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PHYTOECDYSONES OF *Rhaponticum integrifolium*

III. INTEGRISTERONE B

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Continuing a study of the phytoecdysones of the flower heads of *Rhaponticum integrifoliu* C. Winkl., in addition to the ecdysterone (I) and integristerone A (II) isolated previously [1, 2], we have isolated a new ecdysone — integristerone B (III), with the composition $C_{27}H_{44}O$ This compound has a lower chromatographic mobility than the other phytoecdysones obtained fro from this plant source.

In the mass spectrum of the ecdysone under investigation, the peak of the molecular ion is absent. The fragment with the highest mass number, m/e 476, and the ions with m/e 458, 440, and 422 are obviously formed by the loss from the molecular ion of integristerone B (M^+ 512) of from two to five molecules of water.

The fragmentation of the side chain of the new phytoecdysone is described by ions with m/e 143, 125, 99, 81, and 69. These ions, which are characteristic of ecdysones with the side chain of ecdysterone (I) [3,4], including integristerone A (II) [2], suggest the identity of the structure of the C-20—C-27 chains in ecdysones (I), (II), and (III).

The latter assumption is confirmed by the practical coincidence of the values of the chemical shifts of the C-21, C-26, and C-27 methyl groups and of the C-22 proton in the PMR spectra of compounds (II) and (III) (Table 1).

Experiments on the deuterium exchange of (I)-(III) showed that the fragments of the side chain with m/e 143 and 99 formed as the result of C-17—C-20 and C-20—C-22 cleavages, respectively, have a cyclic form (Scheme 1).

The steroid part of the molecule after analogous cleavages must lead to the ions $(M - 180)^+$, by C-17—C-20 cleavage, and $(M - 117)^+$, by C-20—C-22 cleavage [2, 3, 5]. The spectrum of integristerone B does actually have the weak peak of the ion $(M - 180)^+$ with m/e 332, a fragment with m/e 395 $(M - 117)^+$, and also the products of its successive dehydration — ions with m/e 377, 359, 341, and 323 (not shown in Scheme 1). The mass numbers of the ions of this series are displaced by 16 m.u. in the direction of high masses as compared with those of integristerone A [2], which shows the presence of five hydroxy groups in the steroid nucleus of phytoecdysone (III).

In addition to this, the mass spectrum of integristerone B showed strong peaks of fragments with m/e 390, 273, 272, 255, 229, and 228 (see Scheme 1) which are not characteristic of the spectra of ecdysterone and of integristerone A [1-3]. This can be explained by the characteristic decomposition of the hydroxy groups in the steroid part of the molecule of the phytoecdysone (III). We directed our attention to the fact that in the IR spectrum of integristerone B the maximum absorption of the 6-keto group appears at 1680 cm^{-1} and is displaced in comparison with the corresponding maximum of integristerone A (1665 cm^{-1}) by 15 cm^{-1} in the direction of high wave numbers. Such a displacement, which is possible if the C=O and the neighboring hydroxy group are coplanar, shows the presence of a 5β-hydroxy function in the molecule of phytoecdysone (III) [5-8].

