PHYTOECDYSONES OF *Rhaponticum integrifolium*

II. INTEGRISTERONE A

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We have previously [1] reported the isolation from the flower heads of *Rhaponticum integrifolium* C. Winkl of ecdysterone and the detection of at least three more ecdysones in this plant. In the present paper we give information on the structure of a new phytoecdysone — integristerone A (I).

The peak of the molecular ion is absent from the mass spectrum of integristerone A, but the high-mass region of the mass spectrum is characterized by the presence of peaks of dehydration ions with m/e 478 ($M - H_2O$), 460 ($M - 2H_2O$), 442 ($M - 3H_2O$), and 424 ($M - 4H_2O$). In addition, fragments with m/e 379, 361, 343, and 325 show the presence of four hydroxy groups in the steroid nucleus of ecdysone (I) [2-5].

The fragmentation of the side chain of integristerone A is marked by ions with m/e 143, 125, 99, 81, and 69, which are characteristic of the breakdown of the side chain of ecdysterone [6, 7]. Such fragmentation shows that the side chains of integristerone A and ecdysterone have the same structure. This conclusion is confirmed by the good agreement of the values of the chemical shifts (CS's) of the C-21, C-26, and C-27 methyl groups and of the proton at C-22 in the PMR spectra of integristerone A and ecdysterone (Table 1).

Thus, the molecule of the new ecdysone contains seven hydroxy groups, of which three are in the side chain and four in the steroid nucleus.

In the PMR spectrum of integristerone A, a broadened singlet at 4.15 ppm (3 H) belongs to three protons located geminally to secondary hydroxy groups. This assignment is based on the fact that in the PMR spectra of the tetraacetate (II) and the pentaacetate (III) obtained by the acetylation of integristerone A this signal is shifted downfield: 5.25 ppm (3 H).

For compound (III) it was established by the double-resonance method that the one-proton multiplet at 4.74 ppm corresponds to H-22, since it is converted into a narrow singlet on irradiation in the region of methylene protons (1.40 ppm).

It follows from these facts that in the steroid nucleus of integristerone A (I) three of the four hydroxy groups are secondary.

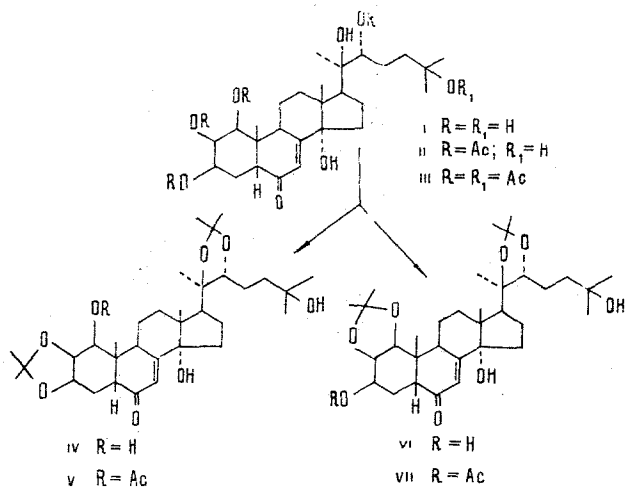

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TABLE 1. Chemical Shifts of the Protons of Integristerone A, Ecdysterone, and Their Derivatives (δ, ppm)

Positions of the protons	Compound								
	I	II	III	IV	V	VI	VII	VIII	IX
H-1	4,15	5,25	5,25	3,69	5,23 d	4,19	4,15 d	—	—
H-2	4,15	5,25	5,25	4,26	4,20 m	4,19	4,29 t	4,01	4,98
H-3	4,15	5,25	5,25	4,26	4,20 m	3,90	5,11 q	4,07	5,26
H-22	3,71	4,77	4,74	3,59	3,60	3,59	3,59	3,70	4,74
H-7	6,14	5,83	5,80	5,76	5,78	5,79	5,78	6,12	5,80
H-9	3,42	3,05	3,05	3,06	—	—	—	3,42	3,12
$OCOCH_3$	—	1,93	1,92	—	2,14	—	2,06	—	1,89
		2,03	2,03						2,06
		2,04	2,06						
CH_3-18	1,07	0,79	0,79	0,71	0,70	0,75	0,75	1,06	0,75
CH_3-19	1,26	0,97	0,95	1,18	1,26	1,18	1,19	0,94	0,97
CH_3-21	1,43	1,19	1,19	1,09	1,07	1,10	1,10	1,44	1,17
CH_3-26	1,24	1,13	1,33	1,18	1,18	1,18	1,18	1,22	1,34
CH_3-27		1,17	1,36	1,27	1,26	1,27	1,29		1,35

Note. The spectrum of compound (I) was taken in C_5D_5N, (II-VII and IX) in $CDCl_3$, and (VIII) in C_5H_5N. Compound (VIII) represents ecdysterone, and (IX) ecdysterone 2,3,22,25-tetraacetate.

