

with 0.2 M sodium dihydrogen phosphate in the butanol-acetone-water (4:5:1) system and by GLC-mass spectrometry in the form of the corresponding aldonitrile peracetates. L-arabinose and 2,4-di-O-methyl-D-xylose were identified.

High-Temperature Hydrogenation of Culcitosides C₂ and C₃. Hydrogenation was performed with a mixture of 3 mg of a glycoside and 300 mg of catalyst [5% Pd/CaCO₃] as described in [8]. 24-Methylcholesterol was hydrogenated similarly. 24-Methylcholestane was identified in the reaction products by GLC and GLC-MS.

SUMMARY

Two new steroid glycosides have been isolated from the starfish Culcita novaeguineae and characterized: 24 ξ -methyl-5 α -cholestane-3 β ,4 β ,6 α ,8,15 β ,16 β , 28-heptaol 28-O-[O-(2,4-di-O-methyl- β -D-xylopyranosyl)-(1 \rightarrow 2)- α -L-arabinopyranoside] - culcitoside C₂ - and its 4-deoxy analogue - culcitoside C₃.

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WITHASTEROIDS OF Physalis.

VII. 14 α -HYDROXYIXOCARPANOLIDE AND 24,25-EPOXYWITHANOLIDE D

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Two new withasteroid have been isolated from Physalis angulata L. - 14 α -hydroxyixocarpanolide and 24,25-epoxywithanolide D. 14 α -hydroxyixocarpanolide - C₂₈H₄₀O₇, mp 245-250°C (from methanol), $[\alpha]_D^{20} +29 \pm 2^\circ$ (chloroform). 24,25-Epoxywithanolide D - C₂₈H₃₈O₇, mp 257-261°C (from methanol), $[\alpha]_D^{20} +23 \pm 2^\circ$ (chloroform). On the basis of its UV, IR, CD, mass, and ¹H and ¹³C NMR spectra the structure of 5 α ,14 α ,20R-trihydroxy-1-oxo-6 α ,7 α -epoxy-22R-witha-2-enolide is suggested for 14 α -hydroxyixocarpanolide. On the basis of spectral characteristics and the preparation of 4 β -acetoxy and 4-oxo derivatives of 24,25-epoxywithanolide D, the structure of 4 β ,20R-dihydroxy-1-oxo-5 β ,6 β ;24S,25S-diepoxy-22R-witha-2-enolide is proposed for it.

We have begun an investigation of the plant Physalis angulata L. (Solanaceae) growing in the territory of Tashkent province. As a result of the chromatographic separation of an extract we have obtained several compounds belonging, according to their spectral characteristics, to the withasteroids.

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TABLE 1. Chemical Shifts* and Spin-Spin Coupling Constants (δ , ppm; J, Hz; C₅D₅N; 0 - TMS) of the Protons of 14 α -Hydroxycarpanolide(I), 24,25-Epoxywithanolide D (II), and 24,25-Epoxywithanolide D Acetate (III).

Proton	Compound		
	I	II	III**
H-2	6.02 dd $^3J=10.1$; $^4J=2.7$	6.47 d $^3J=9.9$	6.50 d
H-3	6.57 ddd $^3J=10.1$; 5.1 & 2.1	7.25 dd $^3J=9.9$ & 6.4	7.2 dd
H-4	2.57 dd $^3J=18.8$; $^3J=5.1$	4.03 d J=6.4	5.05 d
H-4	2.71 dt $^3J=18.8$; $^3J=2.1$; $^4J=2.7$	—	—
H-6	3.09 d $^3J=4.0$	3.25 br.s	3.37 br.s
H-7	3.49 dd $^3J=4.0$ & 1.7	—	—
H-22	4.43 dd $^3J=11.7$ & 2.9	4.67 dd $^3J=11.8$ & 3.4	4.66 m
H-25	2.12 m $^3J=9.3$ & 6.6	—	—
CH ₃ -18	1.42 s	0.96 s	0.94 s
CH ₃ -19	1.27 s	1.84 s	1.70 s
CH ₃ -21	1.42 s	1.47 s	1.45 s
CH ₃ -27	1.17 d $^3J=6.6$	1.64 s	1.66 s
CH ₃ -28	0.88 d $^3J=6.6$	1.34 s	1.33 s
CH ₃ -COO	—	—	1.92 s

*d - doublet; dd - doublet of doublets; dt - doublet of triplets; ddd - doublet of quartets; m - multiplet; s - singlet; br.s - broadened singlet.

