(c 0.80; methanol). $\lambda_{max}^{C_2H_5O}$ 245 nm (log ε 4.0). ν_{max}^{KBr} (cm^{-1}): 3380-3430 (OH); 1655 (Δ^7-6-keto grouping, CD (c 0.12; ethanol); Δε = −1.28 (253 nm); Δε = +1.35 (330 nm).

Mass spectrum, m/z (%): 446 (M^+ − 164; 0.2), 430 (1), 412 (5), 403 (1.5), 398 (2), 395 (2), 379 (2), 361 (4), 343 (3), 332 (4), 314 (4), 311 (4), 299 (7), 285 (8), 284 (15), 264 (7), 251 (8), 234 (6), 233 (6), 185 (7), 149 (100), 135 (11), 129 (1), 99 (15), 81 (27), 69 (30).

Enzymatic Hydrolysis of Sileneoside E (IV). A solution of 20 mg of sileneoside E (IV) in 2 ml of water was treated with 2 ml of the complex enzyme of the snail *Helix plectotropis* and the mixture was left at 36°C for six days. Then another 10 ml of water was added to it and it was extracted with ethyl acetate. After evaporation of the solvent and recrystallization of the residue from aqueous methanol, 12 mg of 2-deoxy-α-ecdysone (I) was obtained with mp 235-236°C (from aqueous ethanol), $[\alpha]_D^{20}$ + 93.3 ± 3° (c 1.5; methanol), identical with an authentic sample with respect both to its R_f value in TLC (system 1) and its IR spectrum.

SUMMARY

A new ecdysteroid, sileneoside E, has been isolated from the epigeal part of *Silene brahuica* Boiss. and has been shown to be 2-deoxy-α-ecdysone 3-O-β-D-glucopyranoside.

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WITHASTEROIDS OF *PHYSALIS*

VI. ^{1}H AND ^{13}C NMR SPECTRA OF WITHASTEROIDS IXOCARPALACTONE A AND IXOCARPANOLIDE

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The ^{1}H and ^{13}C NMR spectra of two withasteroids isolated from *Physalis ixocarpa* Brot. have been studied in detail. Their spectral characteristics are discussed. A comparison of the results obtained has led to the identification of a withasteroid with the composition $C_{28}H_{40}O_8$, mp 291-292°C (from methanol) as ixocarpalactone A, in spite of some difference in its physical constants. The other compound, with mp 252-253°C (from methanol), had the composition $C_{28}H_{40}O_6$, $[\alpha]_D^{20}$ +27 ± 4°, is new and has been called ixocarpanolide. The structure of 5α,20R-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2-enolide has been proposed for it.

It is known that the qualitative and quantitative presence of withasteroids changes according to the chemotype of the plant. This has been shown, in particular, for the case of *Withania somnifera* [1]. *Physalis ixocarpa* Brot. (tomatillo ground-cherry) has been studied by two groups of workers. Physalin B was isolated from a *Physalis* growing in India [2], and ixocarpalactones A and B from a plant growing in Israel [3]. We were interested in what

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TABLE 1. Chemical Shifts (δ, ppm, 0 is TMS) and Spin-Spin Coupling Constants (J, Hz) in the PMR Spectra of Compounds (I) and (IV)

