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SYNTHESIS OF 20-HYDROXYECDYSONE 6-CHLORONICOTINATES

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*New esters **2-5** that contain 6-chloropyridine groups characteristic of the alkaloid epibatidine were prepared by acylation of 20-hydroxyecdysone (**1**) by 6-chloronicotinoylchloride.*

Key words: 20-hydroxyecdysone, 6-chloronicotinates, 6-chloronicotinoylchloride, synthesis, *Serratula sogdiana*.

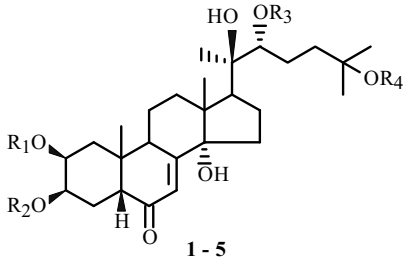
Ecdysteroids are an extensive group of natural steroids united by a common chemical structure and biological activity as shedding and metamorphosis hormones of insects and other invertebrates [1].

The plant *Serratula sogdiana* was previously studied in detail for ecdysteroid content [2-4]. The goal of our work was the chemical modification of natural ecdysteroids to increase the biological activity of the starting compound.

One of the distinguishing features of the ecdysteroid structure is a large number of hydroxyls that differ widely in their reactivity. As a result, ecdysteroids are exceedingly suitable starting materials for preparing new biologically active compounds using various chemical transformations [5].

We have previously synthesized several 3-(6-chloronicotinates) of 3β,5α,6β-trihydroxysteroids and 3β,5-dihydroxy-6-ketosteroids. A distinguishing feature of the compounds resulting from this was the presence in their structures of the α-chloropyridine moiety, which is characteristic of the alkaloid epibatidine, a natural analgesic isolated from the Ecuadorian frog *Epipedobates tricolor* [7]. The α-chloropyridine moiety is an important structural element that is responsible for the high biological activity of several modern neonicotinoid insecticides [8]. The pyridine ring occurs in the structure of the phytoecdysteroid diploclidine, which was isolated from *Diploclisia glaucescens* (Menispermaceae) [9].

In continuation of previous work [6], we synthesized 6-chloronicotinates of 20-hydroxyecdysone (**1**), one of the principal ecdysteroids that is widely distributed in plants and represents a convenient starting material for preparing new biologically active derivatives [1]. Acylation of **1** by 6-chloronicotinoylchloride in Py at room temperature was carried out for 4 d. The acylation catalyst was 4-dimethylaminopyridine. It was found that a mixture of four 6-chloronicotinates was formed under these conditions. Column chromatography could separate them into pure components, producing mono-, di-, tri-, and tetra-6-chloronicotinates, the structures of which were established by analyzing PMR and ^{13}C NMR spectra. The most interesting feature of these was that the labile 14α-hydroxy-Δ^7-6-keto group characteristic of starting 20-hydroxyecdysone (**1**) was retained in all acylation products. PMR spectra of the acylation products could rather simply indicate the number of 6-chloronicotinic acids bonded to the 20-hydroxyecdysone from the number of proton resonances of the pyridine rings.



1 - 5

1: $R_1 = R_2 = R_3 = R_4 = H$
2: $R_1 = Z, R_2 = R_3 = R_4 = H$
3: $R_1 = R_3 = Z, R_2 = R_4 = H$
4: $R_1 = R_2 = R_3 = Z, R_4 = H$
5: $R_1 = R_2 = R_3 = R_4 = Z$

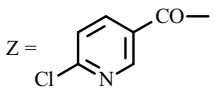


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TABLE 1. Chemical Shifts of C Atoms ($CDCl_3$, δ, ppm) in ^{13}C NMR Spectra of 20-Hydroxyecdysone (**1**) and Its Derivatives

C atom	**1** [1]*	**1** – 2,3,22-triacetate [1]	**3**	**4**	**5**
1	37.8	38.4	32.919	34.420	34.461
2	68.0	67.2	72.990	68.661	68.621
3	68.0	68.7	71.917	70.568	70.338
4	32.2	34.1	31.449	29.247	29.201
5	51.2	51.0	49.837	51.217	51.326
6	203.4	202.2	203.284	201.167	201.292
7	121.5	121.6	121.877	121.708	121.332
8	166.0	164.8	164.703	164.392	164.517
9	34.3	33.7	33.555	33.676	33.736
10	38.5	38.4	38.598	38.617	38.489
11	20.4	20.5	20.544	20.562	20.860
12	31.6	31.2	31.931	31.729	31.268
13	48.0	47.6	47.561	47.606	47.725
14	84.1	84.4	84.595	84.570	84.156
15	31.6	31.4	31.149	31.060	31.225
16	21.3	29.2	29.706	29.702	29.707
17	50.0	49.6	49.494	49.487	49.872
18	17.6	17.5	17.469	17.494	17.335
19	24.3	23.8	23.733	24.010	24.031
20	76.8	77.0	75.146	77.238	77.277
21	21.0	20.5	21.478	21.524	21.499
22	77.4	79.9	81.069	80.977	81.255
23	29.8	24.8	24.765	24.798	24.474
24	42.4	40.4	40.283	40.261	40.151
25	69.6	70.5	70.767	70.300	83.936
26	27.3	28.5	28.448	28.758	24.196
27	29.8	30.3	30.408	30.287	27.006
6-Chloronicotinate			163.956; 165.382; 151.137; 151.256; 155.903; 155.952; 139.760; 139.833; 124.339; 124.385; 171.549	163.555; 163.715; 165.415; 151.061; 151.138; 155.945; 156.048; 156.337; 139.473; 139.600; 139.767; 124.335; 124.384; 124.604	162.953; 163.512; 163.595; 165.296; 150.512; 151.057; 151.143; 155.057; 156.007; 156.052; 156.324; 139.476; 139.608; 139.800; 140.075; 124.297; 124.404; 124.580