**Spectrum taken with the addition of trifluoroacetic acid.

The UV spectrum of withasteroid (I) has an absorption maximum at 225 nm (ϵ 10,370) and in the IR spectrum a band at 1683 cm⁻¹ showing the presence of an α,β -unsaturated carbonyl group. The PMR spectrum (Table 1) has a doublet at 6.02 ppm and a doublet of triplets at 6.57 ppm, which are characteristic for the H-2 and H-3 protons, respectively, at a conjugated double bond in ring A. An analysis of the multiplicities of the signals showed that the H-2 atom interacts vicinally with H-3 ($^3J = 10.0$ Hz) and allylically with one of the protons at C-4 ($^4J = 2.7$ Hz). The proton at C-3 experiences vicinal spin-spin coupling with H-2 ($^3J = 10.1$ Hz) and with the methylene protons at C-4 ($^3J = 5.1$ and 2.1 Hz). These characteristics of the PMR spectrum, and also the presence in the ¹³C NMR spectrum (Table 2) of signals at 203.5 ppm (C-1), 129.2 ppm (C-2), and 140.3 ppm (C-3) unambiguously show the presence of a 1-keto-2,3-ene grouping in ring A [1].

A doublet at 3.09 ppm with $^3J = 4.0$ Hz and a doublet of doublets at 3.49 ppm with $^3J = 4.0$ and 1.7 Hz in the PMR spectrum of withasteroid (I), which are assigned to H-6 and H-7, fall into the region characteristic for protons geminal to an epoxide group. The doublet nature of the signal at 3.09 ppm means that H-6 interacts only with one proton (H-7); there is no proton at C-5. The values of the spin-spin coupling constants, SSCCs, and of the chemical shifts of the H-6 and H-7 signals indicate the α orientation of the epoxy group [2]. The substituent at C-5 is also α -oriented, as is shown by the negative absorption band at 338 nm in the CD spectrum [3].

Although the IR spectrum of the withasteroid (I) has a band at 3575 cm⁻¹ corresponding to the vibrations of the OH groups, compound (I) cannot be acetylated, and in the PMR spectrum there are no signals of protons geminal to hydroxy groups. Consequently, all the hydroxy groups present are tertiary. One of the hydroxy groups is located at C-5, as is shown unambiguously by a singlet at 73.7 ppm in the ¹³C spectrum. This is confirmed by the chemical shifts of doublets at 55.7 and 54.5 ppm (Table 2), belonging to C-6 and C-7, respectively [4]. Thus, the substitution of rings A and B of the steroid moiety of compound (I) and that of isocarpanolide (V), isolated from *Physalis ixocarpa* Brot. [2], are identical.

An absorption band of withasteroid (I) in the IR spectrum at 1735 cm⁻¹, two doublet signals in the PMR spectrum (0.88 and 1.17 ppm), the position of the signals from C-22 and C-26 in the ¹³C NMR spectrum (Tables 1 and 2) [1, 2] and an ion with m/z 127 in the mass spectrum show that the lactone present in the side chain is six-membered and is saturated. The CD spectrum shows a negative Cotton effect at 218 nm ($\Delta\epsilon = -0.98$) due to the n- π^* transition of a saturated lactone [5]. A positive effect at 240 nm ($\Delta\epsilon = +0.27$) shows the R configuration at C-22. All this enabled us to come to the conclusion that the lactones of