All the signals from the methyl groups are singlets; in all cases the H-7 proton appears in the form of a broadened singlet, and H-9 and H-22 respectively as a quartet and a multiplet with broadened lines; with the exception of compounds (V) and (VII) the signals from H-1, H-2, and H-3 form highly broadened singlets or multiplets with a coarsened fine structure.

In solution in dry acetone, integristerone A forms two isomeric diacetonides (IV) and (VI) with the molecular weight of 576. The mass spectra of both derivative show the peaks of ions with m/e 201, 143, 125, 102, 99, and 81, corresponding to the breakdown of the side chain of the diacetonide of ecdysterone [6, 7] and showing the presence of an acetonide grouping at C-20 and C-22. We may also note the good agreement of the parameters of the H-22 and CH_3-21 signals in the PMR spectrum of the diacetonide (IV) with those of the diacetonide (VI) (see Table 1). These facts confirm the identity of the structure of the side chains in compounds (IV) and (VI). It is clear that the difference in the structures of the acetonides (IV) and (VI) is due to different positions of the dioxolane ring in the steroid nucleus of this ecdysone. Consequently, the tetracyclic nucleus of integristerone A must contain a cis-vicinal triol grouping with the hydroxy groups on the 1,2,3- or 2,3,4-carbon atoms.

The choice of one of the alternative structures selected was made on the basis of a study of the parameters of the signals in the PMR spectra of diacetonides (IV) and (VI), the full spectra of which are given in Fig. 1.

As can be seen from Fig. 1, the signals in the most informative part (~3.5-4.5 ppm) are highly broadened and partially overlap one another, which prevents the elucidation of the fine structure of the multiplets of the protons at C-1, C-2, C-3, and C-4 that are of interest to us. In this respect, the spectra obtained by using benzene as solvent for the diacetonide (IV) (Fig. 1c) and deuteropyridine for the diacetonide (VI) (Fig. 1b') proved to be far more useful.

In the PMR spectrum of compound (IV) taken in C_6H_6 at +68°C (Fig. 1c), a one-proton doublet with ^3J = 4.4 Hz (at 3.61 ppm) must be assigned to H-1 for a triol system with hydroxy groups at carbon atoms 1, 2, and 3. In actual fact, in the PMR spectrum of the diacetonide (IV) taken in $CDCl_3$ at +50°C (Fig. 1b) a one-proton signal corresponding in the value of its CS (2.56 ppm) to H-5 is observed [8]. The quartet nature of the splitting of this signal shows that H-5 interacts with the methylene protons at C-4. On the basis of these facts and the spin–spin coupling constants (SSCC's) of the protons, the triplet at 4.08 ppm (1 H) with $^3J_{2,1}$ = 4.4 Hz and $^3J_{2,3}$ = 5.9 Hz was assigned to H-2, and the quartet at 4.28 ppm (1 H) with $^3J_{3,2} = {}^3J_{3,4} = {}^3J_{3,4'} \simeq 5.9$ Hz to H-3 (Fig. 1c).

All this convinced us of the fact that in integristerone A (I) there are hydroxy groups

