VISCOSALACTONE A AND VISCOSALACTONE B, TWO NEW STEROIDAL LACTONES FROM *PHYSALIS VISCOSA*

S. William Pelletier, Gulilat Gebeyehu, Jacek Nowacki, and Naresh V. Mody.

Institute for Natural Products Research and the Department of Chemistry, The University of Georgia, Athens, Georgia 30602, U.S.A.

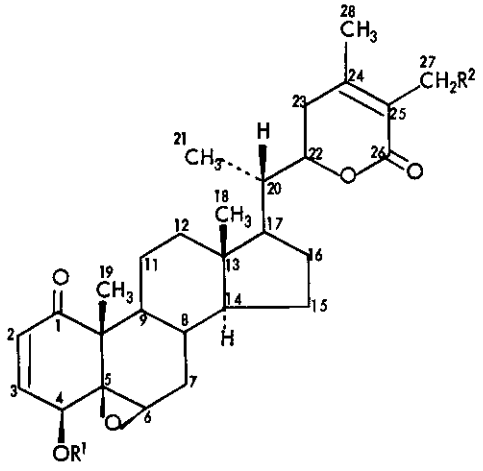
Abstract: Investigation of the extracts of *Physalis viscosa* has led to the isolation and structure elucidation of two new steroidal lactones, viscosalactone A (2) and viscosalactone B (3). The structures of these withanolides were assigned on the basis of chemical correlation with withaferin A and their ^{13}C NMR spectral data.

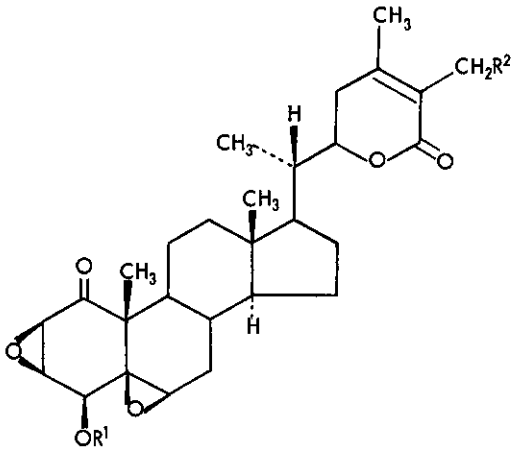
Our search for tumor-inhibitory natural products from plants led us to investigate the chemical constituents of the stems and leaves of *Physalis viscosa*,[1] a plant for which, to our knowledge, no chemical investigation has been reported. A 95% ethanol extract of *P. viscosa* showed confirmed activity against the National Cancer Institute 90 KB (human epidermoid carcinoma of the nasopharynx) test system. The ethanolic extract was partitioned according to the NCI scheme and fractionation was guided by the 90 KB bioassay. Recently, we reported[2] the isolation of the known antineoplastic agent, withaferin A (1)[3], as a major constituent of the most active fraction of this plant. After removal of withaferin A from this fraction, the remaining mother liquor was examined for other steroidal lactones. We wish to report here the isolation and structure determination of two new steroidal lactones, viscosalactone A (2) and viscosalactone B (3).

Viscosalactone A, $C_{28}H_{38}O_7$, crystallized from acetone-hexane, mp. 184-186°C (corrected), $[\alpha]_D$-27.4° (c 0.46 MeOH), and showed infrared absorption at 3615 (hydroxyl), 1715 (α,β-unsaturated δ-lactone) and 1700 (cyclohexanone) cm^{-1} in methylene chloride solution. The 90 MHz ^1H NMR spectrum of viscosalactone A in $CDCl_3$ revealed the presence of three singlets (3H each) for three tertiary methyl groups at δ 0.66, 1.24 and 2.03, a secondary methyl group (3H, doublet) centered at δ 0.97, a broad two-proton singlet at δ 4.33 for the C(25)—CH_2OH group, and various one-proton signals centered at δ 3.06, δ 3.5 (doublet), δ 3.68 (triplet) and δ 3.86 (doublet) ppm.

Viscosalactone B, $C_{28}H_{40}O_7$, $[\alpha]_D$-19.4° (c 0.57 MeOH), mp. 184-186°C (corrected), crystallized from acetone-hexane, and showed infrared absorption at 3690 (intramolecularly bonded hydroxyl), 3610 and 3470 (hydroxyl), 1715 (α,β-unsaturated δ-lactone), and 1695 (cyclohexanone) cm^{-1}. The 90 MHz ^1H NMR spectrum in deuteriochloroform showed three singlets (3H each) at δ 0.66, 1.13 and 2.03 for three tertiary methyl groups, a three-proton doublet centered at δ 0.96 for a secondary methyl group, a one-proton doublet at δ 3.03, a broad undefined signal for one proton at δ 3.25, a doublet of one proton at δ 3.38 and a broad two-proton singlet at δ 4.33 for the C(25)—CH_2OH group.

