PHYTOECDYSTEROIDS OF PLANTS OF THE GENUS Serratula.

AJUGASTERONE C 20,22-MONOACETONIDE FROM Serratura wolffii

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The phytoectysteroids of the epigeal part of the plant Serratula wolffii Andrae have been investigated. The structure of a new ecdysteroid – ajugasterone C 20,22-monoacetonide has been established. Seven known acdysteroids have been isolated and identified.

It has been shown previously [1] that the plant Serratula coronata L. (family Compositae) growing in the Dzhungarian Ala-Tau contains viticosterone E, ecdysterone, and α-ecdysone.

We have studied the phytoecdysteroids of S. wolffii Andrae grown in the Aleksandriya forest reserve of the Ukrainian Academy of Sciences [2, 3]. This plant raw material proved to be extremely rich in various ecdysteroids. From the epigeal part of the plant we isolated nine ecdysteroids, among which seven were known – α-ecdysone (I), ecdysterone (II), polypodin B (III), integristerone A (IV), pterosterone (V), ecdysterone 20,22-monoacetonide (VI), and ecdysterone 2,3:20,22-diacetonide (VII), and two new compounds, A and B. This paper describes the determination of the structure of ecdysteroid A (VIII) (see scheme on top of following page).

TABLE 1. Chemical Shifts of the Protons of the Compounds of (VIII-X) (δ, C_5D_5N, 0 – TMS)*

Proton	Compound		
	VIII	IX [21]	X
1-He	3,41; dd, $J_{1e,1a}$ = 13,5 $J_{1e,2a}$ = 4,5		3,38; dd, $J_{1e,1a}$ = 13,0 $J_{1e,2a}$ = 4,1
2-Ha	4,53; m, $W_{1/2}$ = 22 [a]		4,55; m, $W_{1/2}$ = 22 [d]
3-He	4,18; m, $W_{1/2}$ = 8		4,18; m, $W_{1/2}$ = 9
7-H	6,27; d, $J_{7,9a}$ = 2,7		6,24; $J_{7,9a}$ = 2,8
9-H	3,82; m, $W_{1/2}$ = 16,5 [b]		3,80; m, $W_{1/2}$ = 16,0 [e]
11-Ha	4,53; m, $W_{1/2}$ = 22 [a]		4,55; m, $W_{1/2}$ = 22 [d]
22-H	3,82; m, $W_{1/2}$ = 16,5 [b]		3,80; m, $W_{1/2}$ = 16,0 [e]
18-CH_3	1,05; s	1,21; s	1,22; s
19-CH_3	1,28 [c] s	1,27; s	1,26; s
21-CH_3	1,49; s	1,51; s	1,54; s
26-CH_3	0,77; d, $J_{26,25}$ = 6,5	0,82; d, J = 5	1,32; s
27-CH_3	0,78; d, $J_{27,25}$ = 6,5	0,82; d, J = 5	1,32; s
Protons of the acetonide group	1,45, 1,28 [c]		

*The signals marked with the same superscript letters are superposed on one another. The SSC and $W_{1/2}$ values are given in Hz.

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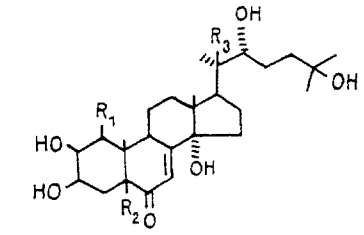
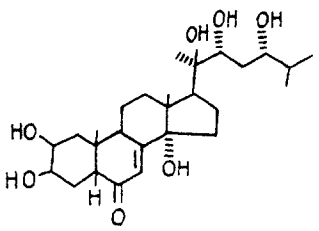