Proton	Compound, solvent		
	I, C_5D_5N	I [3], $CDCl_3$	IV, C_5D_5N
H-2	6,47 d, $^3J=9,9$	6,19 d	6,03 dd, $^3J=10,0$; $^4J=2,8$
H-3	7,26 dd, $^3J=9,9$ and 6,3	6.92 dd	6,57 dddd, $^3J=10,0$; 5,0 and 2,0
H-4	4,06 d, $^3J=6,3$	3,76 d	2,56 dd, $^2J=18,8$; $^3J=5,0$
H-4	—	—	2,69 dt, $^2J=18,8$; $^3J=2,0$; $^4J=2,8$
H-6	3,36 br.t, $^3J=2,4$	3,24	3,12 d, $^3J=3,8$
H-7	2,18 m		3,26 m, $^3J=3,8$ and 2,2
H-8			1,80 m
H-16	4,78 dt, $^3J=7,3$; $\Sigma ^3J=11,9$	4.50	
H-17	1,58 d, $^3J=7,3$		
H-22	4,56 br.s, $W_{1/2}=2,7$	3,96 s	4.39 dd, $^3J=11,5$ and 2,9
H-23	4,86 br.d, $^3J=8,4$ and 0,7	4,43 d.	1,50 m $^2J=14,0$; $^3J=3,8$ and 2,9
H-23	—	—	1,86 m, $^2J=14,0$; $^3J=11,5$ and 9,0
H-24	2,39 m, $^3J=12,0$; 8,4 and 6,8		1,57 m
H-25	3,03 m, $^3J=12,0$ and 7,1		2,18 m, $^3J=9,4$ and 6,7
CH_3-18	1,45 s	1,15 s	1,18 s
CH_3-19	1,73 s	1,43 s	1,21 s
CH_3-21	1,87 s	1.38 s	1,39 s
CH_3-27	1,21 d, $^3J=7,1$	1,23 d	1,22 d., $^3J=6,7$
CH_3-28	1.20 d, $^3J=6,8$	1,17 d	1,06 d, $^3J=6,6$

Note. s) singlet; d) doublet; dd) doublet of doublets; dt) doublet of triplets; t) triplet; m) multiplet; br.) broadened.

TABLE 2. Chemical Shifts of the Carbon Atoms in the ^{13}C NMR Spectra of Ixocarpalactone A (I), Ixocarpanolide (IV), and the Steroid Lactone (V) (δ, ppm, relative to TMS)

C Atom	Compound, solvent			
	I, C_5D_5N	I, $CDCl_3$ [3]	IV, C_5D_5N	V, $CDCl_3$ [4]
1	202.4	202,0	203,7	204,2
2	132,2	132,6	129,3	128,9
3	145,0	143,2	140,5	140,4
4	70,3	73,0	37,8	36,8
5	64,4	64,1	73,9	73,6
6	60,1	61,2	56,2	56,3
7	31,7	31,2	56,2	57,3
8	29.4	29,0	35,7	35,1*
9	44,7	44,2	36,0	35,5*
10	48,5	48,1	51,6	51,1
11	21,5	21,3	22,2	21,8
12	40,3	40,0	40,8	40,3
13	43,6	43,3	44,0	43,8
14	54,6	54,5	52,3	51,9
15	38,0	37,2	23,6	23,8
16	72.6	70,0	22,2	21,8
17	58,0	57,6	55,1	54,4
18	14,5	14,1	14,3	13,9
19	17,1	16,8	14,4	14,8
20	79,6	79,5	75,0	75,0
21	22,6	22,1	20,9	20,8
22	73,8	73,2	81,0	81,5
23	79,1	78,9	31,7	31,6
24	40,6	41,9	31,7	150,2
25	42,3	40,3	40,5	121,7
26	180,4	181,6	175,9	167,3
27	13,1	14,6	14,8	12,4
28	14,9	13,0	21,1	20,7

*Assignment ambiguous within a column.

changes could take place in the qualitative composition of the withasteroids of the same plant introduced into culture in the temperate zone of the territory of the Soviet Union. With this aim, we have investigated the epigeal part of *Physalis ixocarpa* (family Solanaceae growing in Khar'kov province.

It was possible to isolate two compounds in the individual form, and from UV, IR, and mass spectrometric measurements and the results of PMR spectroscopy these have been assigned to the class of withasteroids.

