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SYNTHESIS OF THE *O*-(2-CHLOROPYRIDIN-5-YLMETHYL)OXIME OF 20-HYDROXYECDYSONE

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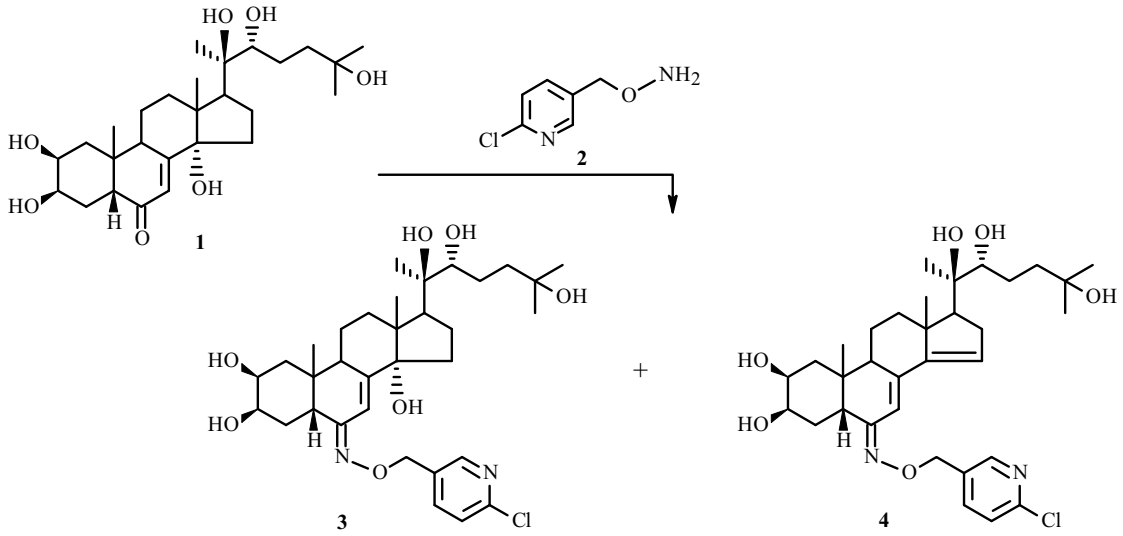
The new ecdysteroid derivative 20-hydroxyecdysone O-*(2-chloropyridin-5-ylmethyl)oxime (**3**) was synthesized via the reaction of 20-hydroxyecdysone (**1**) and* O-*(2-chloropyridin-5-ylmethyl)hydroxylamine (**2**) in Py in the presence of* $SnCl_4$.

Keywords: 20-hydroxyecdysone, *O*-(2-chloropyridin-5-ylmethyl)hydroxylamine, 20-hydroxyecdysone *O*-(2-chloropyridin-5-ylmethyl)oxime, synthesis.

We previously developed a synthetic method for *O*-substituted 6-ketoximes of steroids that also contain in their structure the α-chloropyridine rings that are typical of several modern insecticides of the neonicotinoid series. It seemed advisable to expand this method and produce the corresponding 6-ketoximes of ecdysteroids, in particular, 20-hydroxyecdysone (**1**). This compound is the most common representative of ecdysteroids in the plant and animal worlds [2, 3]. Compound **1** exhibits tonic, adaptogenic, hepatoprotective, and anabolic activities [2, 3]. Because of their broad spectrum of biological activity, **1** and its derivatives are constantly receiving attention as promising active compounds for creating new preparations for medicine and sports and tonic food additives. Recently the synthesis of oximes of **1** and its diacetonide was investigated [4]. The corresponding 6-ketoximes were used for radioimmunoassay of ecdysteroids [2, 5].



It is well known [2] that the 6-ketone in ecdysteroids is sterically hindered and unreactive. Therefore, we followed the previously developed method [1] and carried out the oximation of **1** using *O*-substituted hydroxylamine **2** in Py with heating in the presence of $SnCl_4$ catalyst. According to TLC, this version of the oximation formed a single product that partially decomposed into a second compound during isolation from the reaction mixture and subsequent purification. We found that the content of the impurity in the reaction product increased with time.