I. $R_1 = R_2 = R_3 = H$

II. $R_1 = R_2 = H$; $R_3 = OH$

III. $R_1 = H$; $R_2 = R_3 = OH$

IV. $R_1 = R_3 = OH$; $R_2 = H$



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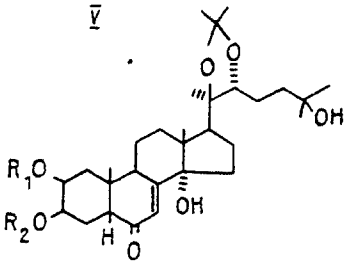
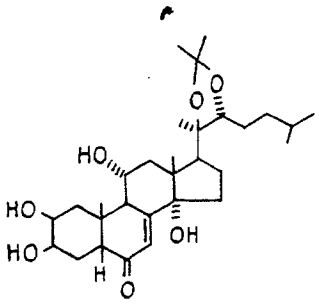
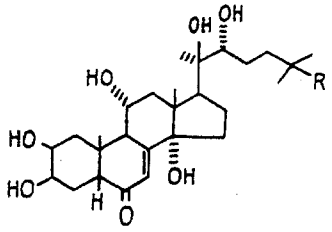


VI. $R_1 = R_2 = H$

VII. $R_1, R_2 = >CMe_2$



VIII



IX. R = H

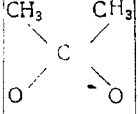
X R = OH

The IR spectrum of compound (VIII) was characteristic for ecdysteroids – it contained absorption bands of hydroxy groups (3340-3400 cm^{-1}) and of a keto group conjugated with a double bond (1675 cm^{-1}). The presence in its mass spectrum of peaks of ions with m/z 379 ($C_{21}H_{31}O_6$) and 361 ($C_{21}H_{24}O_5$) permitted the assumption that there were four hydroxy functions in the steroid nucleus of ecdysteroid (VIII) [4-6]. Furthermore, the peak of the molecular ion, M^+ 520 ($C_{30}H_{48}O_7$) and fragments with m/z 185 ($C_{11}H_{21}O_2$), 127 ($C_8H_{15}O$), and 109 (C_8H_{13}) showed that the two hydroxy groups present in the side chain were included in an isopropylidene grouping [7]. Thus, the molecule of the new phytoecdysteroid contained six hydroxy groups of which four were present in the steroid nucleus and two were in the side chain and were involved in an acetonide group.

Table 1 shows a good agreement of the values of the chemical shifts (CSs) of the signals located in the region from 3.4 to 6.3 ppm for the ecdysteroid (VIII) and turkesterone (X) [6]. This fact showed that the functional elements of the steroid nucleus of the new ecdysteroid were identical in nature and position with those of turkesterone. We may also note the considerable downfield shift of the 1-He signal in the spectra of (VIII) and (X) in comparison with ecdysterone (II) (1-He, δ_{II} – 2.14 [8]; δ_{VIII} – 3.41; δ_X – 3.38 ppm). This effect is explained by the influence of the 11α-OH group on 1-He which appears on the use of pyridine as solvent [9, 10].

A comparative analysis of the ^{13}C NMR spectra (Table 2) of turkesterone (X), ajugasterone C(IX), and compound(VIII) confirmed that in the tetracyclic moiety of the new ecdysteroid the hydroxy groups were located in the 2β, 3β, 11α, and 14α positions.

TABLE 2. Chemical Shifts of the Carbon Atoms of Compounds (VII)-(X) (δ, ppm, C_5D_5N, 0 – TMS)†

Carbon atom	Compound			Carbon atom	Compound		
	VIII	IX [21]	X		VIII	IX	X
1	39,48	39,6	39,87	17	49,72	50,0	50,07
2	68,37	68,2	68,53	18	18,23	19,0	19,00
3	68,69	68,5	68,25	19	24,81	24,9	24,96
4	32,86	32,9	32,95	20	84,81*	76,9	76,95
5	52,43	52,5	52,52	21	22,24**	21,6	21,70
6	203,88	204,2	204,21	22	81,94	76,9	77,60
7	122,37	122,2	122,33	23	27,10	30,3	27,53
8	163,68	164,4	164,49	24	36,86	37,2	42,65
9	42,69	42,8	42,79	25	28,24	28,2	69,77
10	39,81	39,8	39,62	26	22,81**	23,4	30,05
11	68,83	68,9	68,95	27	22,43**	22,5	30,20
12	43,83	44,2	44,20		29,37		
13	47,82	48,3	48,28		106,93		
14	84,13*	84,3	84,32				
15	31,75	31,9	31,96				
16	22,13	21,6	21,65				

†The assignment of the chemical shifts of the carbon atoms the signals of which are marked with the same superscript may be interchanged.

