PHYTOECDYSONES OF *Silene praemixta*.

I. SILENOSTERONE

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In addition to known ecdysteroids (2-deoxy-α-ecdysone and 2-deoxyecdysterone) from *Silene praemixta* (*Caryophyllaceae*) we have isolated new ones — premixisterone and selenosterone (I), $C_{27}H_{42}O_7$, mp 115-117°C (from MeOH), $[\alpha]_D^{28}$ +86.9 ± 2° (c 0.92, MeOH), yield 0.003%. The acetylation of (I) with $(CH_3CO)_2O$ in Py gave 22-acetylselenosterone (II), $C_{29}H_{44}O_6$, mp 210-212°C ($MeOH-C_6H_{14}$), $[\alpha]_D^{27}$ +45.5 ± 3° (c 0.16; MeOH). On the basis of physiocochemical and spectral characteristics it has been established that (I) has the structure of 14α,22R,25-trihydroxy-5β-cholest-7-ene-3,6-dione. The IR, PMR, and mass spectra of (I) and (II) are presented.

Plants of the genus *Silene* (family Caryophyllaceae), which is widespread in the temperate zone of the northern hemisphere (about 400 species are known), are being studied for the first time for their phytoecdysone content. In particular, in *Silene praemixta* M. Pop., growing in Central Asia [1], we have detected a considerable amount of ecdysteroids. The ecdysone-like compounds accumulate mainly in the leaves and inflorescences of the plant, their amount in the roots and stems being somewhat smaller and ranging from 0.1 to 1.6%. The maximum amount of phytoecdysones (about 2%) is found in the epigeal organs in the vegetation phase of the plant. The sum of the ecdysteroids consists of at least six components. By extraction with ethanol followed by chromatography of the extract on a column of alumina and rechromatography on silica gel, we have isolated five substances. Of these, two — deoxy-α-ecdysone (III) and 2-deoxyecdysterone (IV) — are known compounds [2, 3], while two minor products of low polarity proved to be new. We have called them silenosterone (I) and premixisterone.

The present communication is devoted to the determination of the structure of silenosterone. The UV spectrum of this compound has a pronounced maximum at 246 nm (log ε 4.05) which is characteristic for Δ^7-6-ketosteroids. The IR spectrum contains, in addition to absorption at 3420 cm^{-1} (OH) and 1660 cm^{-1} (conjugated ketone), a band with a maximum at 1710 cm^{-1} showing the presence of an additional carbonyl group.

When the mass spectra of ecdysteroids (III) and (I) were compared, it became obvious that the molecular weight of silenosterone (M^+ 446) was two units smaller than that of 2-deoxy-α-ecdysone (M^+ 448).

The fragmentation of the side chain of silenosterone (I), like the decomposition of the corresponding section of the 2-deoxy-α-ecdysone molecule (III) [2], was characterized by high-intensity peaks with m/e 99 and 81. This shows that the side chains of the two compounds have the same structure. This conclusion was confirmed, as shown below, by the good agreement in the PMR spectra of these substances of the chemical shifts of the protons of the C-21, C-26, and C-27 methyl groups and of the proton at C-22.

It stands to reason that if the side chains of silenosterone and of 2-deoxy-α-ecdysone have the same structure, the new phytoecdysone must include three hydroxy groups: one in the steroid nucleus of the molecule and two in the side chain.

When silenosterone (I) was acetylated with acetic anhydride in pyridine we obtained a monoacetate (II). The results of the fragmentation of the monoacetate under the action of electron impact agreed with the hypothesis that in the steroid nucleus a nonacetylatable hydroxy group was present at C-14. The marked decrease in the intensities of the peaks of the ions with m/e 99 and 81 in the spectrum of the acetate (II) showed that the acetoxy group

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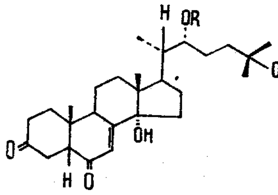
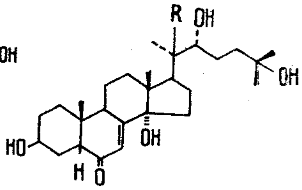
occupied a position at C-22. The absence of additional substituents in the steroid nucleus of silenosterone was also confirmed by the fact that its mass spectrum contained the peak of an ion with m/e 282 — an analog of the ion with m/e 284 in the mass spectrum of 2-deoxy-α-ecdysone (III). This fragment was formed by the cleavage of the C-17—C-20 bond with the simultaneous elimination of H_2O at the expense of the hydroxyl at C-14.

