with those in **1** although the H-22 resonance appeared as a doublet (J = 10.8) at 5.39 ppm that shifted by 1.63 ppm to weaker field. The spectrum exhibited characteristic resonances for ethyl at 0.96 (t, J = 7.5 Hz) and 2.26 ppm (q, J = 7.5 Hz) (Table 1). An analysis of the $^{13}C$ NMR data and DEPT spectra of **8** showed resonances for 30 C atoms. The $^{13}C$ NMR spectrum of **8** showed a weak-field shift by 2.7 ppm for the C-22 resonance (δ 80.74 ppm) as compared with that of **1**. Furthermore, resonances at 10.13, 28.63, and 175.21 ppm for the three propionic-acid C atoms were also found in the spectrum. These results indicated that the ester in **8** was located on C-22.

Compound **2** and succinic anhydride produced the new derivatives 20-hydroxy-2,3-*O*-isopropylidene-22-*O*-succinylecdysone (**9**), acid hydrolysis of which removed the protecting group to give 20-hydroxy-22-*O*-succinylecdysone (**10**).

Acylation of **2** by acetic anhydride occurred primarily at the secondary C-22 and tertiary C-25 hydroxyls to form 22,25-di-*O*-acetyl-20-hydroxy-2,3-*O*-isopropylideneecdysone (**5**) although the reactions with propionic and succinic anhydrides occurred only at secondary C-22 to form 20-hydroxy-2,3-*O*-isopropylidene-22-*O*-alkylecdysones **7** and **9**.

The reactivity for esterification of **2** depended on the carbon radical of the carboxylic-acid anhydride. Whereas $CH_3C(O)$ added readily and formed the diester, $CH_3CH_2C(O)$ added only to the hydroxyl on secondary C-22 to form the ester.

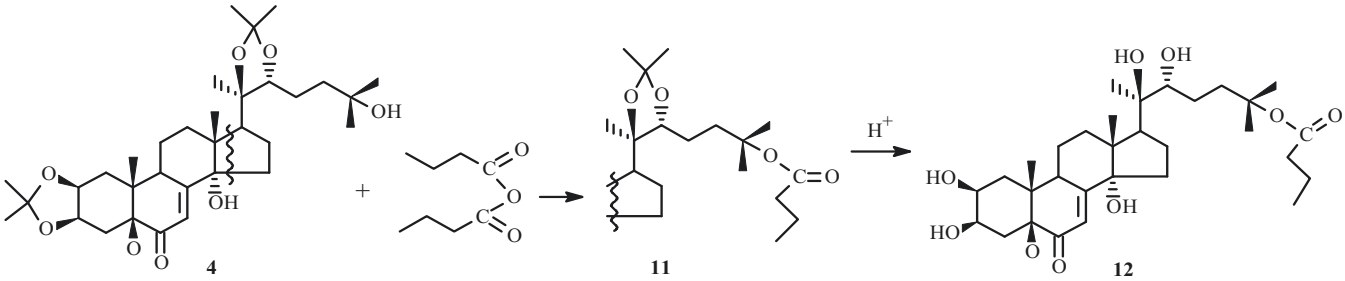
The PMR spectrum of **10** had chemical shifts for H-2, H-3, and H-22 (4.07, 4.11, and 5.45 ppm, respectively) that were about the same as those of **8**. An analysis of the $^{13}C$ NMR spectra of **10** showed a weak-field shift of the C-22 resonance (81.21 ppm). Furthermore, the aliphatic portion of the spectrum had resonances at 31.02 and 30.85 ppm for the two succinic-acid C atoms in addition to those for the steroid. Resonances for the two carbonyl C atoms appeared at weaker field at 173.82 and 176.13 ppm. These results established that the ester formed on C-22.

The reaction of 20-hydroxy-2,3,20,22-di-*O*-isopropylideneecdysone (**4**) with butyric anhydride produced the new derivative 25-*O*-butyryl-20-hydroxy-2,3,20,22-di-*O*-isopropylideneecdysone (**11**); hydrolysis (10% HCl–$H_2O$) of diacetonide **11**, 25-*O*-butyryl-20-hydroxyecdysone (**12**) (Scheme 2).