The multiplicities and CSs of the methyl groups – CH_3-21 (singlet) and CH_3-26/27 (doublets) – and the CS of H-22 (see Table 1), and also the mass spectral details given above, provide a basis for considering that the hydroxy groups of the side chain of ecdysteroid (VIII) are located at carbon atoms 20 and 22 and are included in an acetonide grouping.

A combination of the facts given above led to the conclusion that ecdysteroid (VIII) was ajugasterone C 20,22-monoacetonide.

EXPERIMENTAL

Thin-layer chromatography (TLC) was conducted on Silufol plates. For column chromatography we used alumina, KSK silica gel, and silica gel L 100/160 μm (Czechoslovakia). The following solvent systems were employed: chloroform–methanol (25:1); 2) (15:1); 3) (9:1); 4) (4:1); and 5) (2:1); and 6) chloroform–methanol–water (4:1:0.1). In TLC, the ecdysteroids were revealed by spraying with vanillin/sulfuric acid followed by heating at 110-120°C for 2-5 min [11]. The mass spectra and elementary compositions of the ions were obtained on a MKh-131 instrument at an ionizing voltage of 50 V and a temperature of 100-140°C; IR spectra were obtained on a UR-20 spectrophotometer in KBr.

The PMR spectra of compounds (I)-(VII) were taken on a BS-567 A instrument (100 MHz, Tesla) and the (^{1}H and ^{13}C) NMR spectra of (VIII) and (X) on WM-250 and AM-300 instruments (Bruker) with C_5D_5N as solvent, 0 – TMS. The assignment of the signals in the PMR spectra of compounds (VIII) and (X) was made from the results of selective double homonuclear resonance (difference variant) [12] and also, for (VIII), with the use of COSY spectra and, for (X), with the use of COSY and HET COSY (^{1}H – ^{13}C). The COSY and HET COSY spectra were taken by a standard procedure included in the mathematical instructions of the Bruker firm for ASPECT 2000 and ASPECT 3000 computers (COSY and XHCORRD, respectively).

The assignment of the signals in the ^{13}C NMR spectra of (VIII) and (X) was made with the use of the APT procedure [13], and, for (X) also with the use of HET COSY (^{1}H – ^{13}C).

Isolation of the Phytoecdysteroids. The epigeal part of the Serratula wolffii Andrae introduced into cultivation in the Aleksandriya forest reserve was gathered in the flowering phase in August, 1988. The dried and comminuted raw material (3 kg) was exhaustively extracted with ethanol (18 liters). The extract was concentrated to a volume of 500 ml and was diluted with 250 ml of water; the last traces of ethanol were distilled off. After the aqueous solution had been treated with chloroform (4 × 50 ml), the ecdysteroids were extracted with butanol (4 × 250 ml). The dry residue obtained after the butanol had been distilled off was chromatographed on a column of alumina.

Elution with system 5 gave the total ecdysteroids, the recrystallization of which from ethyl acetate yielded 6.0 g of ecdysterone (II) (here and below, the yields have been calculated on the air-dry raw material – 0.2%), $C_{27}H_{44}O_7$, mp 240-241°C, $[\alpha]_D^{10}$ +59.7 ± 2° (c 0.40; methanol) [11, 14].

The mother solutions obtained after the recrystallization of the ecdysterone were rechromatographed on a column of silica gel. Elution with system 1 yielded 150 mg (0.005%) of compound (VII),, $C_{33}H_{52}O_7$, mp 230-232°C (from acetone–ether), $[\alpha]_D^{20}$ +36.8 ± 2°; (c 0.30; methanol), which was identified from the facts given and also by a direct comparison with an authentic sample in TLC (system 1) as ecdysterone 2,3:20,22-diacetonide [11, 15].

