WITHASTEROIDS OF Physalis.

IX. PHYSANGULIDE – THE FIRST NATURAL 22S-WITHASTEROID

O. E. Vasina, N. D. Abdullaev,
and N. K. Abubakirov

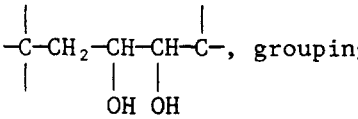
UDC 547.926

A new withasteroid – physangulide – has been isolated from the leaves of Physalis angulata L. It has been shown that physangulide is the first natural 22S-withanolide. Its structure has been determined as 3β,4β,20,24,25-pentahydroxy-1-oxo-5β,6β-epoxy-20R,22S,24S,25R-withanolide. Its ^1H and ^{13}C NMR spectra, confirming this interpretation, are given.

Withasteroids are characterized by very diverse configurations of their chiral centers. Series of 5α- and 5β-withanolides and 14α- and 14β-hydroxywithanolides [1] and compounds with 17α- and 17β-side chains [2] are known. In 1973, a publication [3] appeared on the isolation of a 22S-methanolide, but the report proved to be erroneous [4]. In the present paper we give a proof of the structure of physangulide (I), the first natural 22S-withasteroid [5], isolated from the leaves of Physalis angulata L. (family Solanaceae).

The IR, PMR (Table 1), and CD spectra of compound (I) revealed the absence of the α,β-unsaturated keto group in ring A that is characteristic for the majority of withasteroids. In the IR spectrum, an absorption band at 1710 cm^{-1} and a negative Cotton effect at 288 nm in the CD spectrum indicated the presence of a saturated keto group in the cis-linked rings A and B [6] provided that this keto group was located at C-1. In the ^{13}C NMR spectrum of the withasteroid (I) (the complete assignment of the signals of the carbon atoms is given in Table 2), a resonance signal at 210.3 ppm also confirmed the presence of a keto group at C-1.

In the PMR spectrum of physangulide there were two signals with coincident constants (SSCCs) at 3.20 and 3.35 ppm which can be assigned to a methylene interacting vicinally with only one proton. There were also signals at 3.98 and 4.72 ppm lying in the region of the resonance lines of protons geminal to hydroxy groups. Analysis of the PMR spectrum of the new withasteroid showed that the groups of signals mentioned above belonged to



grouping, which in the steroid series can be located only in ring A. The β-orientation of the hydroxy groups at C3-C4 was determined on the basis of SSCC values. Attempts to obtain a 3,4-diacetyl derivative of physangulide were unsuccessful, which was apparently due to the β,β-orientation of this diol group [7]. A signal at 3.65 ppm in the PMR spectrum of compound (I) must be assigned to a proton at C-6 germinal to an epoxy group [8].

In the PMR spectrum of the product of the Jones oxidation of physangulide (compound (II)) a shift of the H-4 signal to 4.62 ppm (br.s.) and the absence of a signal at 4.72 ppm were due to the appearance of a keto group at C-3.

When the ^{13}C spectra of physangulide (I) and the known compound viscosalactone B (III) [7] (Table 2) were compared, the values of the chemical shifts of the steroid moieties of both withasteroids were found to coincide, on the whole. The slight differences observed for C-2 and C-6 were obviously due to the nature of the solvents used (the spectrum of compound (III) was taken in $CDCl_3$).

Institute of the Chemistry of Plant Substances, Uzbek SSR Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 366-371, May-June, 1990. Original article submitted July 4, 1989.

 0009-3130/90/2603-0304$12.50

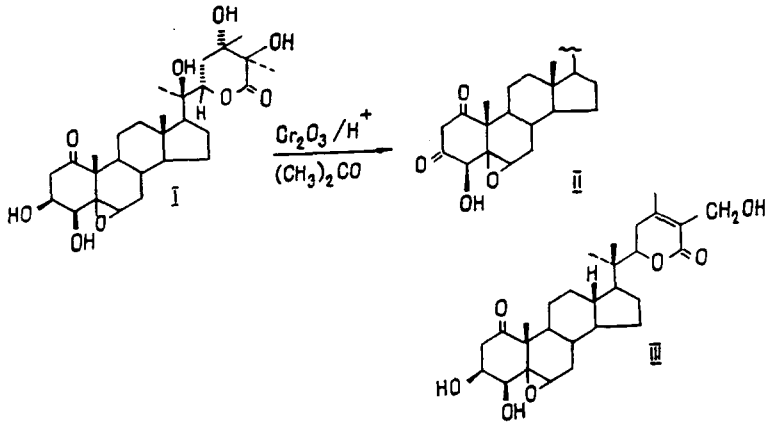
TABLE 1. Characteristics of the PMR Spectrum (C_5D_5N, 0 – TMS) of Physangulide (I)

