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SYNTHESIS OF CYASTERONYLTHIOCARBAMATE DERIVATIVES

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Ecdysterone, cyasterone, ajugalactone, ajugasterone B, 22-acetylcyasterone, and turkesterone were isolated from leaves of Ajuga turkestanica *(Labiatae). Their structures were established using spectral and chemical data. The 2,3,22-triacetate, 2,3,22-tri-*O-p-*iodobenzoylthiocarbamate, and 2-*O-p-*iodobenzoylthiocarbamate derivatives of cyasterone were synthesized.*

Key words: cyasterone, cyasterone-22-acetate, cyasterone-2,3,22-triacetate, *p*-iodobenzoylisothiocyanate, cyasterone-2,3,22-tri-*O-p*-iodobenzoylthiocarbamate, cyasterone 2-*O-p*-iodobenzoylthiocarbamate.

In continuation of research on ecdysteroids from plants of Uzbekistan, we investigated the aerial organs, namely the leaves, of *Ajuga turkestanica* growing in Central Asia. Compounds typical of the genus *Ajuga* were observed (ecdysterone, cyasterone, ajugalactone, ajugasterone B, 22-acetylcyasterone, and turkesterone) [1-4].

New derivatives of cyasteronoyl thiocarbamate (**3**, **4**) were prepared by reacting cyasterone (**1**) with *p*-iodobenzoylisothiocyanate.

The reaction was carried out in dry pyridine at 100-110°C for 6 h. The new cyasteronoyl thiocarbamate derivatives were prepared in rather high yields.

The synthesized compounds were insoluble in water.

The structures of the cyasterone derivatives (**3**, **4**) were confirmed by PMR spectroscopy (Table 1).

Adding three *p*-iodobenzoylthiocarbamate functional groups to the molecule clearly increases the biological activity of the starting compound. Phytoecdysteroids are known to have a definite (rather weak) anti-inflammatory effect [6] because of their stabilizing effect on the membrane [5]. The chemical modification of cyasterone was accompanied by a significant increase in both the antiexudative and antiproliferative properties of the newly prepared derivatives (**3**, **4**) (Tables 2 and 3).

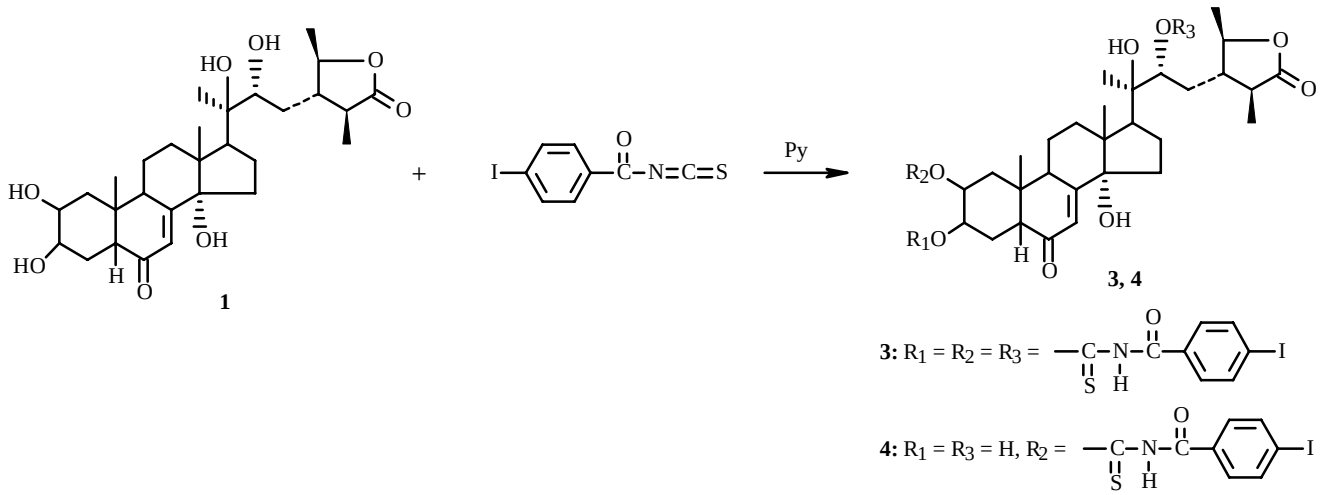

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TABLE 1. Proton Chemical Shifts* in Cyasterone (**1**), Cyasterone-2,3,22-triacetate (**2**), Cyasterone-2,3,22-tri-*O*-*p*-iodobenzoylthiocarbamate (**3**), and Cyasterone-2-*O*-*p*-iodobenzoylthiocarbamate (**4**)

