PREPARATION OF 20-HYDROXYECDYSONE-22-BENZOATE

N. Z. Mamadalieva,[1] N. Sh. Ramazanov,[1] J.-P. Girault,[2] R. Lafont,[3] and Z. Saatov[1]

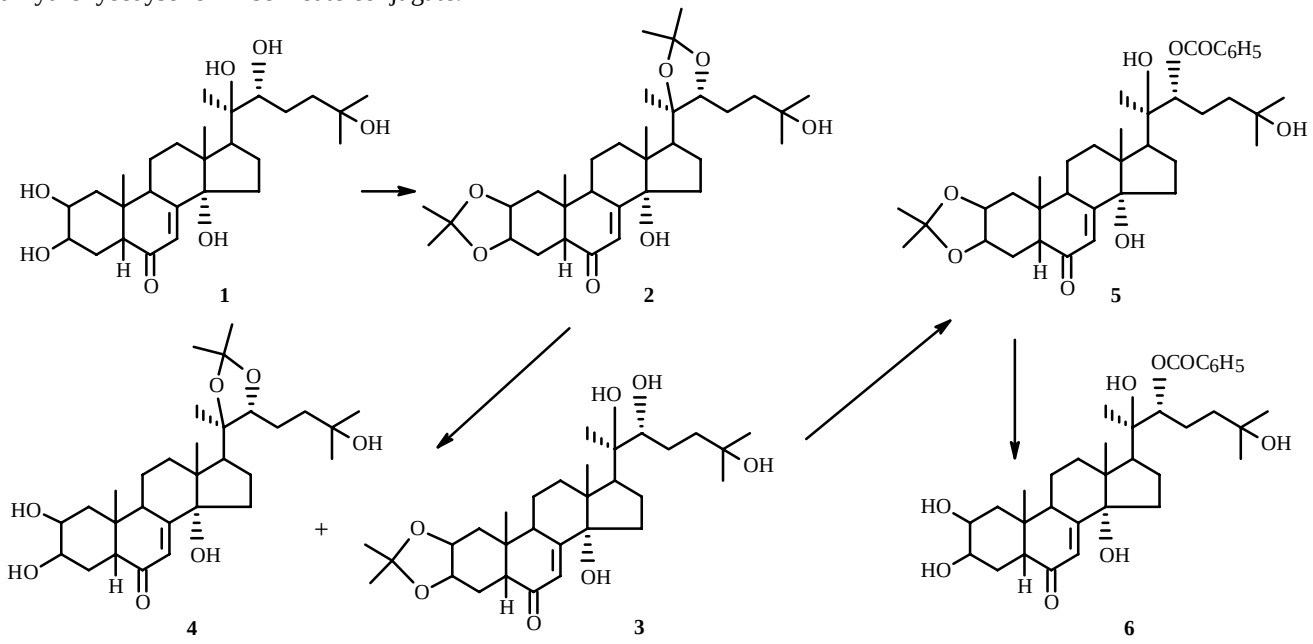
UDC 547.926:591.147

20-Hydroxyecdysone-22-benzoate was for the first time synthesized from 20-hydroxyecdysone.

Key words: ecdysteroids; 20-hydroxyecdysone; diacetonide; monoacetonide; benzoate; IR, UV, mass, and PMR spectra.

Ecdysteroids are natural compounds that exert a strong tonic effect on mammals and humans. At present, 20-hydroxyecdysone is a component of several preparations with tonic, anabolic, and wound-healing action. Ecdysteroid conjugates were recently observed in plants [1-3]. It was theorized that these are inactive reserve forms of the hormone.

We developed a chemical transformation scheme of 20-hydroxyecdysone for further synthesis of the 20-hydroxyecdysone-22-benzoate conjugate.



20-Hydroxyecdysone (**1**) reacted with acetone and phosphomolybdic acid to give ketal **2**. The mass spectrum of the ecdysteroid has peaks for ions characteristic of 20-hydroxyecdysone diacetonide [4]. This was confirmed by the agreement of the mass spectra and PMR spectra (Table 1) and direct comparison using thin-layer chromatography (TLC).