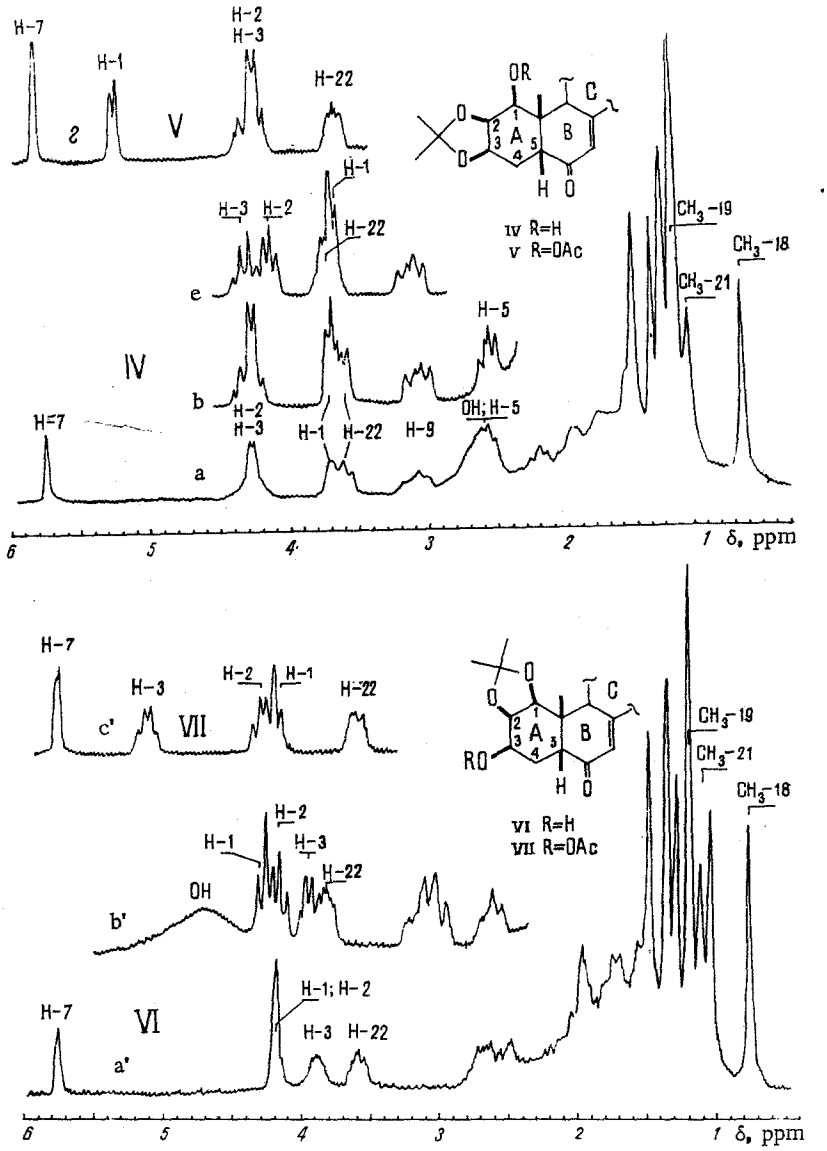

Fig. 1. PMR spectra of the 2,3:20,22-diacetonide of integristerone A (IV) in $CDCl_3$ at 20-22°C (a), at +50°C (b), and in C_6H_6 at +68°C (c), of the 2,3:20,22-diacetonide of integristerone A 1-acetate (V) in $CDCl_3$ at 20-22°C (d), of the 1,2:20,22-diacetonide of integristerone A (VI) in $CDCl_3$ at 20-22°C (a') and in C_5D_5N at +45°C (b'), and of the 1,2:20,22-diacetonide of integristerone A 3-acetate (VII) in $CDCl_3$ at 20-22°C (c').

in ring A on carbon atoms 1, 2, and 3.

Acetylation of the diacetonide (IV) yielded the monoacetate (V). In the PMR spectrum of compound (V) taken in $CDCl_3$ (Fig. 1d), a doublet signal with $^3J_{1,2}$ = 4.0 Hz from H-1 is strongly shifted downfield (δ_V = 5.23 ppm, δ_{IV} = 3.69 ppm; $\Delta\delta$ = 1.54 ppm). This shows that compound (V) is the 1-acetate of the 2,3:20,22-diacetonide of integristerone A. Consequently, substance (IV) is the 2,3:20,22-diacetonide of integristerone A.

Then compound (VI) must be the 1,2:20,22-diacetonide of integristerone A. As was to be expected, the nature of the splitting of the signals of H-1,2,3 in the PMR spectrum of (VI) in C_5D_5N is similar to that for the diacetonide (IV). On this basis, the signals shown in Fig. 1b' may be assigned in the following way: the doublet (1 H) at 4.31 ppm with $^3J_{1,2}$ = 5.6 Hz to H-1; the triplet (1 H) at 4.16 ppm with $^3J_{2,1}$ = 5.6 Hz and $^3J_{2,3}$ = 4.2 Hz to H-2; and the quartet (1 H) at 3.96 ppm with $^3J_{3,2} + {}^3J_{3,4} + {}^3J_{3,4'} \approx 13.2$ to H-3.