TABLE 2. Chemical Shifts of the Carbon Atoms in the ^{13}C NMR Spectra of 14 α -Hydroxyixocarpanolide (I), Ixocarpanolide (V), 24,25-Epoxywithanolide D (II), and Withanolide D (VI) δ , ppm; $\text{C}_5\text{D}_5\text{N}$; 0 - TMS)

C atom	Compound			
	I	V [2]	II	VI [4]*
1	203,5	203,7	202,3	202,2
2	129,2	129,3	132,3	132,5
3	140,3	140,5	144,8**	142,2**
4	37,7	37,8	70,4	69,9
5	73,7	73,9	64,4	64,0
6	55,7	56,2	60,0**	62,1**
7	54,5	56,2	31,6	31,1
8	38,3	35,7	29,7	29,1
9	29,5	36,0	44,6	44,1
10	48,4	51,6	48,5	47,7
11	21,4	22,2	21,6	21,7
12	33,8	40,8	39,8	39,6
13	51,9	44,0	42,7	42,7
14	84,3	52,3	56,7	56,3
15	32,8	23,6	24,2	23,8
16	21,4	22,2	22,4	21,9
17	49,3	55,1	55,2	54,6
18	18,4	14,3	13,8	13,5
19	14,5	14,4	17,0	17,1
20	75,3	75,0	74,7	75,1
21	20,9	20,9	20,9	20,7
22	81,4	81,0	80,1	80,9
23	31,8	31,7	30,6	31,4
24	31,7	31,7	59,5	149,1
25	40,4	40,5	63,2	121,9
26	175,8	175,9	169,9	166,2
27	14,5	14,8	17,7	12,4
28	20,9	21,1	13,8	20,5

*Spectrum taken in CDCl_3 .

**Some of the difference in the chemical shifts in the C-3 and C-6 atoms observed on passing from (II) to (VI) [4] is due to the influence of the solvent.

the side chains of compound (I) and of ixocarpanolide (V) [2] have identical structures.

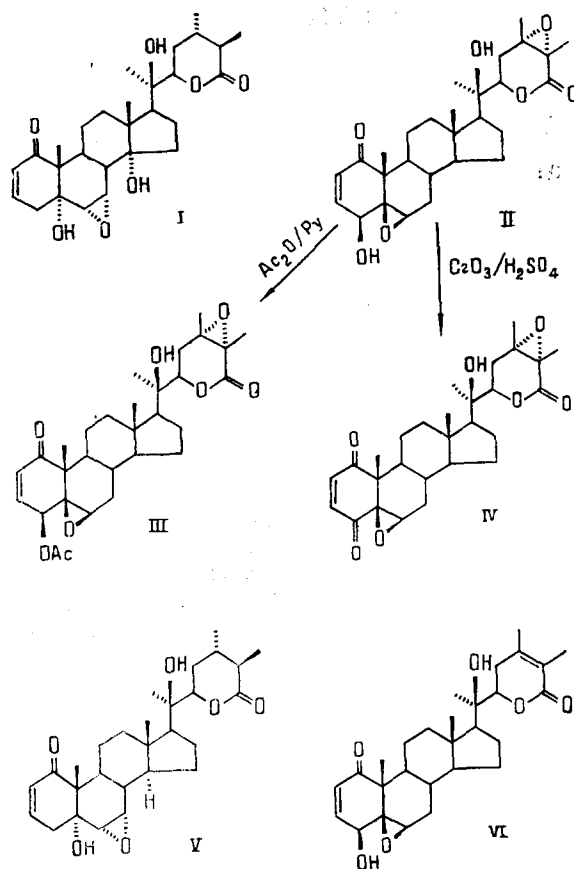
The elimination of the side chain or part of it is typical for the mass spectrometric fragmentation of the withasteroids. In the mass spectrum of withasteroid (I) characteristic ions with m/z 343 (100%) and 325 (85%) and m/z 299 (31.5%) and 282 (63%) are observed. The first pair corresponds to the loss of the lactone grouping and of one and two water molecules. The second corresponds to the loss of the whole side chain (cleavage of the C-17-C-20 bond) and of one and two water molecules, respectively. From this it may be assumed that the steroid part of the molecule contains two hydroxy groups, one of which is, as mentioned above, present at C-5.