Treatment of **2** with dilute acetic acid [5] led to 20-hydroxyecdysone-2,3-monoacetonide (**3**) [6], which was identified using mass and NMR spectra (Table 1).

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75; 2) Universite Rene Descartes, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, France; 3) Universite Pierre et Marie Curie Endocrinologie Moleculaire et Evolution, France. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 401-403, September-October, 2004. Original article submitted June 28, 2004.

 0009-3130/04/4005-0488

TABLE 1. Proton Chemical Shifts in **1-6** (CD_3OD, 0 = TSP-d_4, δ, ppm, J/Hz)

Proton	**1**	**2**	**3**	**4**	**5**	**6**
1ax	1.43					
1eq	1.78					
2ax	3.83 (ddd, J = 12, 3, 3)	4.27 (m, $w_{1/2}$ = 23)	4.27 (m, $w_{1/2}$ = 23)	3.84 (m, $w_{1/2}$ = 22)	4.27 (m, $w_{1/2}$ = 23)	3.83 (m, $w_{1/2}$ = 22)
3eq	3.94 (ddd, J = 3, 3, 3)	4.30 (m, $w_{1/2}$ = 9)	4.30 (m, $w_{1/2}$ = 9)	3.95 (m, $w_{1/2}$ = 9)	4.31 (m, $w_{1/2}$ = 9)	3.95 (m, $w_{1/2}$ = 8)
4ax	1.65					
4eq	1.75					
5	2.38 (dd, J = 12.5)	2.24 (t, J = 8.6)	2.24 (t, J = 8.6)	2.39 (dd, J = 12.5)	2.24 (t, J = 8.6)	2.39 (dd, J = 12.7, 4.4)
7	5.80 (d, J = 2.5)	5.80 (d, J = 2.4)	5.78 (d, J = 2.3)	5.81 (d, J = 2.5)	5.81 (d, J = 2.2)	5.82 (d, J = 2.5)
9ax	3.14 (m, $w_{1/2}$ = 22)	2.93 (m, $w_{1/2}$ = 23)	2.94 (m, $w_{1/2}$ = 23)	3.15 (m, $w_{1/2}$ = 23)	2.95 (m, $w_{1/2}$ = 23)	3.16 (m, $w_{1/2}$ = 23)
11ax	1.65					
11eq	1.78					
12ax	2.13 (ddd, J = 13, 13, 5)	2.10 (ddd, J = 13, 13, 5)	2.12 (ddd, J = 13, 13, 5)	2.12 (ddd, J = 13, 13, 5)	2.16 (ddd, J = 13, 13, 5)	2.18 (ddd, J = 13, 13, 5)
12eq	1.85					
15a*	2.00					
15b*	1.55					
16a*	1.95					
16b*	1.75					
17	2.39 m	2.31 (t, J = 8.7)	2.39 (m, $w_{1/2}$ = 20)	2.32 (t, J = 8.7)	2.48 (t, J = 9.2)	2.48 (t, J = 9)
22	3.33 (dd, J = 11, 2)	3.68 (m, $w_{1/2}$ = 15)	3.30 (dd, J = 11, 2)	3.69 (m, $w_{1/2}$ = 15)	5.15 (dd, J = 11, 2)	5.15 (dd, J = 11, 2)
23a	1.30					
23b	1.65					
24a	1.75					
24b	1.45					
18-Me	0.89 s	0.819 s	0.884 s	0.829 s	0.888 s	0.896 s
19-Me	0.96 s	0.963 s	0.965 s	0.964 s	0.963 s	0.965 s
21-Me	1.18 s	1.179 s	1.190 s	1.184 s	1.426 s	1.430 s
26-Me	1.19 s	1.197 s	1.194 s	1.198 s	1.158 s	1.158 s
27-Me	1.20 s	1.207 s	1.204 s	1.207 s	1.170 s	1.168 s
		1.318 s	1.319 s		1.323 s	
		1.470 s	1.469 s		1.471 s	
		1.318 s		1.320 s	8.08 (2H, ortho)	8.08 (2H, ortho)
		1.389 s		1.391 s	7.60 (1H, para)	7.60 (1H, para)
					7.49 (2H, meta)	7.49 (2H, meta)

s, singlet; d, doublet; t, triplet; m, multiplet.