A comparison of the spectral characteristics and a detailed analysis of the ^1H and ^{13}C NMR spectra of withasteroid (I) and its acetyl derivatives (II) and (III) (Tables 1 and 2), in spite of some difference in chemical constants, led us to a structure agreeing completely with that of ixocarpalactone A [3]. Since we recorded the ^{13}C NMR spectrum of withasteroid (I) in C_5D_5N, we were interested in comparing the characteristics obtained with the chemical shifts of the signals and the results of their assignment in the spectrum taken in $CDCl_3$ [3]. As can be seen from Table 2, on passing from C_5D_5N to the less polar $CDCl_3$ the values of the chemical shifts of the carbon atoms did not change appreciably, with the exception of those of C-4, C-16, C-24, C-27, and C-28. It is known that in the case of withanolide D [4] and withaferin A [5], which have the same structure of the steroid moiety as ixocarpalactone A but do not contain a hydroxy group at C-16, the C-4 carbon atom is characterized by a signal at 69.8-69.9 ppm ($CDCl_3$). Consequently, of the three resonance lines in the spectrum of compound (I) in $CDCl_3$ at 70.0, 73.0, and 73.2 ppm corresponding to carbinol carbon atoms, the first must be assigned to C-4. According to the literature [5], the signals at 40.3 and 41.9 and at 13.4 and 14.6 ppm may also, apparently, be reassigned to C-24 and C-25 and to C-27 and C-28, respectively. These possible corrections were taken into account in the assignment of the signals in the spectrum of (I) taken in C_5D_5N (Table 2).

The withasteroid (IV), which we have called ixocarpanolide, is new. An absorption maximum at 226 nm (log ε 3.9957) in the UV spectrum and a band at 1690 cm^{-1} in the IR spectrum showed the presence of an α,β-unsaturated keto group. An absorption band at 1760 cm^{-1} and an ion with m/z 171 formed by the cleavage of the C-17—C-20 bond in the mass spectrum shows the presence of a six-membered lactone in the side chain. The compound did not undergo acetylation and its PMR spectrum showed no signals from protons geminal to hydroxy groups. Consequently, there are no primary and secondary hydroxy groups.

In the PMR spectrum of withasteroid (IV) signals appear at 6.03 and 6.57 ppm (Table 1) that are typical for H-2 and H-3 protons at a conjugated double bond. It follows from the nature of the splitting of the resonance lines of these protons that H-2 experiences a vicinal spin-spin interaction with H-3 (^3J = 10.0 Hz) and allyl interaction (^4J = 2.8 Hz) with the proton at C-4. The H-3 proton interacts vicinally with H-2 (^3J = 10.0 Hz) and with two hydrogen atoms at C-4 (^3J = 5.0 Hz and 2.0 Hz). It follows from this that there are no substituents at C-4 of the ixocarpanolide molecule. By using the method of selective suppression of spin-spin coupling (Fig. 1a, d, e) it was established that a doublet of doublets with ^2J = 18.8 Hz and ^3J = 5.0 Hz at 2.56 ppm and a doublet of triplets with ^2J = 18.8 Hz, ^3J = 2.0 Hz, and ^4J = 2.8 Hz at 2.69 ppm corresponds to the protons of a methylene group at C-4. They interact only with H-2 and H-3, there being no proton at C-5.

Two other one-protons signals are, from their chemical shifts, characteristic for H-6 and H-7 protons at an epoxide group. They resonate at 3.12 ppm in the form of a doublet with ^3J = 3.8 Hz and of a broadened multiplet at 3.26 ppm. Under double-resonance conditions with saturation of the transitions of the nuclei resonating at 3.12 ppm, the second multiplet at 3.26 ppm was converted into a doublet with ^3J = 2.2 Hz. Consequently, the proton at C-6 interacts vicinally only with H-7, with ^3J = 3.8 Hz, and the latter also with H-8 (with ^3J = 2.2 Hz). This means (taking into account the nature of the splitting of the signals of the protons at C-4) that one of the tertiary hydroxy groups is located at C-5 of the ixocarpanolide molecule.

The spectral characteristics of the H-4, H-6, and H-7 protons also permit us to come to the conclusion of the α-orientation of the epoxide group at C-6 and C-7.