The position of the additional carbonyl group was determined by comparing the PMR spectra of silenosterone (I) and of 2-deoxy-α-ecdysone (III). In our experiments (solvent deuteropyridine, internal standard HMDS, δ scale, ppm) the silenosterone spectrum showed the following chemical shifts (the corresponding figures for (III) are given in parentheses for comparison): H-7, broadened singlet, 6.00 (6.13); H-9, multiplet, 3.45 (3.42); CH_3-18, singlet, 0.60 (0.61) CH_3-19, singlet, 0.94 (0.95); CH_3-21, doublet, 1.15 (1.17); CH_3-26/27, singlet, 1.25 ppm (1.26).

It is not difficult to observe the good agreement of the magnitudes compared. The only exception is the signal of the geminal proton at C-3. In deoxy-α-ecdysone (III) this signal is superposed on the signal of the proton at C-22 and resonates in the form of a complex two-proton multiplet at 4.0 ppm. In silenosterone (I) only the one-proton multiplet of H-22 appears at 3.90 ppm, and the signal of the proton at C-3 is absent.



I. R = H
II. R = Ac



III. R = H
IV. R = OH

Thus, the only difference in structure between the ecdysteroids (I) and (III) is that in the first of them C-3 bears a ketonic function in place of a hydroxy group. It is a fact not causing doubt that for this reason, as well, silenosterone on acetylation gives not a di- but a monoacetate and this single acetate group is, as the mass spectrum shows, located in the side chain. In view of the closeness of the chemical shifts of the main protons it must be assumed that in the type of linkage of rings A and B and in the configuration of the substituents silenosterone (I) and 2-deoxy-α-ecdysone (III) are identical.

Summarizing what has been said, we arrive at the conclusion that silenosterone has the structure of 3-keto-2-deoxy-α-ecdysone, or 14α,22R,25-trihydroxy-5β-cholest-7-ene-3,6-dione (I).

No ecdysone-like compounds with a ketone group at C-3 have been isolated in the native form. 3-Dehydro-α-ecdysone has been identified and characterized chemically in *in vitro* experiments with insect homogenates [4]. 3-Dehydroecdysterone was first detected as a metabolite in the sheep maggot fly *Calliphora erythrocephala* (*C. vicine* R. B.) [5]. Initially, the compound was not purified and reliably identified.

This product has been synthesized enzymatically and chemically comparatively recently [6]. In a biotest for ecdysial activity in the *Calliphora* test the substance proved to be 15 times less active than α-ecdysone.

EXPERIMENTAL

The IR spectra were obtained on a UR-20 spectrophotometer (KBr), the PMR spectrum on a JNM-4H-100 instrument using the δ scale, O–HMDS (in deuteropyridine), and the mass spectra on a MKh-1310 instrument fitted with a system for the direct introduction of the sample into the ion source at an ionizing voltage of 70 V and a temperature of 100-120°C.

For chromatography we used silica gel of types KSK and L (Czechoslovakia), treated in the usual way, and alumina (activity grade IV). The purity of the substances was checked by thin-layer chromatography on KSK silica gel in chloroform–ethanol systems: 1) 4:1; 2) 9:1; 3) 25:1. The phytoecdysones were revealed with vanillin–sulfuric acid.

Isolation of the Ecdysteroids. The plant was collected in May, 1977 in the area at the foot of the low mountain Alim-Tau (Chimkent province, KazSSR). The leaves and inflorescences of the S. praemixta were separated from the stems and the air-dry comminuted raw material (6 kg) was extracted with 30 liters of ethanol. The extract was concentrated, diluted with water, and filtered. After the additional removal of ethanol by vacuum distillation, the aqueous residue was treated with chloroform–benzene (1:1) to remove resins and waxes. The phytoecdysones were exhaustively extracted from the aqueous residue with ethyl acetate. The solvent was distilled off and the combined ecdysterones so obtained were chromatographed on a column of alumina (3 kg). The column was eluted with chloroform, and then with chloroform–ethanol (9:1). The partially purified combined material was rechromatographed on a column of silica gel. Elution of the column with chloroform containing gradientwise increasing amounts of ethanol (2-10%) yielded successively silenosterone (156 mg), premixisterone (122 mg), and the two known ecdysones 2-deoxy-α-ecdysone (7.48 g) and 2-deoxyecdysterone (4.92 g).

The further elution of the column with chloroform–ethanol (4:1) gave 3.9 g of a substance with mp 242-243°C (from acetone).