Atom	Compound			
	1	**2**	**3**	**4**
H-2	3.93	5.10	5.15	5.07
H-3	4.10	5.21	5.27	4.31
H-7	6.13	5.94	6.13	6.09
H-9	3.42	3.06	3.33	3.48
H-22	3.80	4.94	4.83	3.87
CH_3-18	1.08	0.80	0.83	0.90
CH_3-19	0.94	1.0	0.98	0.93
CH_3-21	1.46	1.44	1.42	1.45
CH_3-27/29	1.25	1.21-1.37	1.18	1.22
Protons of substituents	-	CH_3-COO 1.8; 2.0; 2.10	8.9 (2H); 7.7 (3H):8.27 (7H)	8.6 (H); 7.81 (H):8.43 (2H)

*Signals for methyls were singlets; in all instances H-7 appeared as a broad singlet; all other signals were broad multiplets.

TABLE 2. Effects of **1** and **3** on formalin, dextran, and serotonin edema in rat paws

Compound	Growth of rat paws relative to initial, mL	Edema inhibition, %
3 h after formalin administration		
Control	0.47±0.030	-
Cyasterone	0.39±0.024	17.1
P	<0.1	
3	0.25±0.020*	46.8
P	<0.001	
3 h after dextran administration		
Control	0.66±0.028	-
Cyasterone	0.54±0.022	18.2
P	<0.01	
3	0.46±0.018*	30.3
P	<0.001	
3 h after serotonin administration		
Control	0.70±0.048	-
Cyasterone	0.56±0.032	20.0
P	<0.5	
3	0.38±0.028*	45.7
P	<0.001	

Note. Here and in Table 3 the P value is given relative to the control. Asterisks denote reliable values of these quantities between the two experimental groups ($P < 0.05$).

TABLE 3. Effects of **1** and **3** on Formation of Cotton Granuloma in Rats

Compound	Mass of granulation-fibrose tissue, mg	Inhibition effect, %
	3 h after formalin administration	
Control	67.3±3.2	-
Cyasterone	58.4±4.8	13.2
P	<0.25	
3	42.2±2.6*	37.3
P	<0.001	

EXPERIMENTAL

Column and thin-layer chromatography used $CHCl_3$:CH_3OH and $CHCl_3$:CH_3OH:H_2O (9:1, 1; 4:1, 2; 4:1:0.1, 3).

IR spectra were recorded on a Perkin—Elmer System 2000 FT-IR Fourier spectrometer in KBr disks; PMR spectra, on a BS-567 A instrument (100 MHz, Tesla, C_5D_5N, HMDS internal standard, δ 0.05 ppm); mass spectra, in MX-1310 and MX-1303 instruments.

Isolation of Phytoecdysteroids. *Ajuga turkestanica* (Rgl.) Brig. (Labiatae) was collected in 2000 in Surkhandarʹin District of the Republic of Uzbekistan in gorges near Derbent. Dried and ground leaves (1.5 kg) were extracted with ethanol (80%, 8 L).

The combined alcohol extract was condensed and diluted with water to a volume of 0.8 L. The aqueous solution was extracted first with $CHCl_3$ and then butanol. The butanol extract was evaporated to dryness and dissolved in alcohol (0.5 L). The resulting solution was stirred and treated with acetone (1 L). The resulting precipitate was separated.

The mother liquor that contained phytoecdysteroids was evaporated. The total phytoecdysteroids were chromatographed over a column of Al_2O_3 with elution by systems 1 and 2 and then rechromatographed over silica gel. Elution of the column with system 2 isolated ajugalactone (15 mg, 0.0003%) and cyasterone (0.405 g, 0.027%). Elution with system 3 produced 22-acetylcyasterone (2.1 g, 0.14%) (yields here and henceforth are calculated per air-dried raw material).

Cyasterone; $C_{29}H_{44}O_8$; mp 161-162°C (CH_3OH); $[\alpha]_D^{20}$ +60.0 ± 2° (*c* 1.0, pyridine); λ_{max} (EtOH): 245 nm (log ε 4.00); ν_{max} (KBr, cm^{-1}): 3400 (OH), 1750 (γ-lactone), 1660 (7-en-6-ketone) [7, 8].

