Physalis WITHANOLIDES II. WITHAPHYSANOLIDE

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A new withanolide with the composition $C_{28}H_{38}O_7$, which has been called withaphysanolide (I) has been obtained from a chloroform extract of an aqueous extract of the epigeal part of *Physalis viscosa* L. On the basis of a study of the UV, IR, PMR, and mass spectra of (I) and the products of the oxidation of its acetate (II) — a lactone $C_9H_{12}O_3$ (III) and 4β-acetoxy-5α-hydroxy-1,17-dioxoandrosta-2,8(14)-diene (IV) — the structure of 4β,5α,17β,20α-tetrahydroxy-1-oxo-22R-witha-2,8(14),24-trienolide is suggested for (I).

We have previously reported [1] on investigations of the withanolide composition of *Physalis alkekengi* L. that have been begun. It has recently become known [2] that the *Physalis* growing in the foothills of the Kopet-Dagh (Turkmen SSR, village of Kara-Kala) and serving as the object of our study, had been erroneously assigned to the species *Ph. alkekengi*. In actual fact, it is *Physalis viscosa* L. — a species that has apparently been introduced into the territory of Central Asia from outside.

From a chloroform extract of an aqueous extract of the epigeal part of *Ph. viscosa* we have isolated the substance with the composition $C_{28}H_{38}O_7$ which has been assigned to the withanolides on the basis of the UV, IR, PMR, and mass spectra. The new compound has been called withaphysanolide (I).

The presence of a strong maximum at 212 nm (log ε 4.08) in the UV spectrum of compound (I), of an ion with m/e 125 (100%) in the mass spectrum, and of three-proton singlets at δ 1.80 and 1.63 ppm in the PMR spectrum indicates that the side chain of the withanolide includes an unsaturated lactone ring. An intense fragment with m/e 169 arising as the result of the cleavage of the C-17—C-20 bond shows the presence of hydroxy groups at C-17 and C-20.

At three-proton singlet at δ 1.66 ppm confirms the presence of a hydroxy group at C-20. Oxidation of acetylwithaphysanolide (II) by the Jones reagent to the lactone (III) [3] and to an acetoxy-5α-hydroxy-1,17-dioxoandrosta-2,8(14)-diene are further proofs of the presence of a diol grouping at the position of cleavage (C-17 and C-20).

Resonance signals of a vinyl group in the weak field at δ 5.90 and 6.92 ppm correspond to protons at C-2 and C-3 of Δ^2-1-oxosteroids.

A one-proton doublet at δ 4.75 ppm for withaphysanolide (I) undergoes a downfield shift (5.65 ppm) in its acetate (II). This has made it possible to assume the presence of another readily acetylatable hydroxy group at C-4. To prove this, we investigated the PMR spectrum of the acetylandrostadiene (IV). The signal of the olefinic proton at C-3 of this compound (δ 6.54 ppm) has the form of a quartet, which indicates the presence of only two hydrogen atoms in its neighborhood. A study of the multifrequency resonance spectrum showed that with the simultaneous irradiation of the olefinic (at C-2) and gem-acetyl (C-4- protons the signal of the proton at C-3 was converted into a singlet.

Consequently, the quartet nature of the splitting of the signal of the C-3 proton is due to coupling with the hydrogen atom at C-2 (6.02 ppm, $J_{2,3}$ = 5.2 Hz) and with the proton geminal to the acetyl group at C-4 (5.79 ppm, $J_{3,4}$ = 9.2 Hz). According to the Garbisch curve, the value found for $J_{3,4}$ = 9.2 Hz indicates a value of the dihedral angle between the C-3 and C-4 protons of approximately 150° (30°). This corresponds to a quasi-equatorial orientation of the C-4 hydrogen and a quasi-axial orientation of the acetyl group.