In the PMR spectrum ($CDCl_3$, Fig. 1c') of the monoacetate (VII) obtained by the acetyla-

tion of (VI), as was to be expected, the quartet signal from H-3 had shifted downfield (δ = 5.11 ppm), which defines substance (VII) as the 3-acetate of the 1,2:20,22-diacetonide of integristerone A.

It can be seen from Fig. 1c' that the spectrum of compound (VII) shows fairly good resolution of the signals from H-1, 2, and 3. This enabled us to perform an additional check on the assignment of the signals of the protons mentioned by the double-resonance method. When H-3 was irradiated, the triplet from H-2 was converted into a doublet, while the doublet from H-1 remained unchanged. When the H-2 nucleus was saturated, the quartet from H-3 was converted into a triplet with $^3J_{3,4} + {}^3J_{3,4'} = 10.0$ Hz. It may be concluded from this experimental fact that there is no trans-diaxial interaction between H-3 and the neighboring vicinally located protons. In addition to this, the facts unambiguously confirm the correctness of the assignments that we have made of the signals from H-1, 2, 3.

The orientation of the hydroxy groups in ring A of the new ecdysone and of the protons adjacent to them was made in the following way: the circular dichroism (CD) curve of compound (I) shows a negative $\Delta\varepsilon = -3.2$ ($\pi \rightarrow \pi^*$ transition, 245 nm) and a positive $\Delta\varepsilon = +1.4$ ($n \rightarrow \pi^*$ transition, 338 nm) of the Cotton effect. Such a nature of the CD curve shows the presence in the phytoecdysone (I) of a Δ^7-6-keto-14-hydroxy grouping with the cis linkage of rings A/B [9]. In the cis-1,2,3-triol system the presence of which follows so clearly from the formation of the diacetonides (IV) and (V), the hydroxy groups may have either the α or the β orientation. However, in the case of the cis linkages of rings A/B only the β-orientation of the hydroxy groups excludes the diaxial interaction of H-3 with the neighboring protons that we mentioned above.

The experimental material presented leaves no doubt of the fact that integristerone A has hydroxy groups in the 1β, 2β, and 3β positions.

A comparison of the CS values of the C-18 methyl group of integristerone A with ecdysterone and of the pentaacetate (III) with those of ecdysterone tetraacetate (see Table 1) in combination with the indices of the CD curve given previously and the results of mass-spectrometric fragmentation permit us to consider that there is a 14α-hydroxy group in the new phytoecdysone (I).

The identity of the configurations of the hydroxy groups at C-20 and C-22 in integristerone A and ecdysterone follows from the coincidence of the parameters of the CH_3-21 and H-22 signals in the PMR spectra of these ecdysones and their acetates (see Table 1).

Thus, integristerone A is 1β,2β,3β,14α,20R,22R,25-heptahydroxy-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 recorded on an MKh-1303 instrument fitted with a system for direct introduction of the substance into the ion source at an ionizating voltage of 40 V and a temperature of 140-170°C and the PMR spectra were obtained on a JNM-4H-100 instrument using the δ scale with HMDS as zero. The accuracy of the measurements of the CS's was ±0.01 ppm and of the SSCC's ± 0.1 Hz, and the accuracy of the temperature of the sample was ±2°C. For chromatography we used type KSK silica gel. The phytoecdysones were revealed with vanillin/sulfuric acid.

Isolation of Integristerone A (I). The air-dry inflorescences of *Rh. integrifolium* (36 kg) collected in 1975 at the end of flowering (KirgSSR, Fergana range, environs of the village of Pravda) were exhaustively extracted with methanol, the extract was concentrated to 1.0 liter and was diluted with 2 liters of water, and the hydrophobic compounds were extracted with petroleum ether.