Mass spectrum (*m*/*z*, %): 520 (0.9) $[M]^+$, 502 (16), 484 (35), 466 (40), 451 (15), 448 (14), 363 (62), 345 (100), 327 (62), 301 (30), 300 (29), 201 (15), 183 (35), 157 (40), 113 (45), 97 (8), 69 (15).

Literature data for cyasterone: mp 164-166°C (CH_3OH), $[\alpha]_D^{20}$ +64.5° (pyridine) [7].

Cyasterone-2,3,22-triacetate (2). A solution of cyasterone (100 mg) in pyridine (1 mL) was acetylated with acetic anhydride (1 mL) at room temperature for 24 h. The excess of the reagents was removed in vacuo. The solid was chromatographed over silica gel with elution by system 3 to isolate cyasterone acetate (85 mg), which was recrystallized from CH_3OH. $C_{35}H_{50}O_{11}$; mp 252-254°C (CH_3OH); $[\alpha]_D^{20}$ +68.2 ± 2° (*c* 0.37, CH_3OH); ν_{max} (KBr, cm^{-1}): 3460-3480 (OH), 1778 (γ-lactone), 1742 and 1243 cm^{-1} (ester), 1657 (7-en-6-ketone) [4, 9].

Mass spectrum (*m*/*z*, %): 646 (0.3) $[M]^+$, 628 (0.5), 618 (0.8), 610 (2), 568 (5), 550 (55), 539 (25), 455 (5), 385 (10), 334 (100), 232 (56), 201 (5), 183 (7), 113 (25), 69 (97).

IR spectrum of **2** (KBr, ν, cm^{-1}): 1268 (C–O–C), 1673 (C=O).

Literature data for cyasterone triacetate: mp 251-252°C, $[\alpha]_D^{20}$ +69.0° (pyridine) [8].

Synthesis of Cyasterone-2,3,22-tri-*O*-*p*-iodobenzoylthiocarbamate (3) and Cyasterone-2-*O*-*p*-iodobenzoylthiocarbamate (4). Cyasterone (**1**, 0.52 g, 0.001 mol) was placed into a three-necked flask equipped with a condenser with a $CaCl_2$ tube, a stirrer, and a thermometer; treated with freshly distilled triethylamine (2 mL); heated (70-80°C); stirred; treated dropwise with *p*-iodobenzoylisothiocyanate (1.18 g, 0.0041 mol) dissolved in pyridine (10 mL); and stirred for 6 h at 100-110°C. After the reaction was finished, the mixture was transfered to a 0.5-L cylinder and treated with water (80 mL). The resulting yellow crystals were dissolved in butanol and chromatographed over a column of Al_2O_3 with elution by system 2 to produce yellow and white powders. TLC gave spots with R_f 0.78 (**3**) and 0.69 (**4**) (C_2H_5OH:$CHCl_3$, 5:0.5), yield 69.4% (0.96 g) (**3**), mp 267-269°C; 12.4% (0.17 g) (**4**), mp 284-287°C. Products **3** and **4** were soluble in DMF, trifluoroacetic acid, pyridine, and DMSO [10].

IR spectrum (**3**) (KBr, ν, cm^{-1}): 1219 (C–O–C), 1685 (C=O), 1177 (C=S), 1600, 3363 (N–H).

IR spectrum (**4**) (KBr, ν, cm^{-1}): 1243 (C–O–C), 1667 (C=O), 1182 (C=S), 1596, 3339 (N–H).

The antiphlogistic activities of **3** and **4** were evaluated using models of acute inflammation in rats (males, 150-180 g) according to their ability to inhibit paw adema (determined oncometrically) induced by injection under the plantar fascia of formalin (0.2 mL, 1%), dextran (0.1 mL, 6%), and serotonin (0.5%). The results were scaled to the maximum in the corresponding changes in the control.

In addition to the study of the antiexudative effects of the compounds, their antiproliferative properties were also determined. For this, sterile cotton balls (10 mg) were implanted under the skin of the spine. The balls together with the granules that developed around them were extracted and dried to constant weight at 75°C. The mass of the granular-fibrous tissue was determined from the difference between the masses of the dried granules and the implanted balls.

The studied compounds were administered i.p. to animals at doses of 5 mg/kg for the study of the antiexudative properties, on the eve of the experiment and then 2 h before and 30 min after innoculation of the inflammatory agents; for the study of the antiproliferative properties, once a day for seven days. The results were treated statistically using the Student t-criterion.

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