The ease of cleavage of the C-20-C-22 bond and the singlet nature of the signal from the methyl group at 1.42 ppm and also the presence of a signal in the ^{13}C NMR spectrum of withasteroid (I) at 75.3 ppm are proofs of the fact that another hydroxy group is attached at C-20. Thus, the side chain of withasteroid (I) and the substitution of ring A and B are analogous to those for isocarpanolide [2].

The molecular ion of compound (I) is 16 units heavier than the molecular ion of ixocarpanolide (V). Consequently, the withasteroids being compared differ from one another by one hydroxy group located in ring C or ring D. The ^{13}C NMR spectra of withasteroids (I) and (V) (Table 2) differ by a considerable change in the chemical shift of the C-14 atom. Thus, in the spectrum of compound (I) this carbon atom is characterized by a singlet at 84.3 ppm while, in the case of compound (V) it appears in the form of a doublet at 52.3 ppm. There is no doubt that such a substantial paramagnetic shift is caused by the presence of a 14-OH group [4]. A diamagnetic shift of the C-7, C-9, and C-12 signals is observed simultaneously, which is completely explicable by the action of an axial hydroxy group.

On the basis of what has been said, withasteroid (I) has the structure of 5 α ,14 α ,20R-trihydroxy-1-oxo-6 α ,7 α -epoxy-22R-witha-2-enolide. It differs from ixocarpanolide by the

presence of an additional hydroxy group at C-14 and has therefore been called 14-hydroxy-ixocarpanolide. In plants of the genus *Physalis* compounds with α,β -unsaturated six-membered lactone rings are found most frequently [6], and there are only occasional withasteroids with saturated lactone rings [7, 8].



Withasteroid (II), $C_{28}H_{38}O_7$, which has been called 24,25-epoxywithanolide D, has in the UV spectrum a maximum at 203 nm ($\epsilon = 16,500$), in the IR spectrum an absorption band at 1660 cm^{-1} , and in the PMR spectrum the signals of vinyl protons at 6.47 and 7.25 ppm (Table 1). All these facts show the presence of an α,β -unsaturated carbonyl group in ring A. The nature of the splitting of the H-3 signal (doublet of doublets) and its SSCCs ($^3J = 9.9$ and 6.4 Hz) show the presence of a substituent at C-4. The PMR spectrum of compound (II) has a doublet at 4.03 ppm with $^3J = 6.4\text{ Hz}$. In the acetyl derivative (III), this doublet has shifted downfield by 1.02 ppm, while in the product of the Jones oxidation of withasteroid (II) (compound (IV)), it has disappeared. In the spectrum of substance (IV), as compared with the spectrum of withasteroid (II), the signals of the vinyl protons have changed and appear in the form of a two-proton singlet at 6.95 ppm. The signal of the proton geminal to the epoxy group has shifted to 3.60 ppm, and the signal for the 19- CH_3 group has undergone a considerable downfield shift (to 1.25 ppm). The changes in the PMR spectrum that have been mentioned unambiguously show the formation of a dihydroquinoid structure in ring A of product (IV). This is possible only if there is a C-4 hydroxy group in compound (II). According to its SSCC, it occupies the β position.

The position and splitting of the signal at 3.25 ppm in the spectrum of compound (II) is typical for protons at C-6 when a $5\beta,6\beta$ -epoxide grouping is present in the molecule [9]. Thus, rings A and B of compound (II) have the type of substitution that is most common in the withasteroids: 4β -hydroxy-1-oxo- $5\beta,6\beta$ -epoxy-2,3-ene.