The aqueous methanolic fraction was extracted repeatedly with butanol. After elimination of the solvent, the dry residue was chromatographed on a column of silica gel. Elution was performed with chloroform–methanol (9:1 and 4:1). This led to the isolation of, together with 36.0 g (0.1%) of ecdysterone, 450 mg (0.0013%) of integristerone A (I), $C_{27}H_{44}O_8$, with mp 244-246°C (ethyl acetate–methanol), $[\alpha]_D^{20}$ + 33.7 ±2° (c 0.92; methanol) $\lambda_{max}^{C_2H_5OH}$ 245 nm (log ε 4.12); ν_{max}^{KBr} 3300-3500 (OH), 1605 cm^{-1} (Δ^7-6-keto grouping); CD (c 0.10; dioxane), $\Delta\varepsilon = -3.2$

(245 nm), $\Delta\varepsilon$ = +1.4 (338 nm). Mass spectrum (m/e, %): 478 (14, 460(20), 442 (94), 424 (33), 409 (10), 391 (6), 379 (15), 374 (12), 368 (42), 361 (100), 343 (90), 325 (30), 316 (25), 301 (15), 283 (24), 143 (12), 125 (12), 99 (49), 81 (27), 69 (27).

Integristerone A 1,2,3,22-Tetraacetate (II) and 1,2,3,22,25-Pentaacetate (III). Integristerone A (I) (138 mg) in 3 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for 4 days. Then the reaction mixture was diluted with water and the precipitate that deposited was filtered off and was chromatographed through a column of silica gel. Elution with chloroform–methanol (20:1) gave 38 mg of the pentaacetate (III), $C_{37}H_{54}O_{13}$, mp 150-152°C (chloroform–methanol), $[\alpha]_D^{20}$ + 43.4 ±2° (c 0.55; methanol). ν_{max}^{KBr}, cm^{-1}: 3500-3520 (OH), 1735, 1250 (ester grouping), 1670 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): 610 (19), 568 (60), 550 (100), 535 (24), 507 (27), 495 (30), 392 (15), 265 (18), 99 (19), 81 (33), 69 (58).

Further washing of the column with the same mixture of solvents yielded 28 mg of the tetraacetate (II), $C_{33}H_{52}O_{12}$, mp 170-172°C (chloroform–methanol), $[\alpha]_D^{20}$ + 71.7 ±2° (c 0.6; methanol). ν_{max}^{KBr}, cm^{-1}: 3470-3520 (OH), 1745, 1250 (ester grouping), 1765 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): 586 (10), 568((49), 553 (20), 550 (8), 510 (8), 495 (8), 443 (9), 392 (23), 350 (10), 152 (82), 99 (32), 81 (52), 69 (100).

Integristerone A 2,3:20,22-Diacetonide (IV) and 1,2:20,22-Diacetonide (VI). A suspension of 196 mg of integristerone A (I) in 7 ml of dry acetone was treated with 5 mg of molybdophosphoric acid, and the reaction mixture was shaken at room temperature until dissolution was complete. After 20 h, the reaction mixture was diluted with 50 ml of water and neutralized with sodium bicarbonate. The neutral solution was extracted with ether, and the extract was chromatographed on a column of silica gel. Elution with the chloroform–methanol (85:15) system yielded 60 mg of the 2,3:20,22-diacetonide (IV), $C_{33}H_{52}O_8$, mp 228-230°C (chloroform–methanol), $[\alpha]_D^{20}$ + 91.3 ±2° (c 0.46; methanol). ν_{max}^{KBr}, cm^{-1}: 3400-3520 (OH), 1665 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): M^+ 576 (3), 561 (6), 558 (2), 543 (6), 540 (1), 525 (2), 518 (1), 500 (7), 485 (12), 473 (15), 467 (5), 465 (5), 460 (5), 442 (15), 431 (5), 425 (11), 419 (44), 402 (22), 384 (17), 376 (11), 373 (6), 361 (48), 357 (35), 351 (8), 343 (28), 318 (24), 299 (11), 201 (26), 143 (82), 125 (78), 102 (93), 99 (100), 82 (28), 81 (30).

By continuing the elution of the column with the same mixture of solvents we isolated 35 mg of the 1,2:20,22-diacetonide (VI), $C_{33}H_{52}O_8$, with mp 238-240°C (chloroform–methanol), $[\alpha]_D^{20}$ + 58.7 ±2° (c 0.46; methanol), ν_{max}^{KBr}, cm^{-1}: 3400-3520 (OH), 1665 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): M^+ 568 (1), 561 (5), 558 (1), 543 (1), 540 (0.4), 525 (1), 518 (0.7), 500 (1), 485 (6), 483 (10), 477 (2), 460 (4), 442 (3), 431 (1), 425 (5), 419 (51), 402 (11), 385 (5), 376 (3), 361 (32), 356 (7), 351 (6), 343 (19), 325 (7), 318 (16), 306 (3), 301 (5), 283 (4), 201 (15) , 183 (14), 158 (9), 143 (49), 125 (41), 102 (100), 99 (97), 81 (22).