The yields of phytoecdysones (% on the weight of the air-dry raw material) were as follows: silenosterone, 0.003; premixisterone, 0.002; 2-deoxy-α-ecdysone, 0.12; 2-deoxyecdysterone, 0.082.

Silenosterone (I), $C_{27}H_{42}O_5$, mp 115-117°C (from methanol), $[\alpha]_D^{28}$ +86.9 + 2° (c 0.92; CH_3OH), R_f ~ 0.46 (system 2, TLC on silica gel); $\lambda_{max}^{C_2H_5OH}$ 246 nm (log ε 4.05); ν_{max}^{KBr} 3420 cm^{-1} (OH), 1710 (C=O), 1660 cm^{-1} (Δ^7-6-keto grouping). Mass spectrum, m/e (%): 446 (M^+; 0,3), 428 (5), 413 (4), 410 (8), 400 (3), 395 (10), 377 (3), 359 (3), 341 (8), 330 (7), 312 (10), 297 (10), 283 (14), 282 (16), 261 (10), 233 (33), 232 (25), 231 (17), 99 (100), 81 (92).

The substance was readily soluble in ethanol, acetone, and chloroform and sparingly soluble in benzene and water.

22-Acetylsilenosterone (II). At room temperature, 18.4 mg of silenosterone in 1.6 ml of pyridine was acetylated with 0.8 ml of acetic anhydride for 4 h. The excess of the reagents was driven off in vacuum. The residue was chromatographed on silica gel (8 g). Elution of the column with chloroform–ethanol (50:1) yielded 4.2 mg of the acetate (II), $C_{29}H_{44}O_6$, mp 210-212°C (from methanol–hexane), $[\alpha]_D^{27}$ +43.5 ± 3° (c 0.16; CH_3OH); $\lambda_{max}^{C_2H_5OH}$ 243 nm (log ε 4.07); ν_{max}^{KBr}(cm^{-1}) 3480 (OH), 1740, 1735 (C=O), 1670 (Δ^7-6-keto grouping), 1250 (C–O–C). Mass spectrum, m/e (%): 488 (M^+, 1), 470 (6), 452 (3), 428 (1), 410 (16), 395 (29), 392 (21), 377 (13), 330 (2), 329 (4), 312 (8), 311 (14), 301 (10), 300 (13), 283 (39), 282 (57), 281 (20), 232 (100), 231 (92), 99 (20), 81 (20).

2-Deoxy-α-ecdysone (III). $C_{27}H_{44}O_5$, mp 235-236°C (from aqueous ethanol), $[\alpha]_D^{23}$ +93.3 ± 3° (c 1.5; CH_3OH); R_f ~0.63 (system 1, TLC on silica gel); $\lambda_{max}^{C_2H_5OH}$ 245 nm (log ε 4.12); ν_{max}^{KBr} 3440 cm^{-1} (OH), 1645 cm^{-1} (Δ^7-6-keto grouping). Mass spectrum, m/e (%): 448 (M^+; 2), 430 (13), 415 (14), 412 (11), 402 (16), 397 (16), 379 (3), 361 (7), 343 (6), 332 (33), 314 (28), 303 (3), 299 (16), 285 (12), 284 (12), 263 (38), 235 (26), 234 (50), 233 (32), 215 (10), 99 (100), 81 (63).

Literature information [2]: mp 231-232°C, $\lambda_{max}^{C_2H_5OH}$ 244 nm (ε 12,900); ν_{max}^{KBr} 3450, 1650 cm^{-1}; M^+ 448.

3,22-Diacetyl-2-deoxy-α-ecdysone. At room temperature, 0.35 g of the ecdysteroid (III) in 7.0 ml of pyridine was acetylated with 3.0 ml of acetic anhydride for 24 h. After the elimination of the excess of the reagents in vacuum, the residue (0.36 g) was chromatographed on silica gel (12.0 g). Elution of the column with chloroform gave 0.31 g of the diacetate $C_{31}H_{48}O_7$, mp 245-246°C (from chloroform–ethanol), $[\alpha]_D^{24}$ +39.0 ± 3° (c 0.22; CH_3OH); $\lambda_{max}^{C_2H_5OH}$ 243 nm (log ε 4.04); ν_{max}^{KBr} (cm^{-1}) 3460 (OH), 1740 (C=O), 1660 (Δ^7-6-keto grouping), 1250 (C–O–C). Mass spectrum, m/e (%): 532 (M^+; 1), 514 (4), 504 (8), 499 (2), 496 (2), 486 (10), 472 (3), 454 (18), 444 (14), 439 (20), 436 (17), 426 (60), 412 (4), 394 (14), 379 (19), 374 (6), 373 (5), 361 (4), 345 (3), 344 (5), 327 (37), 326 (80), 314 (7), 313 (11), 276 (89), 275 (86), 267 (42), 266 (48), 216 (100), 215 (99), 99 (34), 81 (88).