The presence of a lactone moiety in withasteroid (II) was confirmed by an absorption band in the IR spectrum at 1720 cm^{-1} and by its characteristic breakdown in the mass spectrum: ions with m/z 185, 283, and 345 corresponding to the side chain, to the loss by the steroid nucleus of the side chain and a molecule of water, and to the loss of the lactone grouping. Thus, of the seven oxygen functions in withasteroid (II), three are present in rings A and B, and four in the side chain.

In the strong-field region of the PMR spectrum of compound (II) there are five singlet lines from the 18-, 19-, 21-, 27-, and 28-methyl groups; the tertiary nature of the last three is due to the presence of substituents at C-20-, C-24, and C-25. The magnitude of the chemical shift of the signal from the C-21-CH₃ group shows the presence of a hydroxy group at C-20. The singlets at 1.46 and 1.63 ppm, which have remained unassigned, are shifted upfield in comparison with the signals of methyl groups at a double bond [9]. It may be concluded that the lactone grouping in compound (II) has a saturated nature. The mass spectrum and the PMR spectrum suggest the presence of an epoxide group at C-24, C-25 in the side-chain lactone grouping of withasteroid (II) [4, 10].

An analysis of the ¹³C NMR spectrum of compound (II) confirmed the position of the substituents in rings A and B and of an oxirane ring in the lactone moiety [1, 4]. In these circumstances, we turned our attention to the fact that the spectral characteristics of the withasteroid that we are considering are close to the analogous characteristics of the steroid moiety of the known compound withanolide D (VI), isolated from Withania somnifera, chemotype II [4, 11].

The CD spectrum of compound (II) has a positive band at 211 nm ($\Delta\epsilon = +6.15$) and a negative band at 236 nm ($\Delta\epsilon = -1.06$), which, according to [12], means the 22R,24S,25S configuration of the chiral centers.

Thus, withasteroid (II) is 4 β ,20R-dihydroxy-1-oxo-5 β ,6 β ;24S,25S-diepoxy-22R-witha-2-enolide.

EXPERIMENTAL

General Remarks. For thin-layer chromatography (TLC) we used Silufol UV-254 plates, and for column chromatography and TLC the following solvent systems: 1) chloroform-acetone (5:1); 2) chloroform-methanol (20:1); 3) hexane-acetone (1:1); and 4) ethyl acetate-benzene (1:1). The spectral characteristics were obtained on the instruments described in [2].

The fact that the plant under investigation belonged to the species Physalis angulata L. was established by S. S. Kovalevskaya of the Institute of Botany of the Academy of Sciences of the Uzbek SSR.

Isolation of the Withasteroids. The extraction of the air-dry epigeal part of Physalis angulata L. (7 kg) was performed by the method described in [13]. The combined extractive substances were separated into two parts: chloroform-soluble (37 g) and chloroform-insoluble. The first part was transferred to a column containing 2.5 liters of KSK silica gel (<56 μ) and was eluted with system 1. Fractions 9-12 (3.1 g) contained 24,25-epoxy-withanolide D (II) and fractions 27-42 (6.31 g) 14 α -hydroxyxocarpanolide (I).

The chromatography in system 3 of the fractions containing compound (I) yielded 0.88 g of an individual product. In system 3 it appeared in the form of a crimson spot with R_f 0.60. Its yield was 0.0126% calculated on the weight of the air-dry raw material.

The rechromatography of fractions 9-12 in system 2 gave 1.81 g of 24,25-epoxywithanolide D (II). In system 2 it appeared in the form of a pink spot R_f 0.61. Its yield amounted to 0.026%. The results of an investigation of the intermediate and following fractions will be published separately.