*Solvent: C_5D_5N.

PMR spectra proved that the structure of the monoacylation product of **1** was the 2-(6-chloronicotinate) (**2**). Resonances of methine protons H-3α and H-22 in the spectrum recorded in C_5D_5N appeared at 4.523 and 3.921 ppm, respectively. The resonance of H-2α was overlapped by the strong resonance of water protons and was not observed. Chemical shifts of methine proton H-22 in spectra of **2** and **1** (δ 3.908 ppm) practically coincided. This led to the conclusion that the former had a free 22-hydroxyl. The resonance of equatorial H-3α in the spectrum of **2** was shifted to weak field by about 0.32 ppm compared with the position of the analogous proton in the spectrum of **1** (δ 4.20 ppm). Such an insignificant shift indicated that the 6-chloronicotinic acid in **2** was located on C-2 and not C-3.

PMR spectra proved reliably that the structure of diester **3** was the 2,22-di-(6-chloronicotinate). Resonances of H-2α and H-22 appeared at weak field of δ 5.279 and 5.166 ppm, respectively. This indicated that they were geminal to 6-chloronicotinate groups. However, the chemical shift of H-3α (δ 4.393 ppm) in the PMR spectrum indicated that **3** had a free 3β-hydroxyl.

PMR spectra also established unambiguously that the structure of triester **4** was the 2,3,22-tri-(6-nicotinate). Resonances of methine protons H-2α, H-3α, and H-22 at weak field of δ 5.480, 5.771, and 5.176 ppm, respectively, were important for proving the structure. This indicated unambiguously that the esters in **4** were situated at the 2-, 3-, and 22-positions of the steroid.

Resonances of H-2α, H-3α, and H-22 had approximately the same chemical shifts in the PMR spectrum of tetraester **5**. This established that three of the four 6-chloronicotinic acids were bonded at C-2, C-3, and C-22. Comparison of the chemical shifts of resonances for the 26- and 27-methyls in the PMR spectrum of **5** with analogous spectral characteristics of **4** was useful for determining the site of attachment of the fourth ester. It was informative that resonances of the 26- and 27-methyls in the spectrum of **5** were shifted significantly to weak field compared with their positions in the spectrum of triester **4**. Therefore, the ester in **5** was located at the 25-position. Thus, this compound was 20-hydroxyecdysone 2,3,22,25-tetra-(6-chloronicotinate).

Conclusions about the structures of **3-5** that were made based on the analysis of the PMR spectra were also confirmed by the ^{13}C NMR spectra, data for which are given in Table 1.

EXPERIMENTAL

PMR and ^{13}C NMR spectra in deuterated solvents were obtained on a Bruker Avance 500 spectrometer (operating frequency 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C). Chemical shifts are given relative to TMS as an internal standard. The course of reactions and purity of products were monitored using Kieselgel 60F_{254} plates (Merck).

Isolation of Ecdysteroids from *S. sogdiana*. *S. sogdiana* Bge. (Compositae) was collected in Namangan Oblast′, Republic of Uzbekistan, during the growth period (2006). Dried and ground aerial part of *S. sogdiana* (1.0 kg) was extracted with MeOH (6 × 8 L). The extract was concentrated to 0.3 L and diluted with an equal volume of water. The resulting precipitate was removed by filtration. The MeOH was evaporated. The remaining aqueous part was treated successively with $CHCl_3$ (1.5 L), EtOAc (0.6 L), and BuOH (0.6 L). Evaporation of solvents in vacuo produced EtOAc (8.5 g) and BuOH (57.3 g) fractions.

The BuOH fraction (57.3 g) was separated over a column of silica gel with elution by $CHCl_3$:CH_3OH (15:1, 9:1, 4:1) and $CHCl_3$:CH_3OH:H_2O (70:23:3) to afford 20-hydroxyecdysone and purified fractions of 20-hydroxyecdysone, 2-deoxyecdysterone, and viticosterone E [10].