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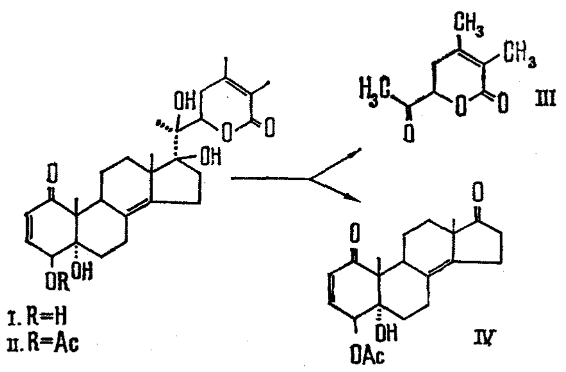
The doublet of the C-4 proton and the position of the signal of the 19-CH_3 hydrogen atoms in a comparatively weak field (δ 1.63 ppm) determine the position of the third hydroxy at C-5 [5].

On the basis of the coincidence of the changes in the chemical shift of the 19-CH_3 group with a change in the solvent ($\Delta_{CDCL_3-C_5D_5N}$ = −0.06 ppm), the α-orientation has been assigned to the 5-hydroxy group. This was confirmed by the well-defined negative Cotton effect found on measuring the circular dichroism ($\Delta\varepsilon_{340}$ = −1.88).



The β-orientation of the 17-OH group of withaphysanolide was established from a study of the PMR spectrum of 4-O-acetylwithaphysanolide (II). According to an x-ray structural analysis [4], the distance between 18-CH_3 and 17β-OH groups is considerably less than between 18-CH_3 and 17α-OH groups. Consequently, a 17β-OH group has a greater descreening influence and the signals of the protons of the 18-CH_3 group undergo a downfield shift. The changes in the shifts of the signals of the 18-CH_3 and 22-H protons due to the influence of the solvent in withaphysanolide acetate (II) ($\Delta_{CDCL_3-C_5D_5N}$ = −0.17 ppm for the 18-CH_3 protons and $\Delta_{CDCl_3-C_5D_5N}$ = −0.3 ppm for the 22-H proton) agrees well with the shifts of the same protons for 4β-hydroxywithanolide E (V) [6], which contains a 17β-OH (solvent $CDCl_3$, in square brackets, C_5D_5N):

Compound	2-H d	3-H q	4-H d	22-H m	18-CH_3 s	19-CH_3 s	21-CH_3 s	27 and 28-CH_3 s
I	[5.9]	[6.92]	[4.75]	[5.15]	[1.29]	[1.63]	[1.66]	[1.64; 1.80]
II	6.00	6.67	5.70	4.85	1.12	1.39	1.36	1.86; 1.92
	[5.98]	[6.70]	[5.65]	[5.15]	[1.29]	[1.45]	[1.65]	[1.67; 1.86]
IV	[6.02]	[6.54]	[5.79]		[0.91]	[1.41]		
V [6]	6.30	7.08	6.40	4.78	1.08	1.41	1.41	1.89; 1.95
	[6.48]	[7.18]	[5.00]	[5.15]	[1.32]	[1.72]	[1.73]	[1.82; 1.91]

According to the empirical formula, withaphysanolide contains, in addition to the Δ^2 another double bond in the steroid part of the molecule. The absence of other vinyl protons permitted us to put forward the hypothesis that it is tetrasubstituted and, by analogy with other withanolides [1, 5], is located between C-8 and C-14.

On the basis of the facts given, for withaphysanolide we suggest the structure of 4β,5α-17β,20R-tetrahydroxy-1-oxo-22R-witha-2,8(14),24-trienolide (I).

The R configuration at C-22 is given on the basis of the circular dichroism curve ($\Delta\varepsilon_{245}$ +5.85).

EXPERIMENTAL

For thin-layer chromatography (TLC) we used type KSK silica gel containing 5% of gypsum in the following systems: 1) chloroform–methanol (10:1); 2) hexane–acetone (1:1); and 3) chloroform–benzene–methanol (5:5:1). Revealing agent — a saturated chloroform solution of $SbCl_3$ (with heating). The UV spectra were taken on a Specord UV-VIS spectrophotometer, IR spectra on a UR-20 instrument, mass spectra on a MKh-1303 mass spectrometer at an ionizing voltage of 40 eV, and PMR spectra on a JNM-4H 100/100 MHz instrument, 0 — HMDS, δ-scale.

Isolation of Withaphysanolide (I). A dry chloroform extract was obtained as described previously [1].