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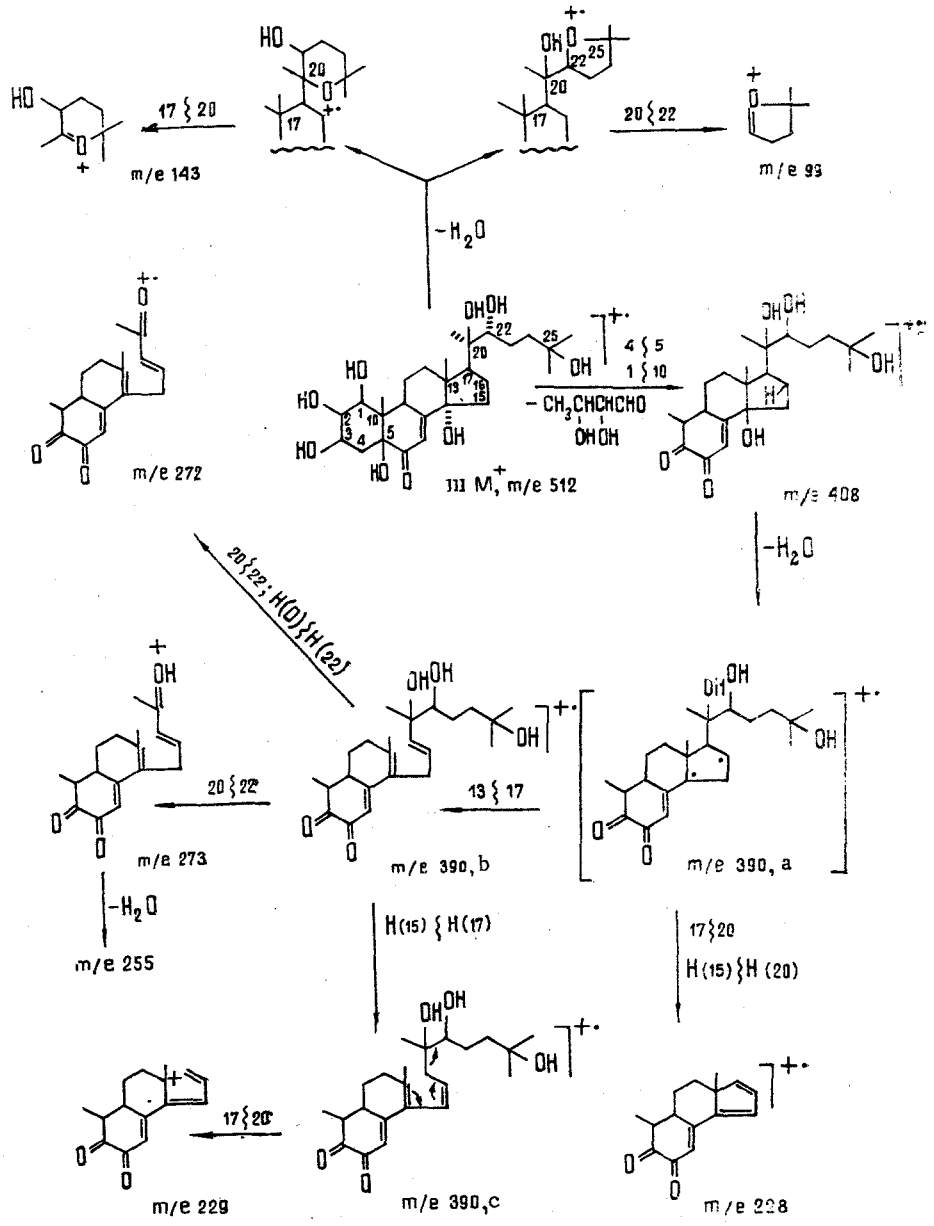


Scheme 1

The circular dichroism (CD) curve of integristerone B shows, by analogy with the curve of ecdysterone A, positive (n → π* transition) and negative (π → π* transition) Cotton effects. However, in the case considered a hypsochromic shift of the n → π* band from 338 nm (integristerone A) to 328 nm (integristerone B) is observed with a disappearance of its fine structure and an increase in the molecular ellipticity from +1.4 to +2.1. Furthermore, the extremum corresponding to the negative Cotton effect (π → π* transition) undergoes a bathochromic shift from 245 nm in the case of the phytoecdysone (II) to 255 nm for compound (III). The phenomena mentioned are also characteristic for 5β-hydroxy ecdysones [6-8].

As can be seen from Table 1 and Fig. 1 (a, a'), the characteristics of the PMR spectrum of integristerone B are close to those of integristerone A. An exception is the signal from the C-19 methyl group which is shifted downfield under the influence of the 5β-hydroxyl [6, 7].