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TABLE 1. Chemical Shifts of C Atoms (δ, ppm) in ^{13}C NMR Spectra of 20-Hydroxyecdysone (20*E*) 6-Ketoximes

C atom	**3**		**4**		C atom	**3**		**4**	
1	38.4	37.9	40.6		18	18.0	18.2	18.6	18.7
2	68.8	69.3	68.7	69.3	19	21.8	21.0	20.8	
3	68.1	68.7	68.0	68.4	20	76.9	78.0	76.2	77.3
4	32.0	32.7	31.1		21	24.6	24.6	24.3	24.4
5	42.2	42.6			22	77.5	78.4	77.6	78.5
6	157.9	158.9	161.2	161.4	23	27.5	27.3	27.3	27.2
7	109.5	110.6	120.6	124.8	24	42.7	42.4	42.4	42.3
8	155.8	155.4	158.6	151.2	25	69.6	71.3		71.3
9	35.1	35.5	36.7		26	30.0	29.0	29.8	
10	37.3	37.9	36.8	37.3	27	30.1	29.7	30.2	
11	21.2	21.6			-CH_2ON=	72.2	72.7	72.4	73.0
12	34.8	34.9	37.9		2_{Py}	150.7	151.4		151.2
13	48.2		48.0		3_{Py}	124.3	125.3	123.8	125.4
14	84.6	85.9	142.2	143.5	4_{Py}	139.2	140.7		140.7
15	32.2	32.1	116.5	116.6	5_{Py}	134.2	135.3		135.2
16	21.6	21.6	31.4		6_{Py}	150.5	150.2		150.0
17	50.1	50.5	58.2	58.9	Solvent	C_5D_5N	CD_3OD	C_5D_5N	CD_3OD

The structures of the reaction product as 20-hydroxyecdysone *O*-(2-chloropyridin-5-ylmethyl)oxime (**3**) and the impurity as stachysterone B *O*-(2-chloropyridin-5-ylmethyl)oxime (**4**) were established using spectral data. For this, a comparison of spectral data of the reaction product with the corresponding spectral characteristics of 6-ketoximes of 20-hydroxyecdysone and its derivatives [4] was decisive. Thus, the PMR spectra indicated that **3** and **4** contained fragments of starting compounds **1** and **2**. Table 1 presents ^{13}C NMR spectral data that led to an analogous conclusion. The good agreement of chemical shifts for C resonances in spectra in CD_3OD of the steroidal parts of 20-hydroxyecdysone (6*E*)-oxime [4] and ecdysteroid derivative **3** was especially interesting. It could be rather convincingly concluded from this that the oxime in **3** had the (*E*)-geometry.

The structure of minor product **4** was also rather reliably determined using NMR spectral data. Thus, the presence of a 7,14-diene group in this steroid was proved by the presence in the PMR spectrum in CD_3OD of resonances for vinyl protons H-7 and H-15 with chemical shifts δ 6.11 and 5.78 ppm, respectively. We were unable to identify all resonances in the ^{13}C NMR spectra that corresponded to C resonances in **4** because of the low content in the mixture. However, resonances for C-6, C-7, C-8, C-14, and C-15 in the spectrum in CD_3OD had chemical shifts similar to those of 14,15-dehydro-20-hydroxyecdysone (6*Z*)-oxime [4]. The insignificant differences could be explained by differences in the geometry of the oxime in these compounds.

We concluded that the oxime in **4** had the (*E*)-geometry based on a comparison of the chemical shifts of the C-5 resonances in ^{13}C NMR spectra of ecdysteroid derivatives presented in Table 1. It is well known [2] that the allylic 14α-hydroxy group in ecdysteroids tends to undergo facile elimination under both acidic and basic conditions. The presence in **3** of a pyridine ring, which possesses basic properties, probably favors such degradation with formation of dehydro-derivative **4** and thereby is responsible for the relative lability of the initial oximation reaction product.