Integristerone A 2,3:20,22-Diacetonide 1-Acetate (V). A solution of 42 mg of the 2,3:20,22-diacetonide (IV) in 4 ml of pyridine was treated with 1 ml of acetic anhydride. The reaction mixture was left at room temperature for 24 h and was then diluted with 50 ml of water and extracted with ether. After the solvent had been distilled off, the dry residue was separated on a column of silica gel. Washing with the chloroform–methanol (30:1) system yielded 16 mg of the 2,3:20,22-diacetonide 1-acetate (V), $C_{35}H_{54}O_9$, with mp 218-220°C (chloroform–methanol), $[\alpha]_D^{20}$ + 54.3 ±2° (c 0.74; methanol). ν_{max}^{KBr}, cm^{-1}: 3450-3520 (OH), 1745, 1250 (ester grouping), 1675 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): M^+ 618 (9), 603 (12), 600 (1), 585 (2), 542 (2), 525 (5), 461 (12), 443 (7), 418 (4), 403 (3), 201 (51), 143 (100), 125 (48), 102 (72), 99 (83), 81 (24).

Integristerone A 1,2:20,22-Diacetonide 3-Acetate (VII). The 1,2:20,22-diacetonide (VII) (25 mg) was acetylated in 5 ml of pyridine with 1 ml of acetic anhydride at room temperature for 48 h. Then the reaction mixture was diluted with 50 ml of water and the reaction product was extracted with ether. The residue obtained after the distillation of the solvent was chromatographed on a column of silica gel. Elution with the chloroform–methanol (30:1) system yielded 13.5 mg of the 1,2:20,22-diacetonide 3-acetate (VII), $C_{35}H_{54}O_9$, mp 226-229°C (chloroform–methanol), $[\alpha]_D^{20}$ + 50.0 ±2° (c 0.37; methanol); ν_{max}^{KBr}, cm^{-1}: 3500-3510 (OH), 1745, 1260 (ester grouping), 1665 (Δ^7-6-keto grouping). Mass spectrum (m/e, %): M^+ 618 (1), 603

(11), 585 (1), 542 (2), 525 (10), 507 (5), 484 (3), 467 (6), 461 (22), 443 (72), 418 (3), 403 (11), 385 (25), 343 (32), 325 (13), 201 (14), 143 (51), 125 (33), 102 (100), 99 (96), 81 (23), 69 (22).

SUMMARY

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

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ISOLATION OF CHOLESTEROL ISONONATRIACONTANATE FROM SHEEPS' WOOL WAX

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UDC 547.922

In a study of the chemical composition of wool wax by chromatography on silica gel and alumina, we have succeeded in isolating an ester of cholesterol (I) with a higher fatty acid in a yield of about 23%, calculated on the wax. The IR spectrum of this ester shows an absorption band with ν_{max} 1728 cm^{-1}. The alkaline hydrolysis of the ester (I) gave cholesterol, identified by comparison with an authentic sample, and a fatty acid (II) with mp 77-80°C which was characterized in the form of its methyl ester (III).

According to its mass spectrum, the methyl ester (III) had the empirical formula $C_{40}H_{82}O_2$ (M^+ 592) and the spectrum contained peaks corresponding to the formation of the fragments $M^+ - 29$ (m/e 563), $M^+ - OCH_3$ (m/e 561), and $M^+ - 43$ (m/e 449), and also the peaks of fragments with m/e 535, 521, 507, 493, 479, 465, 451, 437, 423, 409, 395, 381, 367, 353, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199.

According to the PMR spectrum taken in $CDCl_3$, the methyl ester (III) contains no olefinic protons and has a gem-dimethyl group (presence of two three-proton singlets at 0.84 and 0.90 ppm, J = 6 Hz) and also a $-CH_2COOCH_3$ grouping [triplet with its center at 2.31 ppm (2 H) and singlet at 3.67 ppm (3 H)]. The absence of other characteristic signals in the PMR spectrum of the methyl ester (III) and the nature of its fragmentation on electron impact permit this compound to be assigned the structure of methyl isononatriacontanate and, correspondingly, the natural ester (I) the structure of cholesterol isononatriacontanate. Similar esters containing fatty acids with an iso structure but with a shorter chain have been detected in the wool wax degras [1].

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