The mass spectrum of **3** has a peak for an ion with *m/z* 404, characteristic of 20-hydroxyecdysone monoacetonide [7, 8]. This indicates that the isopropylidene group is bonded to the steroid part of the molecule and replaces the C-2—C-3 diol.

In addition to 20-hydroxyecdysone-2,3-monoacetonide, a compound identified as 20-hydroxyecdysone-20,22-monoacetonide (**4**) was also isolated from the reaction mixture [9] (Table 1). Treatment of the 2,3-monoacetonide with benzoyl chloride in dry pyridine produced 20-hydroxyecdysone-2,3-monoacetonide-22-benzoate (**5**) [1].

The UV spectrum of **5** had an absorption maximum at 235 nm (log ε 4.02). The IR spectrum of **5** showed absorption bands at 3460 cm^{-1} (OH) and 1650 cm^{-1}, corresponding with absorption of a ketone conjugated to a double bond. Furthermore, the IR spectrum exhibits absorptions at 1725, 1710, and 1255 (esters) and 1610, 1585, and 720 cm^{-1} (benzene ring).

The mass spectrum of **5** at high mass numbers exhibits a peak for the molecular ion with *m*/*z* 624 and several peaks formed from loss of several waters and benzoic acid: *m*/*z* 642 [M + NH_4], 625 [MH], 607 [MH - H_2O], 589 [MH - $2H_2O$], 571 [MH - $3H_2O$], 520 [M + NH_4 - $C_7H_6O_2$], 503, 502, 485, 467.

According to IR, mass, and PMR spectra, **5** was identified as 20-hydroxyecdysone-2,3-monoacetonide-22-benzoate, which was synthesized previously from 20-hydroxyecdysone-22-benzoate [1].

Hydrolysis of **5** with dilute acetic acid produced **6**, the IR spectrum of which had absorption bands at 1710 and 1285 (ester) and 1610, 1587, and 720 cm^{-1} (benzene ring). This is also consistent with signals for five aromatic protons in the PMR spectrum (Table 1).

Taken together, the results indicate that the benzoic acid is bonded to the C-22 hydroxyl.

Thus, **6** is 20-hydroxyecdysone-22-benzoate. This compound was synthesized for the first time [1].

EXPERIMENTAL

PMR spectra were recorded on a Bruker WM 250 MHz instrument in CD_3OD using TSP-d_4 as a standard; IR spectra, on a Perkin—Elmer System 2000 FTIR Fourier-spectrometer in KBr disks; mass spectra, on a JEOL JMS-700 instrument using ammonia as the reactive gas.

The following solvent systems were used for chromatography: $CHCl_3$:CH_3OH (25:1, 1; 50:1, 2; 9:1, 3).

2,3;20,22-Diacetonide-20-hydroxyecdysone (2). A suspension of **1** (1.2 g) in anhydrous acetone (170 mL) was treated with phosphomolybdic acid (24 mg), shaken at room temperature until **1** had completely dissolved, concentrated in vacuum to a small volume, diluted with water, and neutralized with $NaHCO_3$ solution. The neutral solution was extracted with ethylacetate. The extract was chromatographed over a silica-gel column. Elution using system 1 afforded **2** (0.95 g, 70%), $C_{33}H_{52}O_7$, mp 230-232°C (ether:acetone), $[\alpha]_D^{20}$ +36.8 ± 2° (*c* 0.30, CH_3OH). IR spectrum (KBr, ν, cm^{-1}): 3460 (OH), 1670 (7-en-6-ketone). Mass spectrum (*m*/*z*): 578 [M + NH_4], 561 [MH], 543 [MH - H_2O], 538 [M + NH_4 - C_2H_6O], 521 [MH - C_2H_6O], 503 [MH - C_2H_6O - H_2O], 480, 447, 445, 380, 364, 304, 279, 234, 193, 160, 132, 78.