The conclusions based on the PMR results were confirmed completely in a study of the ^{13}C NMR spectra of withasteroid (IV) recorded under the conditions of complete and partial suppression of spin-spin coupling with protons. By a comparative analysis of the values of the chemical shifts with the carbon atoms of ixocarpanolide (Table 2) and related compounds [4, 6, 7], it was found that the chemical shifts of the carbon atoms of the steroid moiety of compound (IV) were identical with those of a steroid lactone having the structure of 5α,20R-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2,24-dienolide (V) [4, 8].

The facts given in the present paper enable the structure of the steroid moiety of ixocarpanolide to be determined as 5α-hydroxy-1-oxo-6α,7α-epoxyandrost-2-ene.

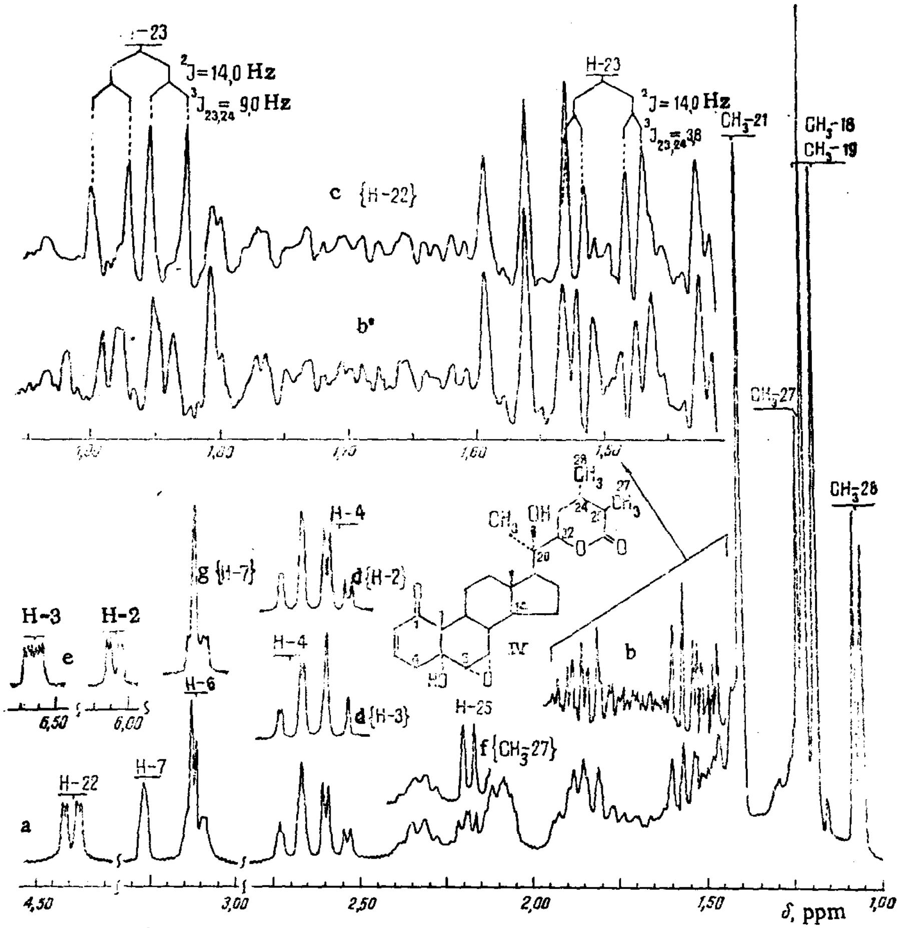
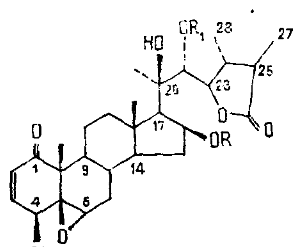


Fig. 1. ^1H NMR spectrum of a solution of ixocarpanolide (IV) in C_5D_5N: a, b) on the normal scale; b') on an expanded scale; c–g) sections of the double $^1H_{\{^1H\}}$ NMR spectrum. The protons irradiated are shown in parentheses.