Protons	σ, ppm; J, Hz
H-2	3.20; dd ^2J=16.0; ^3J=3.7
H-2	3.35 dd ^2J=16.0; ^3J=8.0
H-3	4.72 m
H-4	3.98 br.s
H-6	3.65 br.s
H-22	4.62 br.s
H-23	2.26 dd ^2J=13.0; ^3J=2
H-23	2.87 br.d ^2J=13.0; $^3J\leqslant 1$
CH_3-18	0.68 s
CH_3-21	1.24 s
CH_3-19	1.75 s
CH_3-27	1.87 s
CH_3-28	1.55 s

TABLE 2. Chemical Shifts of the Carbon Atoms in the ^{13}C NMR Spectra of Physangulide (I), Viscosalactone B [7] ($CDCl_3$), and 14α-Hydroxyisocarpanolide [9](III and IV, respectively)

C atom	I	III	IV	C atom	I	III	IV
1	210.3	210.5	203.5	15	24.2	24.4	32.8
2	44.1	42.6	129.2	16	23.8	39.2*	21.4
3	69.2	68.5	140.3	17	54.9	52.0	49.3
4	78.8	77.1	37.7	18	14.6	11.6	18.4
5	65.2	64.6	73.7	19	15.4	15.8	14.5
6	58.7	60.0	55.7	20	78.0	38.9	75.3
7	31.6	29.8	54.5	21	20.1	13.4	20.9
8	29.7	31.1	38.3	22	83.9	78.9	81.4
9	43.2	42.9	29.5	23	37.5	29.3	31.8
10	51.0	50.5	48.4	24	81.9	153.4	31.7
11	21.3	21.7	21.4	25	88.5	125.7	40.4
12	38.7	27.4*	33.8	26	175.6	167.2	175.8
13	41.9	42.8	51.9	27	19.6	57.4	14.5
14	56.2	56.1	84.3	28	23.3	20.1	20.9

*Signals marked with asterisks can in our opinion change places.



Scheme 1

The facts given above unambiguously showed that the structure of the steroid moiety of the physangulide molecule is characterized by the presence of 3β,4β-diol and 5β,5β-epoxy groups and a keto group at C-1; there are no substituents in rings C and D.

The existence of a saturated lactone ring in the side chain of withanolide (I) followed from the general pattern of mass-spectrometric fragmentation. The peaks of ions with m/z 185, 159, 143, and 124 corresponded to the side-chain fragment without a molecule of water (C-17–C 20 cleavage), to the lactone of the side chain (C-20–C-22 cleavage), to the lactone fragment without one molecule of water, and to the lactone fragment without two molecules of water. The singlet nature of the signals of the methyl groups indicated the presence of substituents at C-20, C-24, and C-25.

A hydroxy group is located at C-20. This was shown by a signal at 1.24 ppm in the PMR spectrum and by a characteristic resonance signal at 78.0 ppm in the ^{13}C NMR spectrum.

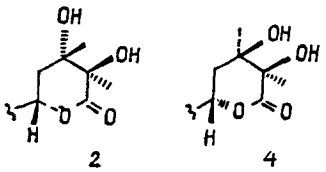
The establishment of the structure of the steroid moiety of compound (I) permitted the isolation in its ^{13}C NMR spectrum of resonance lines relating to a side chain of carbon atoms: 19.6, 20.1, and 23.3 ppm ($-CH_3$); 37.5 ppm ($-CH_2-$); 83.9 ppm (–CH<); 78.0; 81.9; and 88.5 ppm (>C<), and 175.6 ppm (–O–C=O). Consequently, the lactone residue consists of a saturated six-membered ring (chemical shift of 175.6 ppm for C-26 [9]) with heteroatoms at C-24 and C-25 (81.9 and 88.5 ppm, respectively). In contrast to 24,25-epoxywithanolide D [9], in which the same carbon atoms are characterized by chemical shifts of 59.5 and 63.2 ppm, two hydroxy groups are located in the lactone ring of withasteroid (I); at C-24 and C-25.

To determine the stereochemistry of the C-22 chiral center, let us dwell in more detail on the assignment of the signals at 4.62, 2.87, and 2.26 ppm in the PMR spectrum of physangulide.

The H-22 proton resonated at 4.62 ppm and appeared in the form of a broadened singlet with $W_{1/2}$ = 5 Hz. The positions and multiplicities of the two remaining signals corresponded to the resonance of methylene protons at C-23 [10]. The SSCC values (Table 1) showed that, in contrast to all known withasteroid derivatives, in which the proton at C-22 has the α-orientation (22R), in physangulide it is β-oriented. This determined the configuration of the C-22 chiral center as 22S. In those cases where the C-22 chiral center has the R-configuration, H-22 is represented in the PMR spectrum in the form of a double doublet with two SSCCs characteristic for axial-axial and axial-equatorial interaction with H-23 [10].

Thus, physangulide – the first natural withasteroid having the 22S-configuration – contains in the side chain a saturated lactone group hydroxylated at C-24 and C-25.