Acylation of 20-Hydroxyecdysone (1) by 6-Chloronicotinoylchloride. A solution of **1** (24 mg, 0.05 mmol), 6-chloronicotinoylchloride (70.4 mg, 0.40 mmol) (prepared by the literature method [6]), and 4-dimethylaminopyridine (2 mg) in Py (1.5 mL) was held at room temperature for 4 d. The Py was removed from the mixture by repeated evaporation with benzene in a rotary evaporator under vacuum from a water aspirator and at a bath temperature of less than 30°C. The dry solid was separated into components by chromatography over a column of silica gel with elution by CH_2Cl_2:CH_3OH mixtures with gradually increasing CH_3OH content from 0.5% to 10%. Fractions containing pure products were combined and purified additionally from an impurity of 6-chloronicotinic acid by repeated chromatography over columns of silica gel with elution by CH_2Cl_2:CH_3OH mixtures to afford **5** (7.1 mg, 13.7%), **4** (9.3 mg, 20.7%), **3** (7.1 mg, 18.7%), and **2** (10.1 mg, 32.6%).

20-Hydroxyecdyson-2,3,22,25-tetra-(6-chloronicotinate) (5). Yield 7.1 mg, 13.7%. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.913 (3H, s, 18-Me), 1.134 (3H, s, 19-Me), 1.410 (3H, s, 21-Me), 1.600 (3H, s, 26-Me), 1.656 (3H, s, 27-Me), 3.306 (1H, m, W/2 = 24, H-9α), 5.232 (1H, d, J = 9.5, H-22), 5.472 (1H, dt, H-2α), 5.785 (1H, m, H-3α), 5.988 (1H, d, J = 2.5, H-7), 7.365 (1H, d, J = 8.0, H-5′), 7.470 (3H, m, H-5″, H-5‴, H-5⁗), 8.076 (1H, dd, J_1 = 8.0, J_2 = 2.5, H-4′), 8.242 (2H, J_1 = 8.0, J_2 = 2.5, H-4″, H-4‴), 8.286 (1H, J_1 = 8.0, J_2 = 2.5, H-4⁗), 8.793 (1H, d, J = 2.5, H-2′), 8.872 (1H, d, J = 2.5, H-2″), 9.032 (1H, d, J = 2.5, H-2‴), 9.043 (1H, d, J = 2.5, H-2⁗).

20-Hydroxyecdyson-2,3,22-tri-(6-chloronicotinate) (4). Yield 9.3 mg, 20.7%. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.898 (3H, s, 18-Me), 1.133 (3H, s, 19-Me), 1.246 (3H, s, 26-Me), 1.252 (3H, s, 27-Me), 1.397 (3H, s, 21-Me), 3.263 (1H, m, W/2 = 24, H-9α), 5.176 (1H, br.d, J = 11, H-22), 5.480 (1H, m, W/2 = 22, H-2α), 5.771 (1H, m, W/2 = 9.5, H-3α), 5.945 (1H, d, J = 2, H-7), 7.363 (1H, d, J = 8.5, H-5′), 7.454 (1H, d, J = 8.5, H-5″), 7.478 (1H, d, J = 8.5, H-5‴), 8.083 (1H, dd, J_1 = 8.5, J_2 = 2.5, H-4′), 8.233 (1H, dd, J_1 = 8.5, J_2 = 2.5, H-4″), 8.272 (1H, dd, J_1 = 8.5, J_2 = 2.5, H-4‴), 8.800 (1H, s, H-2′), 9.022 (2H, br.s, H-2″, H-2‴).

20-Hydroxyecdyson-2,22-di-(6-chloronicotinate) (3). Yield 7.1 mg, 18.7%. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.879 (3H, s, 18-Me), 1.040 (3H, s, 19-Me), 1.215 (3H, s, 26-Me), 1.238 (3H, s, 27-Me), 1.384 (3H, s, 21-Me), 3.192 (1H, m, W/2 = 23, H-9α), 4.303 (1H, m, W/2 = 10, H-3α), 5.166 (1H, br.d, J = 10.5, H-22), 5.279 (1H, m, W/2 = 22, H-2α), 5.903 (1H, d, J = 2.5, H-7), 7.436 (1H, d, J = 8.0, H-5′), 7.443 (1H, d, J = 8.0, H-5″), 8.265 (2H, dd, J_1 = 8.0, J_2 = 2.0, H-4′, H-4″), 9.015 (1H, d, J = 2.0, H-2′), 9.080 (1H, d, J = 2.0, H-2″).

20-Hydroxyecdyson-2-(6-chloronicotinate) (2). Yield 10.1 mg, 32.6%. PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 1.158 (3H, s, 19-Me), 1.252 (3H, s, 18-Me), 1.431 (6H, s, 26-Me, 27-Me), 1.630 (3H, s, 21-Me), 3.752 (1H, m, W/2 = 24, H-9α), 3.921 (1H, br.d, J = 10, H-22), 4.523 (1H, m, W/2 = 9, H-3α), 6.313 (1H, d, J = 1.5, H-7), 7.461 (1H, d, J = 8.0, H-5′), 8.366 (1H, dd, J_1 = 8.0, J2 = 2.5, H-4′), 9.227 (1H, d, J = 2.5, H-2′).

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