14 α -Hydroxyxocarpanolide (I) [14]. C₂₈H₄₀O₇, mp 245-250°C from methanol, $[\alpha]_D^{20} + 29.1 \pm 2^\circ$ (c 1.18; chloroform), $\lambda_{\max}^{C_2H_5OH}$: 225 nm (ϵ 10370); ν_{\max}^{KBr} , cm⁻¹: 3575, 3480-3450, 2950, 1735, 1685; CD (c 1.20; methanol): $\Delta\epsilon = -1.37$ (338 nm); $\Delta\epsilon = +0.27$ (240 nm); $\Delta\epsilon = -0.98$ (218 nm). Mass spectrum (m/z, %): M⁺ 488 (\sim 1); 470 (2.5); 452 (10); 343 (100); 325 (85); 299 (31.5); 282 (63); 172 (8); 145 (58.5); 128 (41).

24,25-Epoxywithanolide D (II). C₂₈H₃₈O₇, mp 257-261°C (from methanol), $[\alpha]_D^{20} + 23.3 \pm 2^\circ$ (c 1.18; chloroform); $\lambda_{\max}^{C_2H_5OH}$: 203 nm (ϵ 16100) ν_{\max}^{KBr} (cm⁻¹): 3430, 2927, 2959, 1720, 1660; KD (c 0.95; methanol): $\Delta\epsilon = +$; +096 (343 nm), -0(290 nm), $\Delta\epsilon = -1.06$ (236 nm), $\Delta\epsilon = +6.51$ (211 nm). Mass spectrum (m/z, %): M⁺ 486 (3.8); 468 (5.4); 345 (88.5); 283 (31.8); 185 (46.2); 124 (100).

24,25-Epoxywithanolide D 4-Acetate (III) (from II). A solution of 200 mg of compound (II) in 2 ml of pyridine was treated with 1.5 ml of acetic anhydride, and the mixture was

left at room temperature for 24 h. After the usual working up procedure, product (III) (175 mg) was obtained, and it was recrystallized from acetone: $C_{30}H_{40}O_8$ mp 253-256°, ν_{\max}^{KBr} , cm^{-1} : 3555, 2930, 1745 (broadening), 1690, 1250. Mass-spectrum (m/z, %): M^+ 528, 486 (1.4); 468 (1.9); 387 (26.2); 327 (37.1), 309 (26.2); 283 (11.9); 185 (38.1); 141 (16.7); 124 (100).

4-Dehydro-24,25-epoxywithanolide D (IV) from (II). The Jones reagent was added dropwise in the cold over 1.5 h to a mixture of 150 mg of compound (II) and 10 ml of acetone. The process was monitored by TLC in systems 2 and 4. After the disappearance of the spot of the initial compound, the reaction was stopped by pouring the reaction mixture into water. The reaction product was extracted with chloroform (4 × 50 ml), and the solvent was evaporated off. The residue was recrystallized from acetone giving 116 mg of compound (IV): $C_{28}H_{36}O_7$, yellow crystals, mp 245-250°C with decomposition, $\lambda_{\max}^{C_2H_5OH}$: 225 nm (ϵ 8500); ν_{\max}^{KBr} , cm^{-1} : 3555, 2970-2930, 2875, 1735, 1690. Mass spectrum (m/z, %): M^+ 484 (2.5); 395 (100); 343 (40.5); 281 (55.5); 185 (66.6); 171 (44.4); 129 (72.2); 124 (36.1). PMR spectrum (100 MHz, δ , ppm, C_5D_5H : 0 - HMDS): 6.81 s (H-2 and H-3), 4.52 m (H-22), 3.46 br. s (H-6); 1.50 s and 1.35 s (27, 28- CH_3), 1.25 s (19- CH_3), 1.20 s (21- CH_3), 0.82 s (18- CH_3).

SUMMARY

Two new withasteroids have been isolated from *Physalis angulata* L. One of them - 14 α -hydroxyixocarpanolide - has the structure of 5 α ,14 α ,20R-trihydroxy-1-oxo-6 α ,7 α -epoxy-22R-witha-2-enolide, and the other - 24,25-epoxywithanolide D - that of 4 β ,20R-dihydroxy-1-oxo-5 β ,6 β ;24S,25S-diepoxy-22R-witha-2-enolide.

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