Theoretically, a saturated six-membered ring can adopt six most stable conformations [11]: two distorted boat conformationations and four distorted chain conformations. In view of the positive sign of the Cotton effect at 228 nm, it may be assumed that, in contrast to ixocarpanolide [10], having a distorted boat conformation with the diequatorial arrangement of the methyl groups ($\Delta\varepsilon_{221} = -1.56$), the lactone group of physangulide adopts a distorted chain confirmation. According to the S-configuration of the chiral center at C-22, the steroid moiety of the molecule is oriented through the C-20–C-22 bond axially, and the proton at C-22 equatorially. The four possible half-chair conformations have the following orientations of the methyl groups: 1 – 24ax,25ax; 2 – 24eq,25eq; 3 – 24eq,25ax, and 4 – 24ax,25eq. Knowing that the equatorial orientation of the methyl group at C-25 is more stable [11], we retain for physangulide the two most probable conformations 2 and 4.



2 4

Scheme 2

It is known that with a change in the pH of the medium [12] the CD spectrum of 14α-hydroxyixocarpanolide [9], like that of other saturated lactones and epoxylactones, shows a positive Cotton effect at 227 nm. This confirms the stability of the saturated six-membered lactone in a conformation similar to that of physangulide. In view of the facts given in [11] according to which conformation 2 is more stable, the lactone moiety of physangulide must be assigned the distorted chain conformation with the equatorial location of the methyl groups at C-24 and C-25 (the 24S,25R-configuration of the chiral centers).

The structure of physangulide – the first natural 22S-withanolide – has been determined as 3β,4β,20,24S,25R-pentahydroxy-1-oxo-5β,6β-epoxy-20R-withanolide (I), which refines the structure of the 22S-methanolide given in [5].

EXPERIMENTAL

General Observations. For thin-layer chromatography (TLC) we used Silufol UV-254 plates (Czechoslovakia), and for column chromatography and TLC the systems: 1) chloroform–acetone (5:1) and 2) chloroform–methanol (10:1).

UV spectra were taken on a Specord UV-VIS-75 spectrometer, IR spectra on a UR-20 instrument, mass spectra on a MKh-1303 mass spectrometer at an ionizing energy of 50 eV, and CD spectra on a JASCO J-20 spectropolarimeter. ^1H and ^{13}C NMR spectra were obtained on WM-500 (Bruker) and CFT-20 (Varian) instruments in Py-d_5, 0 – TMS.

Isolation of Physangulide. As shown previously [9], the chromatography of an extract of Physalis angulata L. on a column of KSK silica gel in system 1 gave several fractions having uniform compositions. The subsequent rechromatography of the combined fractions 18-28 (850 mg) in system 2 led to the isolation of physangulide (580 mg). The yield amounted to 0.0083% calculated on the weight of the air-dry raw material.

Physangulide (I). $C_{28}H_{42}O_9$, mp 265-270°C (from methanol). UV spectrum, $\lambda_{max}^{C_2H_5OH}$, nm: 206 (ε = 5600). IR spectrum. ν_{max}^{KBr} (cm^{-1}): 3520–3400, 2980–2855, 1735, 1710, 1105, 1040; CD spectrum (c 1 mg/ml; methanol), nm: $\Delta\varepsilon$ –4.94 (288); $\Delta\varepsilon$ +3.64 (228). Mass spectrum, m/z (%): 504 ($M^+ - H_2O$) (4.8); 486(2.4); 476(6.4); 468(4.8); 457(14.3); 415(7.0); 397(100); 379(30.0); 371(25.7); 317(85.7); 299(32.9); 273(25.7); 213(21.4); 185(54.3); 143(28.6); 142(28.6); 125(32.9); 124(35.7) 114(100); 85(42.9).

Jones Oxidation of Physangulide. A solution of 150 mg of compound (I) in 30 ml of acetone was treated with the Jones reagent until the appearance of a permanent orange coloration (0.2 ml). The reaction mixture was worked up in the usual way. The yield of crude product (II) was 130 mg. When it was chromatographed on a column of silica gel, part of the reaction product decomposed. The unchanged residue was recrystallized from chloroform–methanol, giving 50 mg of compound (II).

4,20,24,25-Tetrahydroxy-1,3-dioxo-5β,6β-epoxy withanolide (II). $C_{28}H_{40}O_9$, amorphous yellow powder. IR spectrum ν_{max}^{KBr} (cm^{-1}): 3560–3400, 2990–2810, 1725, 1680–1665, 1630.

The ^1H NMR spectrum was taken on a Tesla PS-567A/100 MHz instrument in C_5D_5N solution, 0 – HMDS (δ scale, ppm): H-2 and H'-2–2.74 d, H-4–4.47 s, H-6–3.02 br. s; H-22–4.47 s, H-23 and H-23'–2.0 m and 2.60 m; $CH_3$18–0.61 s; CH_3-27 and CH_3-28–1.72 s and 1.43 s.

Mass spectrum, m/z (δ): 501(0.5), 484(28.4), 395(100), 377(9.0), 317(0.61), 299(4.5), 297(22.4), 185(5.7), 159(4.5), 133(6.0), 125(3.0), 114(15.0).

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