The similarity of the signals from H-1, H-2, and H-3 in phytoecdysone II with the three-proton resonance signal at 4.15 ppm in the PMR spectrum of integristerone B permits the assumption that the phytoecdysone (III) also contains hydroxy groups at C-1, C-2, and C-3.

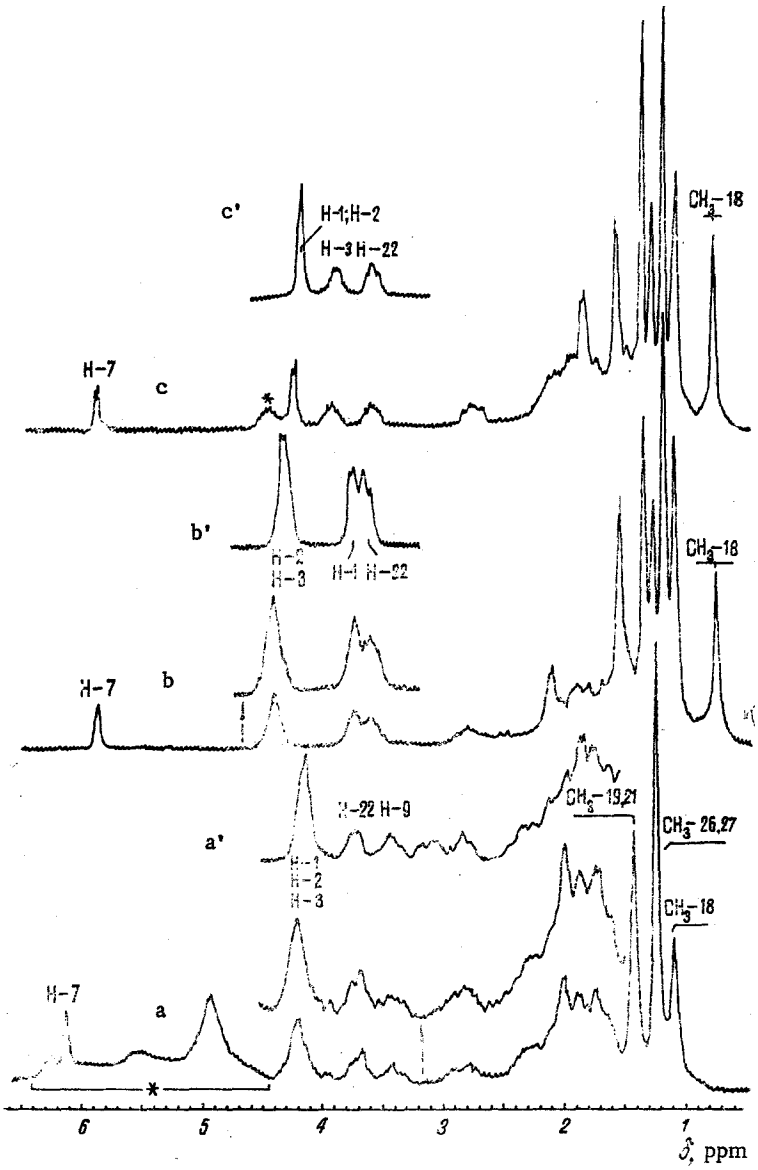
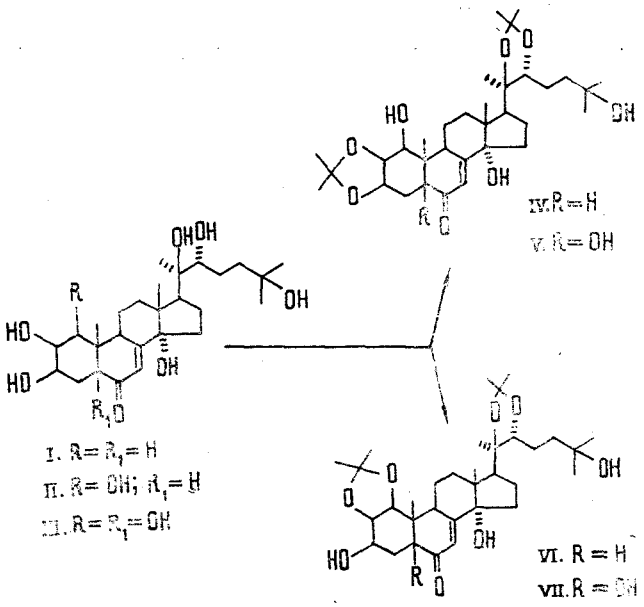