A powder of the extract (10 g) was chromatographed on a column containing 500 g of silica gel. Elution was carried out with mixtures of chloroform and methanol containing gradually increasing concentrations of methanol from 1 to 5%; 300-ml fractions were collected. The process was monitored by TLC in systems 1 and 2. The fractions containing the withaphysanolide (chloroform—3% of methanol) were collected and were rechromatographed on 300 g of silica gel with elution by hexane—acetone (2:1). Fractions 40-55 were the main withaphysanolide-containing fractions, as was shown by their blue-green color. Yield 1.5 g (0.07% on the weight of the air-dry plant). The substance was recrystallized from methanol. Composition $C_{28}H_{38}O_7$, mp 215°C, $[\alpha]_D^{20}$ +95.0 ± 2° (c 2.63, dioxane, $\lambda_{max}^{C_2H_5OH}$ 212 nm (log ε 4.11); ν_{max}^{KBr}: 3400, 1695, 1675, 1600, 1140 cm^{-1}. Mass spectrum, m/e (%): 468 (8), M — H_2O; 450 (30), M — $2H_2O$; 343 (8) 468 — 125; 325 (16); 309 (15); 299 (21) 468 — 169; 238 (41), 225 (25); 169 (43), 151 (43), 125 (100). Ce (c 1.59; methanol): $\Delta\varepsilon_{215}$ +15.3, $\Delta\varepsilon_{245}$ +5.85, $\Delta\varepsilon_{340}$ −1.88.

4-O-Acetylwithaphysanolide (II). A solution of 300 mg of withaphysanolide (I) in a mixture of 5 ml of dry pyridine and 5 ml of acetic anhydride was left in a dark place for a day. Then the solvent was distilled off in vacuum, the residue was dissolved in 5 ml of methanol, and this solution was poured onto ice. The flocculent precipitate that deposited was filtered off and was recrystallized from aqueous methanol. Composition $C_{30}H_{40}O_8$, $[\alpha]_D^{22}$ +95.9 ± 2° (c 1.74; methanol); ν_{max}^{KBr}: 3450, 1735, 1710, 1695, 1230, 1140 cm^{-1}. Mass spectrum, m/e (%): 510 (3), M — H_2O, 468 (100) M — CH_3COOH, 450 (62), 343 (17), 325 (40) 309 (42), 299 (71), 281 (90), 238 (96), 169 (85), 125 (70).

Oxidation of 4-O-Acetylwithaphysanolide. A solution of 300 mg of the acetate (II) in 15 ml of acetone was placed in an ice bath, and the Jones reagent was added dropwise. The process was monitored continuously by TLC in system 3. After the disappearance of the initial compound on a chromatogram, the reaction mixture was diluted with 20 ml of water and extracted with chloroform. The chloroform extract was washed with water and dried over Na_2SO_4. Distillation of the solvent left 210 mg of residue in the form of a light yellow viscous oil. TLC in system 3 showed the presence of two compounds (chromogenic agent, iodine). The reaction products were subjected to preparative separation on plates with a thin layer of silica gel in the same system. Two substances were obtained: a light-yellow oil (III) with the composition $C_9H_{12}O_3$, $[\alpha]_D^{22}$ +151.0 ± 3° (c 1.30, chloroform), mass spectrum, m/e (%): 125 (100), 100 (38), 71 (23), 43 (38); and crystals (IV) with the composition $C_{21}H_{26}O_5$, mp 139°C, $[\alpha]_D^{22}$ +115.5 ± 5° (c 1.31, methanol), $\lambda_{max}^{C_2H_5OH}$ 210 nm (log ε 3.6), ν_{max}^{KBr}: 3450, 1725, 1680, 1670, 1245 cm^{-1}, mass spectrum, m/e (%): M^+ 358 (4), 340 (4), 298 (100) (M — CH_3COOH), 280 (92), 265 (25), 233 (48), 209 (22), 171 (90), CD (c 1.60, methanol), $\Delta\varepsilon_{218}$ = +39.2, $\Delta\varepsilon_{285}$ = +2.14, $\Delta\varepsilon_{343}$ = −2.96.

SUMMARY

A new withanolide, which has been called withanophysanolide, has been isolated from *Physalis viscosa* L. It has the structure of 4β,5α,17β,20R-tetrahydroxy-1-oxo-22R-witha-2,8(14),24-trienolide.

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