The ^{13}C NMR spectrum of compound (IV) has a singlet at 75.0 ppm (C-20), while the signals of the C-17, C-21, and C-22 carbon atoms appear at 55.1, 20.9, and 81.0 ppm, respectively. Consequently, there is another tertiary hydroxy group at C-20 [4]. This conclusion was confirmed by the singlet nature of the signal from the C-21 methyl group in the PMR spectrum of ixocarpanolide.

An absorption band in the IR spectrum at 1760 cm^{-1} can be assigned to a five-membered lactone ring, as, for example, in perulactone [9]. However, the carbon atom of the lactone carbonyl group, C-26, resonated at 175.9 ppm. This value of the chemical shift indicates that the lactone ring in the side chain of withasteroid (IV) is five-membered [5].

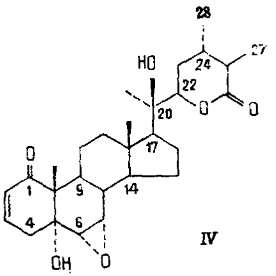
In order to obtain a more definite answer to this disputed question, we made a detailed analysis of the spin-spin interactions of the protons of the side chain using the method of multifrequency resonance with the simultaneous improvement of the resolution of the spectrum by contracting the lines of the signals. The necessity for combining these two methods was due to the closeness of the chemical shifts of the protons under discussion, which leads to mutually overlapping multiplets from them. When the spin-spin coupling constants of the multiply connected protons at C-24, C-25, and C-28 were determined (Fig. 1a, b, b', c, f) it was found that H-24 interacted vicinally not only with the protons at C-25 (^3J = 9.4 Hz) and C-28 (^3J = 6.7 Hz) but also with the two protons at C-23, with ^3J = 9 and 3.8 Hz. Consequently, the carbon atoms C-22 to C-26 participate in the formation of the lactone. In addition to this, the chemical shifts of the carbon atoms of the side chain of compound (IV) correlated well with those for 27-deoxy-2,3,24,25-tetrahydrowithaferin A [5] obtained in the course of a study of the NMR spectra of withaferin A and its derivatives. This confirmed the presence in ixocarpanolide of a saturated five-membered lactone in the side chain.



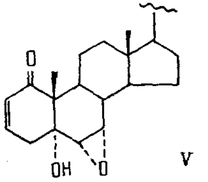
I. R = R₁ = H

II. R = Ac; R₁ = H

III. R = R₁ = Ac



IV



V

The CD spectrum of compound (IV) showed a positive Cotton effect at 242 nm (Δε +0.29), confirming the R configuration at C-22. A negative Cotton effect at 221 nm (Δε − 1.56) — the region of the n → π* transition of a saturated lactone — indicated the half-boat conformation [10]. In this case, the most energetically suitable is the half-boat conformation with the diequatorial arrangement of the methyl groups, which have the 24α and 24β orientations.

The facts given permit the structure of 5α,20R-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2-enolide to be proposed for ixocarpanolide (IV).

EXPERIMENTAL

General Remarks. For thin-layer chromatography (TLC) we used Silufol UV 254 plates and the solvent systems: 1) hexane—acetone (2:1), 2) hexane—acetone (1:1), and 3) hexane—acetone (1:2). The spots were revealed with a saturated chloroform solution of $SbCl_3$ with heating.

UV spectra were taken on a Hitachi EPS-3T spectrophotometer; IR spectra on a UR-20 instrument; mass spectra on a MKh-1303 mass spectrometer at ionizing voltages of 40 and 50 eV; and CD spectra on a Jasco J-20 spectropolarimeter.

^1H and ^{13}C NMR spectra were obtained in the pulsed regime with Fourier transformation on Varian SC-300 (300 MHz) and CFT-20 spectrometers fitted with 620/L computers, in deuterated pyridine with TMS as internal standard. The contraction of the lines in the ^1H NMR spectra was brought about by the use of a procedure introduced into the program of the mathematical equipment of the SC-300 spectrometer by specialists of the M. M. Shemyakin Institute of Bioorganic Chemistry of the USSR Academy of Sciences, A. Z. Gurevich and V. A. Afanas'ev.