Fig. 1. PMR spectra: a) integristerone B (III); b) 2,3:20,22-diacetonide of integristerone B (V); c) 1,2:20,22-diacetonide of integristerone B (VII); a', c', b') the corresponding sections of the spectrum of integristerone A (II), of the 2,3:20,22-diacetonide of integristerone A (IV), and of the 1,2:20,22-diacetonide of integristerone A (VI). The asterisks denote the signals from the hydroxy groups.

The presence of hydroxy groups at C-1, C-2, C-3, and C-5 well explains the distribution of the intensities of the peaks in the mass spectrum of integristerone B, which is not characteristic of ecdysones (I) and (II). Such a combination of hydroxy functions increases the possibility of the cleavage of C-4—C-5 and C-1—C-10 bonds, which leads to the elimination of the elements of ring A and to the formation of a fragment with m/e 408 (see Scheme 1). Its stabilization in the form of ions with m/e 390 (b or c) takes place as the result of the elimination of a molecule of water. The subsequent stage is that connecting link which permits the interpretation of the following processes of the elimination of the side chain. As shown in Scheme 1, the different forms (a-c) of the ion with m/e 390 give fragments with m/e 273, 272, 229, and 228 stable as a consequence of conjugation. The origin of the fragmentary ions of this series is confirmed by measurements of their elementary compositions:

Found	Calculated	Compositon
390.2346	390.2406	$C_{23}H_{34}O_5$
272.1366	272.1412	$C_{17}H_{20}O_3$
255.1411	255.1385	$C_{17}H_{19}O_2$
228.1204	228.1150	$C_{15}H_{16}O_2$

In solution in triacetone, integristerone B, like integristerone A, forms isomeric acetonides (V) and (VII) with a molecular weight of 592 (Scheme 2). The peaks with m/e 201, 143, 125, 102, 99, and 81 observed in the mass spectra of compounds (V) and (VII) show the presence in the side chains of these derivatives of a dioxolane ring C-20—C-22 [2-4].



Scheme 2

TABLE 1. Chemical Shifts of the Protons of Integristerone A, Integristerone B, and Their Acetonides (δ, ppm)

Positions of the protons	Compound					
	II	III	IV	V	VI	VII
H-1	4.15	4.23	3,69	3,71	4,19	4,25
H-2	4.15	4.23	4,26	4.39	4.19	4,25
H-3	4.15	4.23	4,26	4.39	3.90	3,96
H-22	3.71	3,73	3,59	3.57	3,59	3.60
H-7	6,14	6.17	5,76	5.87	5.79	5,91
H-9	3,42	3,45	3.06	2,79	—	—
CH_3-18	1,07	1,09	0,71	0.75	0.75	0.77
CH_3-19	1.26	1.43	1,18	1.34	1.18	1,19
CH_3-21	1.43	1.43	1,09	1,08	1,10	1.08
CH_3-26	1,24	1,25	1.18	1.17	1,18	1.19
CH_3-27			1.27	1,26	1.27	1.27

The spectra of compounds (II) and (III) were taken in C_5D_5N and those of the others in $CDCl_3$. All the signals for the methyl groups have a singlet nature; in all cases the H-7 proton appears in the form of a broadened singlet and other signals as multiplets.