TABLE 1. $^{13}C$ NMR and PMR Chemical Shifts of **6**, **8**, **10**, and **12** (Py-$D_5$, δ, ppm, J/Hz)

| C atom | **6** | **8** | **10** | **12** |
|---|---|---|---|---|
| 1 | 38.46 | 38.47 | 38.47 | 38.47 |
| 2 | 68.65 | 68.68 | 68.68 | 68.67 |
| 3 | 68.56 | 68.57 | 68.57 | 68.56 |
| 4 | 32.98 | 32.97 | 32.98 | 32.95 |
| 5 | 51.89 | 51.91 | 51.91 | 51.90 |
| 6 | 203.97 | 204.03 | 204.05 | 204.02 |
| 7 | 122.29 | 122.25 | 122.24 | 122.17 |
| 8 | 166.36 | 166.45 | 166.47 | 166.62 |
| 9 | 34.87 | 34.89 | 34.89 | 34.92 |
| 10 | 39.18 | 39.18 | 39.18 | 39.17 |
| 11 | 21.57 | 21.58 | 21.57 | 21.62 |
| 12 | 32.52 | 32.50 | 32.51 | 32.52 |
| 13 | 48.57 | 48.64 | 48.65 | 48.60 |
| 14 | 84.60 | 84.64 | 84.64 | 84.66 |
| 15 | 32.18 | 32.21 | 32.20 | 32.28 |
| 16 | 22.06 | 22.10 | 22.05 | 21.99 |
| 17 | 51.01 | 50.96 | 50.89 | 50.60 |
| 18 | 18.39 | 18.40 | 18.42 | 18.44 |
| 19 | 24.93 | 24.95 | 24.96 | 24.97 |
| 20 | 76.62 | 76.84 | 76.88 | 77.34 |
| 21 | 21.77 | 22.88 | 22.96 | 22.19 |
| 22 | 80.56 | 80.74 | 81.21 | 77.77 |
| 23 | 25.93 | 26.62 | 26.58 | 24.23 |
| 24 | 38.54 | 42.24 | 42.07 | 43.22 |
| 25 | 82.35 | 69.77 | 69.82 | 80.76 |
| 26 | 22.74 | 30.61 | 30.67 | 27.28 |
| 27 | 22.74 | 30.18 | 30.13 | 27.14 |
| 1′ | 170.74; 171.76 | 175.21 | 173.82 | 173.36 |
| 2′ | 26.52; 26.66 | 28.63 | 31.02 | 40.48 |
| 3′ | | 10.13 | 30.85 | 27.55 |
| 4′ | | | 176.13 | 12.08 |

| C atom | **6** | **8** | **10** | **12** |
|---|---|---|---|---|
| 2 | 4.05 (br.d, J = 11.0) | 4.08 (br.d, J = 10.9) | 4.07 (dd, J = 11.4, 3.4) | 4.05 (dd, J = 12.0, 3.6) |
| 3 | 4.12 (br.s) | 4.12 (br.s) | 4.11 (br.s) | 4.11 (br.s) |
| 5 | 2.89 (dd, J = 13.1, 3.6) | 2.89 (m) | – | 2.89 (dd, J = 13.2, 3.6) |
| 7 | 6.10 (d J = 2.3) | 6.12 (d, J = 2.4) | 6.11 (d, J = 2.3) | 6.13 (d, J = 2.0) |
| 9 | 3.46 (br.t, J = 8.5) | 3.46 (br.t, J = 8.9) | 3.45 (br.t, J = 8.6) | 3.46 (br.t, J = 8.6) |
| 17 | 2.82 (t, J = 9.0) | 2.87 (t, J = 9.1) | – | 2.84 (t, J = 8.6) |
| 18 | 1.04 (s) | 1.04 (s) | 1.03 (s) | 1.09 (s) |
| 19 | 0.93 (s) | 0.93 (s) | 0.92 (s) | 0.94 (s) |
| 21 | 1.52 (s) | 1.52 (s) | 1.52 (s) | 1.45 (s) |
| 22 | 5.32 (d, J = 9.9) | 5.39 (d, J = 10.8) | 5.45 (dd J = 10.8, 1.9) | 3.73 (dd, J = 9.9, 4.0) |
| 26 | 1.28 (s) | 1.22 (s) | 1.23 (s) | 1.17 (s) |
| 27 | 1.30 (s) | 1.23 (s) | 1.21 (s) | 1.19 (s) |
| 2′ | 1.83 (s); 1.95 (s) | 2.26 (q, J = 7.5) | – | – |
| 3′ | – | 0.96 (t, J = 7.5) | – | – |
| 4′ | – | – | – | 0.68 (t, J = 7.4) |
| OH | 5.42 (s) | 5.25 (s) | – | 5.95 (br.s) |
| OH | 5.94 (br.s) | 5.59 (br.s) | – | 6.25 (s) |
| OH | 6.05 (br.s) | 5.97 (br.s) | – | – |
| OH | 6.33 (s) | 6.04 (br.s) | – | – |
| OH | – | 6.33 (s) | – | – |