Oximation of 20-hydroxyecdysone with hydroxylamine in Py produced a mixture of (6*E*)- and (6*Z*)-oximes with predominance of the latter [4]. However, in our instance the reaction of ecdysteroid **1** with *O*-substituted hydroxylamine **2** formed only (6*E*)-oxime **3** and its dehydration product **4**. These differences in the oximation product mixture could be due to differences in the reagents and the different reaction conditions (e.g., use of $SnCl_4$ as the catalyst).

EXPERIMENTAL

PMR and ^{13}C NMR spectra were obtained in deuterated solvents on a Bruker Avance 500 NMR spectrometer (operating frequency 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C). Chemical shifts are given relative to TMS internal standard. Mass spectra were recorded on an Accela HPLC with an LCQ-Fleet mass-detector (three-dimensional ion trap) using chemical ionization at atmospheric pressure (APCI) (detection of positive ions). The reactive gas was N_2. The *m*/*z* values are given for

the strongest peaks. UV spectra were recorded in EtOH on a Specord M500 spectrophotometer. The course of reactions and purity of products were monitored using Kieselgel $60F_{254}$ plates (Merck).

20-Hydroxyecdysone *O*-(2-Chloropyridin-5-ylmethyl)oxime (3) and Stachysterone B *O*-(2-Chloropyridin-5-ylmethyl)oxime (4). A solution of *O*-(2-chloropyridin-5-ylmethyl)hydroxylamine (**2**, 0.157 g) (prepared as before [1]) in Py (0.5 mL) was treated with a solution of 20-hydroxyecdysone (**1**, 0.160 g) in Py (0.5 mL) and $SnCl_4$ (two drops). The mixture was heated on an oil bath at 60°C with stirring on a magnetic stirrer for 3 d until starting **1** disappeared and then was co-evaporated with benzene. The product was dried in vacuo until traces of Py disappeared completely. The solid was placed on silica gel L100/400 and chromatographed with elution by dichloroethane:methanol mixtures of increasing polarity (20:1, 15:1, 10:1) to afford **3** containing an impurity of **4** as an oil that could not be crystallized. UV spectrum (λ_{max}, nm): 268.

PMR spectrum (C_5D_5N, δ, ppm, J/Hz): for **3**: 1.02 (s, 19-Me), 1.23 (s, 18-Me), 1.38 (s, 26-,27-Me), 1.60 (s, 21-Me), 3.02 (t, J = 9, H-17), 3.08 (dd, J_1 = 13, J_2 = 3.5, H-5), 3.47 (m, H-9α), 3.89 (d, J = 10, H-22), 4.21 (m, H-2α), 4.23 (m, H-3α), 5.27 (s, -CH_2ON=), 7.04 (d, J = 2, H-7), 7.34 (d, J = 8.0, H-3_{Py}), 7.76 (dd, J_1 = 8.0, J_2 = 2, H-4_{Py}), 8.61 (d, J = 2, H-6_{Py}); for **4**: 5.83 (br.s, H-15), 6.51 (d, J = 2, H-7).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): for **3**: 0.82 (s, 18-Me), 0.86 (s, 19-Me), 1.19 (s, 26-,27-Me), 1.20 (s, 21-Me), 2.94 (m, H-9α), 3.33 (m, H-22), 3.81 (m, W/2 = 22, H-2α), 3.90 (m, W/2 = 9, H-3α), 5.08 (s, -CH_2ON=), 6.49 (d, J = 2.5, H-7), 7.43 (d, J = 8.0, H-3_{Py}), 7.78 (dd, J_1 = 8.0, J_2 = 2, H-4_{Py}), 8.32 (d, J = 2, H-6_{Py}); for **4**: 0.90 (s, 19-Me), 1.08 (s, 18-Me), 5.12 (s, -CH_2ON=), 5.78 (br.s, H-15), 6.11 (d, J = 2, H-7), 7.45 (d, J = 8.0, H-3_{Py}), 7.84 (dd, J_1 = 8.0, J_2 = 2, H-4_{Py}), 8.37 (d, J = 2, H-6_{Py}).

Mass spectrum (*m*/*z*): 621, 623 $[M + H]^+$, 603, 605 $[M + H - H_2O]^+$, 585, 587 $[M + H - 2H_2O]^+$, 567, 569 $[M + H - 3H_2O]^+$.

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