There is no doubt that the difference in the structure of compounds (V) and (VII) is due to a different position of the acetonide grouping in the steroid nucleus. The choice between the possible structures was made on the basis of a comparison of the PMR spectra of the diacetonides (V) and (VII) with the corresponding derivatives of integristerone A — (IV) and (VI). As can be seen from Table 1 and Fig. 1b, b', the parameters of the signals from the H-1, H-2, and H-3 protons in the PMR spectrum of the 2,3:20,22-diacetonide of integristerone A (IV) are close in the magnitude of their chemical shifts and in the nature of their multiplicities to the analogous indices of the diacetonide (V). At the same time, the parameters of the signals from the protons of interest to us in the 1,2:20,22-diacetonide of integristerone A (VI) are similar to those of the diacetonide (VII) (see Table 1 and Fig. 1c, c').

Consequently, derivatives (V) and (VII) are, respectively, the 2,3:20,22- and 1,2:20,22-diacetonides of integristerone B. At the same time, it follows from the comparison made that in the molecule of integristerone B, as in intergristerone A, the hydroxy functions at C-1, C-2, and C-3 have the β orientation.

The combination of the facts given convincingly shows that the phytoecdysone (III) has hydroxy groups in the 1β, 2β, 3β, and 5β positions.

The practical coincidence of the values of the chemical shifts of the C-18 methyl groups of integristerone B, integristerone A (see Table 1), and ecdysterone [2], in combination with the results of mass-spectrometric fragmentation and the characteristics of the CD curve given above, show that the phytoecdysone (III) also contains a 14α-hydroxy group.

Thus, integristerone B is 1β,2β,3β,5β,14α,20R,22R,25-octahydroxy-5β-cholest-7-en-6-one.

EXPERIMENTAL

The IR spectra were obtained on a UR-20 spectrophotometer (KBr). The circular dichroism was determined on a J-20 spectropolarimeter. The mass spectra were taken on an MKh-1303 instrument fitted with a system for the direct introduction of the substance into the ion source at an ionizing voltage of 40 V and a temperature of 140-170°C. The elementary compositions of the ions were measured on a Varian MAT-311 instrument. The PMR spectra were taken on a JNM-4H-100 instrument, δ scale, 0 — HMDS, temperature of the sample 22±2°C. The KSK silica gel was used for chromatography.

Isolation of Integristerone B (III). The air-dry inflorescences of *Rh. integrifolium* (25 kg) collected in 1976 at the end of the flowering period (KirgSSR, Fergana Range, environs of the village of Charvak) were exhaustively extracted with methanol, the extract was concentrated to 2 liters and was diluted with an equal volume of water, and the hydrophobic compounds were extracted with petroleum ether. The purified aqueous methanolic fraction was extracted several times with butanol. The butanol was distilled off in vacuum and the dry residue was dissolved in 1200 ml of methanol and poured into 15 liters of ethyl acetate. After separation from the flocculent precipitate that had deposited, the ethyl acetate solution was evaporated, giving 66 g of a crystalline mass consisting of the combined phytoecdysones.

The combined material (4.6 g) was chromatographed on a column of silica gel. Elution was carried out with chloroform—methanol—water (60:32:6). In addition to 3.85 g of ecdysterone (I) and 40 mg of integristerone A (II), this gave 5 mg (0.0003%) of integristerone B, $C_{27}H_{44}O_9$, with mp 186-190°C (ethyl acetate—methanol), $[\alpha]_D^{20}$ +43.6±2° (c 0.55 methanol) $\lambda_{max}^{C_2H_5OH}$ 240 nm (log ε 4.10). ν_{max}^{KBr}, cm^{-1}: 3300-3500 (OH); 1635, 1680 (Δ^7-6-oxo grouping). CD (c 0.10, dioxane); Δε = −1.6 (255 nm), Δε = +2.1 (328 nm). Mass spectrum, m/e (%): 476 $(M - 2H_2O)^+$ (3), 458 (11), 440 (13), 422 (5), 416 (5), 395 (3), 390 (65), 384 (6), 377 (26), 372 (10), 359 (17), 354 (19), 341 (19), 332 (7), 324 (21), 323 (4), 273 (65), 272 (74), 256 (47), 255 (94), 229 (40), 228 (53), 227 (38), 143 (18), 125 (20), 99 (100), 81 (53), 69 (48).