Scheme 2

The structure of **12** was elucidated by analyzing the PMR and $^{13}C$ NMR spectra and a DEPT experiment (Table 1). The PMR spectrum showed resonances for methine protons H-2, H-3, and H-22 at 4.05, 4.11, and 3.73 ppm, respectively. These spectral data led to the conclusion that **12** contained free 2,3,22-OH groups and that the butyric acid was located on C-25. The methyl of the butyric acid resonated at stronger field as a triplet (J = 7.4 Hz) at 0.68 ppm. The $^{13}C$ NMR spectrum of **12** displayed a weak-field shift for C-25 (80.76 ppm) as compared with that in **1**. The chemical shifts of the other hydroxyl-containing C atoms did not change significantly. The other C atoms of the butyric-acid moiety appeared in the aliphatic portion of the spectrum at 12.08, 27.55, and 40.48 ppm. The weak-field portion of the spectrum showed resonances for the steroid and the carbonyl C atom at 173.36 ppm. All these results indicated that the butyric-acid moiety of **12** was situated on C-25 of 20-hydroxyecdysone.

## EXPERIMENTAL

Melting points were determined on an M 5000 melting point meter (Kruss, Germany). UV spectra were recorded on a Lambda-16 spectrophotometer (PerkinElmer). IR spectra were taken from KBr pellets on a System 2000 FTIR spectrometer (PerkinElmer). NMR spectra were recorded in Py-$D_5$ on a Unity 400plus spectrometer (Varian) at operating frequency 400 MHz. TLC used Silufol UV 254 chromatography plates with detection by vanillin (3%) in EtOH and solvent systems $CHCl_3$–MeOH (50:1, 1; 30:1, 2; 20:1, 3; 15:1, 4; 12:1, 5; 10:1, 6; 8:1, 7; 6:1, 8).

**20-Hydroxy-2,3-*O*-isopropylideneecdysone (2).** A suspension of 20-hydroxyecdysone (**1**, 10.0 g, 2.08 mol) in $Me_2CO$ (200 mL) was treated with phosphomolybdic acid (PMA, 3.0 g, 0.16 mol), stirred at room temperature until homogeneous and green (~5 min), evaporated to ~25 mL, treated with $NaHCO_3$ solution (0.1%, 15 mL), and extracted with EtOAc (3 × 100 mL). The extract was evaporated and chromatographed over a column of $SiO_2$ (40 g, eluent system 4) to afford **2** (2.4 g, 22%), mp 242–243°C, $[\alpha]_D^{20}$ +29.6° (*c* 2.53, MeOH).

In addition to target **2**, 20-hydroxy-20,22-*O*-isopropylideneecdysone (**3**, 27% yield, mp 222–223°C) and 20-hydroxy-2,3,20,22-di-*O*-isopropylideneecdysone (**4**, 39% yield, mp 231–232°C) were isolated from the total reaction products.

**22,25-Di-*O*-acetyl-20-hydroxyecdysone (6).** A solution of **2** (0.2 g) in anhydrous Py (5 mL) was treated with acetic anhydride (5 mL), held at room temperature for 1 d, diluted with $H_2O$, and extracted with EtOAc. The extract was chromatographed over a column using system 2 to produce 22,25-di-*O*-acetyl-20-hydroxy-2,3-*O*-isopropylideneecdysone (**5**, 0.135 g, 67.5% yield), $R_f$ 0.56 (system 7), mp 186.8°C.