20-Hydroxyecdysone-2,3-monoacetonide (3) and 20-Hydroxycdysone-20,22-monoaceonide (4). Compound **2** (0.9 g) was hydrolyzed with acetic acid (70 mL) in aqueous CH_3OH (12:19) at room temperature for 4 h. The reaction mixture was diluted with water and neutralized with $NaHCO_3$ solution. The neutral solution was extracted with ethylacetate. The extract was chromatographed over a column using system 2 to afford **3** (135 mg, 15%), $C_{30}H_{48}O_7$, mp 242-244°C (ethylacetate), $[\alpha]_D^{20}$ +59.2 ± 2° (*c* 0.32, CH_3OH). UV spectrum (EtOH, λ_{max}, nm): 246 nm (log ε 4.00).

IR spectrum (KBr, ν, cm^{-1}): 3400-3450 (OH), 1650 (7-en-6-ketone). Mass spectrum (*m*/*z*): 538 [M + NH_4], 521 [MH], 503 [MH - H_2O], 485 [MH - $2H_2O$], 467 [MH - $3H_2O$], 404, 387, 330, 318, 279, 232, 186, 156, 130, 101, 99, 84.

Further elution of the column using system 2 afforded **4** (155 mg, 17%) [9]. Mass spectrum (*m*/*z*): 505, 502, 487, 469, 462, 445, 429, 427, 411, 409, 363, 345, 329, 327, 301, 300, 201, 161, 143, 125, 102, 99, 81.

20-Hydroxyecdysone-2,3-monoacetonide-22-benzoate (5). Compound **3** (125 mg) was dissolved in dry pyridine (20 mL) and benzoylated with benzoyl chloride (3 mL) at room temperature for 24 h. After solvent was removed, the solid was chromatographed over a silica-gel column with elution using system 2 to afford **5** (55 mg, 37%), $C_{37}H_{52}O_8$, mp 242-244°C ($CHCl_3$). Mass spectrum (*m*/*z*): 642 [M + NH_4], 625 [MH], 607, 589, 571, 520, 503, 502, 485, 467, 420, 409, 362, 282, 247, 203, 163, 99, 78.

20-Hydroxyecdysone-22-benzoate (6). Compound **5** (45 mg) was hydrolyzed with acetic acid (6 mL) in aqueous CH_3OH (2.6 mL) at room temperature for 3 h. The reaction mixture was worked up as described above. Chromatography over a column using system 3 afforded **6** (24 mg, 56%) [1], $C_{34}H_{48}O_8$, mp 202-205°C. Mass spectrum (*m*/*z*): 602 [M + NH_4], 585 [MH], 567 [MH - H_2O], 549 [MH - $2H_2O$], 531 [MH - $3H_2O$], 520 [M + NH_4 - $C_7H_6O_2$], 502, 486, 467, 445, 444, 427, 409, 353, 346, 282, 247, 240, 166, 140, 105, 99, 78.

REFERENCES

1. Z. Saatov, M. B. Gorovits, N. K. Melibaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 77 (1986).
2. N. Sh. Ramazanov, E. S. Maksimov, Z. Saatov, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 59 (1996).

3. N. Z. Mamadalieva, N. Sh. Ramazanov, L. N. Dainen, and Z. Saatov, *Khim. Prir. Soedin.*, 405 (2000).
4. K. Miladera, Z. Saatov, Yu. D. Kholodova, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 71 (1992).
5. I. L. Zatsny, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 175 (1973).
6. U. A. Baltaev and N. K. Abubakirov, *Khim. Prir. Soedin.*, 681 (1987).
7. M. N. Galbraith and D. H. S. Horn, *Aust. J. Chem.*, **22**, 1045 (1969).
8. I. L. Zatsny, M. B. Gorovits, Ya. V. Rashkes, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 155 (1975).
9. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 767 (1987).