2,3:20,22-Diacetonide (V) and 1,2:20,22-Diacetonide (VII) of Integristerone B. A solution of 69 mg of integristerone B (III) in 11 ml of dry acetone was treated with 5 mg of phosphomolybdic acid and was left at room temperature for 48 h. Then the reaction mixture was diluted with 50 ml of water and neutralized with sodium bicarbonate, the acetone was evaporated off, and the residual aqueous solution was extracted with ether. After the elimination of the solvent, the extract was chromatographed on a column of silica gel. Elution with chloroform—methanol (85:15) gave 18 mg of the 2,3:20,22-diacetonide (V), $C_{33}H_{52}O_9$, mp 128-130°C (chloroform—methanol), $[\alpha]_D^{20}$ +109.5±2° (c 0.36; methanol). Mass spectrum, m/e: 574 $(M - H_2O)^+$ 559, 556, 516, 498, 483, 480, 458, 440, 432, 417, 400, 382, 372, 354, 342, 314, 296, 228, 201, 143, 125, 102, 99, 81.

On continuing elution, the same system gave 10 mg of the 1,2:20,22-diacetonide (VII), $C_{33}H_{52}O_9$, with mp 115-118°C (chloroform–methanol), $[\alpha]_D^{20}$ + 96.0±2° (c 0.46; methanol). Mass spectrum, m/e: 592 M^+, 577, 574, 559, 556, 516, 498, 483, 480, 458, 440, 432, 417, 400, 382, 372, 354, 342, 314, 296, 228, 201, 143, 125, 102, 98, 81.

SUMMARY

From the flower heads of *Rhaponticum integrifolium* we have isolated a new phytoecdysone — integristerone B. It has been shown that it is 1β,2β,3β,5β,14α,20R,22R,25-octahydroxy-5β-cholest-7-en-6-one.

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PHYTOECDYSONES OF *Rhaponticum integrifolium*

IV. 24(28)-DEHYDROMAKISTERONE A

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We have previously reported the isolation from the flower heads of *Rhaponticum integrifolium* C. Winkl. of ecdysterone, integristerone A, and integristerone B [1-3]. Subsequently, from the mother liquors remaining after the separation of these compounds, we have isolated a new phytoecdysone (I) with a molecular weight of 492 and the composition $C_{28}H_{44}O_7$.

In the region of high mass numbers of the mass spectrum of the phytoecdysone (I) there are the peaks of dehydration ions with m/e 474 $(M - H_2O)^+$, 456 $(M - 2H_2O)^+$, and 438 $(M - 3H_2O)^+$, and also ions with m/e 363 (II), 345, and 327, which are characteristic of ecdysones with a 20,22-diol grouping and the steroid nucleus of ecdysterone [4, 5].

The ion with m/e 99 that is characteristic for the mass spectrum of ecdysterone [5] and is formed from the elements of the side chain as the result of the cleavage of the C-20–C-22 bond and the elimination of a molecule of water is shifted by 12 m.u. in the spectrum of (I) (m/e 111, $C_7H_{11}O$). The absence of an isotopic shift for the ion with m/e 111 in the spectrum of the D0 analog of the ecdysone (I) is evidence that it has the cyclic structure (III) [3]. The decomposition of the ion (III) taking place with the elimination of a C=O molecule leads to the strong peak of a fragment with m/e 83 (IV, C_6H_{11}). The increase in density of the latter can be explained by its high stability.

The formation of ions with m/e 111 and 83 shows the presence of a hydroxy function at C-25 and, at the same time, the presence in the side chain of the phytoecdysone (I) of an additional carbon atom as compared with ecdysterone.

Thus, the results of mass-spectrometric fragmentation lead us to the conclusion that the substance isolated belongs to the ecdysones of the C-28 series.

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