The extract (0.1 g) was treated with $H_2O$ (30 mL), acidified to pH 4 with HCl solution (10%), left at room temperature until starting **5** disappeared (course of reaction monitored by TLC), poured into $H_2O$, and extracted with EtOAc (2 × 25 mL). The solvent was removed. The dry residue was chromatographed over a column of silica gel with elution by system 1 to afford **6** (0.0585 g, 52.5%), $R_f$ 0.43 (system 7), mp 107.10°C. UV spectrum ($C_2H_3OH$, $\lambda_{max}$, nm): 243.63 (log ε 3.10). IR spectrum (KBr, ν, $cm^{-1}$): 3445.34 (OH), 1652.93 (7-en-6-ketone), 1731.15 (ester), 1254.09, 1147.15 (C–O–C). Table 1 lists characteristic resonances in the PMR and $^{13}C$ NMR spectra of **6**.

**22-*O*-Propionyl-20-hydroxyecdysone (8).** A solution of propanoic anhydride (0.52 g, 4.0 mmol) in Py (8 mL) was stirred on a magnetic stirrer, cooled with cold water, treated with **2** (2.0 g, 3.85 mmol) to produce 20-hydroxy-22-*O*-propionyl-2,3-*O*-isopropylideneecdysone (**7**, 1.01 g, 49.9% yield), $R_f$ 0.37 (system 7), mp 176.4°C. UV spectrum ($C_2H_3OH$, $\lambda_{max}$, nm) (log ε): 243.01 (4.01). The extract (1.0 g) was treated with $H_2O$ (30 mL), acidified to pH 4 with HCl solution (10%), left at room temperature until starting **7** disappeared, poured into $H_2O$, and extracted with EtOAc. The extract was dried over

$MgSO_4$ to produce **8** (0.59 g, 53%), $R_f$ 0.25 (system 7), mp 110.20°C. UV spectrum: 242.84 nm (log ε 3.2). IR spectrum ($\nu_{max}$, $cm^{-1}$): 3433.67 (OH), 1650.97 (7-en-6-ketone), 1716.71 (ester), 1226.37, 1149.45 (C–O–C). Table 1 lists characteristic resonances in the PMR and $^{13}C$ NMR spectra of **8**.

**20-Hydroxy-22-*O*-succinylecdysone (10).** A solution of succinic anhydride (3.6 g, 36 mmol) in Py (8 mL) was stirred on a magnetic stirrer, cooled with cold water, treated with **2** (2.0 g, 3.85 mmol) and worked up by a method analogous to that used above for **6**. The dry residue was purified over silica gel with elution by hexane–EtOAc to afford **9** (2.7 g, 32.7%), $R_f$ 0.25 (system 7), mp 177.4°C.

The isopropylidene protection was removed from **9** (1.0 g) in AcOH (70%, 10 mL) at 45°C over 2 h to produce **10** (0.56 g, 50.1%), $R_f$ 0.11 (system 7), mp 146.9°C. UV spectrum: 242.41 nm (log ε 3.9). IR spectrum (ν, $cm^{-1}$): 3379.33 (OH), 1638.18 (7-en-6-ketone), 1714.56 (ester), 1232.06, 1115.01 (C–O–C). Table 1 lists characteristic resonances in the PMR and $^{13}C$ NMR spectra of **10**.

**25-*O*-Butyryl-20-hydroxyecdysone (12).** A solution of butyric anhydride (0.064 g, 4.0 mmol) in Py (8 mL) was stirred on a magnetic stirrer, cooled with cold water, treated with 20-hydroxy-2,3,20,22-di-*O*-isopropylideneecdysone (**4**, 0.056 g, 1.00 mmol), stirred at room temperature until the starting compound disappeared, treated with $H_2O$ (1 mL), stirred for 1 h, treated with $H_2O$, and evaporated in a rotary evaporator to remove the Py and afford **11** (0.037 g, 58.67%), $R_f$ 0.38 (system 7), mp 204.1°C.

Compound **11** (0.037 g) was treated with $H_2O$ (30 mL), acidified to pH 4 with HCl solution (10%), left at room temperature until starting **11** disappeared, poured into $H_2O$, and extracted with EtOAc (2 × 25 mL) to afford **12** (0.028 g, 44.4%), $R_f$ 0.25 (system 7), mp 150.8°C. UV spectrum: 245.03 nm (log ε 3.8). IR spectrum ($\nu_{max}$, $cm^{-1}$): 3363.84 (OH), 1653.06 (7-en-6-ketone), 1686.31 (ester), 1211.12, 1110.12 (C–O–C). Table 1 lists characteristic resonances in the PMR and $^{13}C$ NMR spectra.

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