2-Deoxyecdysterone (IV). $C_{27}H_{44}O_6$, mp 254-255°C (from aqueous ethanol), $[\alpha]_D^{23}$ +82.0 ± 3° (c 1.3; CH_3OH), R_f ~0.54 (system 1, TLC on silica gel), $\lambda_{max}^{C_2H_5OH}$ 246 nm (log ε 4.10);

ν_{max}^{KBr} 3370 cm^{-1} (OH), 1645 cm^{-1} (Δ^7-6-keto grouping). Mass spectrum, m/e (%): 464 (M^+; 1), 446 (3), 428 (10), 413 (8), 410 (14), 395 (6), 392 (6), 377 (5), 347 (35), 329 (90), 312 (33), 311 (28), 303 (9), 302 (11), 297 (13), 295 (14), 285 (30), 284 (35), 234 (15), 233 (15), 99 (100), 81 (73).

Literature information [2, 3]: mp 250-252°C, $\lambda_{max}^{C_2H_5OH}$ 243 nm (ε 12,100), ν_{max}^{KBr} 3450, 1645 cm^{-1}; M^+ 464.

3,22-Diacetyl-2-deoxyecdysterone. The ecdysterone (IV) (0.38 g) was acetylated with acetic anhydride under the same conditions as were given for the preparation of the diacetate of the preceding compound. The column with the crude product was eluted with chloroform-methanol (100:1). This gave 0.31 g of the diacetyl derivatives $C_{31}H_{48}O_8$, mp 185-186°C (from methanol), $[\alpha]_D^{24}$ +79.3 ± 3° (c 0.50; CH_3OH); $\lambda_{max}^{C_2H_5OH}$ 243 nm (log ε 4.10); ν_{max}^{KBr} (cm^{-1}) 3460 (OH), 1740 (C=O), 1665 (Δ^7-6-keto grouping), 1265 (C—O—C). Mass spectrum, m/e (%): 548 (M^+; 0,2), 530 (0,5), 515 (1), 512 (0,5), 497 (2), 488 (2), 470 (15), 460 (5), 455 (10), 452 (33), 442 (6), 437 (22), 389 (80), 371 (45), 346 (13), 341 (11), 329 (80), 327 (23), 326 (18), 311 (100), 276 (32), 275 (11), 216 (36), 215 (27), 99 (48), 81 (84).

SUMMARY

Five phytoecdysones have been isolated from Silene praemixta M. Pop. (family Caryophyllaceae), among them the known 2-deoxy-α-ecdysone and 2-deoxyecdysterone and the new silenosterone and premixisterone.

Silenosterone has the structure of 14α,22R,25-trihydroxy-5β-cholest-7-ene-3,6-dione. This is the first time that a native ecdysterone has been found with a ketone group at C-3.

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PHYTOECDYSONES OF Silene praemixta.

II. Premixisterone

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The structure of premixisterone (I) — a new ecdysteroid from S. praemixta M. Pop. (Caryophyllaceae) — has been established. Compound (I) has the composition $C_{27}H_{44}O_5$, mp 110-112°C (from $C_2H_5OH + H_2O$), $[\alpha]_D^{24}$ 0 ± 4° (c 0.85; MeOH), $\lambda_{max}^{C_2H_5OH}$ 202 nm (log ε 3.35), ν_{max}^{KBr} 3415 cm^{-1} (OH), 1710 cm^{-1} (C=O), and does not contain the Δ^7-6-keto grouping that is characteristic of natural ecdysteroids. The acetylation of (I) with $(CH_3CO)_2$ in Py gave the amorphous 3,22-diacetylpremixisterone (II), $C_{21}H_{48}O_7$. Compound (I) has the structure of 3β,14α,22R,25-tetrahydroxy-5β-cholest-8-en-6-one. The IR, PMR, and mass spectra of (I) and (II) are given.

We have previously [1] reported that five phytoecdysones have been found in Silene praemixta M. Pop., family Caryophyllaceae, including two new ones — silenosterone and premixi-

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