The plant material was kindly provided by the Ukrainian Scientific Research Institute of Vegetable and Gourd Growing (its species affiliation was determined by A. Volodarskaya).

Isolation of the Withasteroids. The extraction of the air-dry epigeal part of *Physalis ixocarpa* (1.5 kg) was performed by the method given in [11]. The total extractive substances (11.9 g) were chromatographed on a column containing 500 g of type L 40/100 silica gel (Chemapol). Elution was performed successively with hexane and with solvent systems 2 and 3, with monitoring by TLC in system 2. Chromatography of the fractions containing compound (I) (2.11 g) in the chloroform—methanol (19:1) system gave 1.25 g of ixocarpalactone A, R_f in system 2 0.36; revealed by a red-brown color. Yield 0.08% (on the weight of the air-dry raw material).

Rechromatography of the fractions containing compound (IV) (1.19 g) with elution by chloroform and then by system 1 gave 140 mg of ixocarpanolide. R_f in system 1 0.70; revealed by a blue color. Yield 0.009%.

Ixocarpalactone A (I): $C_{28}H_{40}O_8$, mp 291-292°C (from methanol), $[\alpha]_D^{20}$ +68.9 ± 3° (c 1.93; CH_3CN); $\lambda_{max}^{C_2H_5OH}$: 216 nm (ε 10710); ν_{max}^{KBr}, cm^{-1}: 3450, 1770, 1670; mass spectrum, m/z (%): M^+ 504, 487 (2.8), 373 (55), 361 (100), 343 (80), 325 (72), 143 (8.5), 113 (4). Literature information [3].

Compound (I) (100 mg) was dissolved in 1.5 ml of pyridine, 2 ml of acetic anhydride was added, and the mixture was left at room temperature for 60 h. After the usual working up, the reaction product (90 mg) was chromatographed in system 1. This gave 30.0 mg of the diacetate (II) and 26.6 mg of the triacetate (III).

Ixocarpalactone A 4,16-Diacetate (II): $C_{32}H_{44}O_{10}$, mp 272-273°C (from aqueous acetone), $[\alpha]_D^{20}$ +148 ± 4° (c 0.1; chloroform—acetone (1:1)); ν_{max}^{KBr}, cm^{-1}: 3560, 1780, 1743, 1690, 1230; mass spectrum; m/z (%): M^+ 588, 570 (0.6), 528 (1.1), 510 (3), 468 (3.4), 403 (19), 385 (100).

Ixocarpalactone A 4,16,22-Triacetate (III): $C_{33}H_{46}O_{11}$, mp 244-245°C (aqueous acetone), ν_{max}^{KBr}, cm^{-1}: 3565, 1783, 1752, 1748, 1745, 1685, 1232, 1235, 1240; mass spectrum m/z (%): M^+ 630 (0.3), 570 (3.4), 510 (4.0), 492 (3.4); 445 (73.1), 385 (77), 126 (100). Literature information [3].

Ixocarpanolide (IV): $C_{28}H_{10}O_6$, mp 252-253°C (from methanol), α_D^{20} +27 ± 4° (c 0.83; chloroform), $\lambda_{max}^{C_2H_5OH}$: 226 nm (ε 9907), ν_{max}^{KBr}, cm^{-1}: 3590, 3440, 1760, 1690 CD (c 1.08; methanol): $\Delta\varepsilon_{221}$ − 1.56; $\Delta\varepsilon_{242}$ + 0.29; $\Delta\varepsilon_{339}$ − 2.39; mass spectrum m/z (%): M^+ 472 (0.7), 454 (0.7), 327 (27), 283 (17), 171 (100), 127 (0.5).

SUMMARY

Two withasteroids have been isolated from *Physalis ixocarpa* Brot. Their ^1H and ^{13}C NMR spectra have been studied. One of them has proved to be ixocarpalactone A. The other, which has been called ixocarpanolide, is new, and is 5α,20R-dihydroxy-1-oxo-6α,7α-epoxy-22R-witha-2-enolide.

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