Washing the column with system 1 led to 10 mg (0.0003%) of substance (V), $C_{27}H_{44}O_7$, mp 217-218°C (from methanol–water), $[\alpha]_D^{20}$ +7.0 ± 2° (c 0.30; methanol), identical in its physicochemical constants and its mass and PMR spectra with pterosterone [16-18].

Ajugasterone C 20,22-Monoacetonide (VIII). The use of system 3 for elution led to a fraction containing a mixture of two ecdysteroids. The recrystallization of this mixture from aqueous ethanol gave 20 mg (0.00066%) of ajugasterone C 20,22-acetonide (compound A), $C_{30}H_{48}O_7$, mp 287-288°C, ν_{max}^{KBr} (cm^{-1}): 3400-3440 (OH), 1675 (Δ^7-6-keto grouping).

Mass spectrum, m/z (%): 520 (M^+; 10), 505(20), 502(5), 487(10), 469(4), 462(98), 445(20), 444(28), 427(33), 426(32), 420(33), 411(10), 409(20), 402(13), 379(20), 361(17), 299(12), 281(7), 266(33), 185(100), 142(58), 127(83), 114(33), 109(33), 84(60).

The mother solution remaining after the isolation of the ajugasterone C monoacetonide was rechromatographed on a column of silica gel with elution by system 3. This led to the isolation of 50 mg (0.018%) of ecdysterone 20,22-monoacetonide (VI), $C_{30}H_{48}O_7$, mp 220-222°C (from ethyl acetate–hexane), $[\alpha]_D^{20}$ +58.9 ± 2° (c 0.32; methanol) [19].

Further elution of the main column with system 4 led to 30 mg (0.001%) of polypodin B (III), $C_{27}H_{44}O_8$, mp 250-252°C (from acetone), $[\alpha]_D^{20}$ +93.2 ± 2° (c 0.28; methanol) [14, 20], and 40 mg (0.001%) of α-acdysone (II), $C_{27}H_{44}O_6$, mp 236-237°C, $[\alpha]_D^{20}$ +63.6 ± 2° (c 0.83; methanol) [1].

The use of system 6 permitted the isolation of 20 mg (0.0006%) of integristerone A (IV), $C_{27}H_{44}O_8$, mp 246-248°C (ethyl acetate–methanol), $[\alpha]_D^{20}$ +36.0 ± 2° (c 0.22; methanol) [5, 14], and 8 mg of an acdysteroid (compound B) of unestablished structure.

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CIRCULAR DICHROISM OF STEROIDS WITH A LACTONE RING B. BRASSINOSTEROIDS AND COMPOUNDS RELATED TO THEM

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The circular dichroism spectra of steroids having the lactone group in ring B that is characteristic for brassinosteroids, and their isomers, have been studied. It has been shown that in the spectra of the isomeric B-homo-7-oxa-6-keto-steroids and B-homo-6-oxa-7-ketosteroids differences are observed in the sign of the Cotton effect of the $n-\pi^*$ transition of the carboxy group, which can be used for proving their structures.

The brassinosteroids, plant growth regulators, are, chemically, $C_{27}-C_{29}$-polyhydroxy-steroids usually having in ring B a 6-keto group or a lactonic B-homo-7-oxa-6-keto group [1]. The presence of a large number of functional groups with strictly determined stereochemistry in the molecules of the brassinosteroids makes it quite unavoidable to use modern physico-chemical methods to establish their structures. These include the circular dichroism method, which has been used for proving the presence of a 6-keto group and a trans-A/B linkage in the molecules of the brassinosteroids castasterone [2] and 2-deoxycastasterone [3]. At the same time, there is no information on studies of the circular dichroism of brassinosteroids with a lactone ring B, which include, for example, 24-epibrassinolide (I). As a result of the realization of our program on the synthesis of various analogs of brassinosteroids from

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