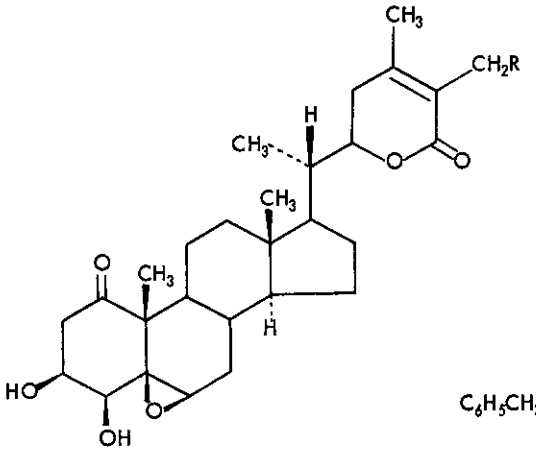
The spectral data of lactones 2 and 3 are similar to those of withaferin A[3] except for the absence of the signals associated with the vinylic protons of ring A. Comparison of the ^{13}C NMR spectra of viscosalactone A and

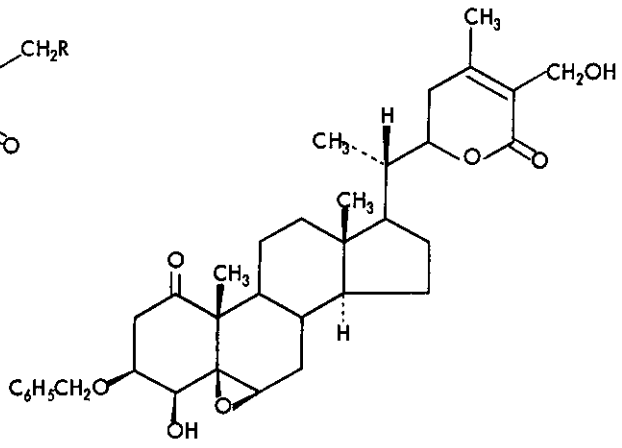




1 R^1 = H; R^2 = OH Withaferin A
8 R^1 = Ac; R^2 = OAc

2 R^1 = H; R^2 = OH Viscosalactone A
4 R^1 = Ac; R^2 = OAc
5 R^1 = Ac; R^2 = H





3 R = OH Viscosalactone B
6 R = H

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Table 1. Chemical Shifts and Assignments of Viscosalactone-A, Viscosalactone-B and their Derivatives.[a,b]

	1	2	4[c]	5[d]	3	6
C(1)	202.3	206.9	206.2	205.8	210.5	210.8
C(2)	132.3	54.9	54.9	54.9	42.6	42.6
C(3)	142.5	55.8	53.7	53.6	68.5	68.5
C(4)	69.8	74.6	76.8	76.7	77.1	76.9
C(5)	63.9	64.0	60.7	60.6	64.6	64.6
C(6)	61.7	59.6	59.2	59.2	60.0	60.1
C(7)	29.8	29.6	30.1	29.6	29.8	29.7
C(8)	31.1	31.0	30.9	30.9	31.1	31.0
C(9)	44.0	42.7	42.7	38.9	42.9	42.9
C(10)	47.8	48.6	49.2	49.1	50.5	50.4
C(11)	21.8	20.0	20.2	20.2	21.7	21.4
C(12)	27.2	27.3	27.3	27.3	27.4	27.3
C(13)	42.5	42.7	42.7	42.6	42.8	42.7
C(14)	56.0	56.1	56.1	56.0	56.1	56.2
C(15)	24.2	24.3	24.3	24.2	24.4	24.3
C(16)	39.2	40.7	40.8	40.8	39.2	39.2
C(17)	51.8	51.9	51.9	51.9	52.0	52.1
C(18)	11.6	11.5	11.5	11.5	11.6	11.6
C(19)	17.0	14.5	13.9	13.9	15.8	15.7
C(20)	38.7	38.9	38.2	38.9	38.9	38.9
C(21)	13.3	13.3	13.3	13.3	13.4	13.4
C(22)	78.7	78.7	78.3	78.2	78.9	78.5
C(23)	29.8	29.8	29.4	29.4	29.3	29.2
C(24)	153.5	153.8	157.3	149.0	153.4	149.2
C(25)	125.6	125.8	122.1	122.0	125.7	122.2
C(26)	167.0	167.4	165.6	167.0	167.2	167.6
C(27)	57.0	57.2	58.1	12.5	57.4	12.5
C(28)	20.0	20.1	20.6	20.5	20.1	20.6

[a] Chemical shifts are recorded as ppm downfield from TMS. The spectra were taken in $CDCl_3$.

[b] Carbon-13 NMR spectra were taken at 15.03 MHz in the Fourier mode using a JEOL FX-60 spectrometer in conjunction with a JEC-980 computer.

[c] Chemical shifts of the C(4) acetyl group occur at 20.9 and 170.3 ppm and signals at 21.1 and 171.1 ppm are assigned to the C(27) acetyl group.

[d] Chemical shifts of the C(4) acetyl group appear at 20.7 and 170.1 ppm.

viscosalactone B was made with the published ^{13}C NMR spectra of withaferin A and its derivatives[2] (Table 1). The chemical shifts pattern of compounds 2 and 3 is similar to that of withaferin A, 2,3-dihydrowithaferin A and compound 6 except for a few changes in the chemical shifts of the ring A carbons. The appearance of two signals at 54.9 and 55.8 ppm, the downfield shifts of the C(1) and C(4) signals, and the disappearance of the two doublets of the double bond in the ^{13}C NMR spectrum of viscosalactone A in comparison with that of withaferin A, revealed the presence of an epoxy function between C(2) and C(3) in viscosalactone A (2). The presence of a C(2)-C(3) epoxy group was confirmed by preparing viscosalactone A from withaferin A. The latter was treated with 30% hydrogen peroxide and anhydrous sodium carbonate in methanol at 5°C for 2 hours to afford a product which was identical (mp, ^1H and ^{13}C NMR) with viscosalactone A. On the basis of the known mechanism[4] for epoxidation of conformationally fixed allylic alcohols, a β configuration was assigned to the C(2)—C(3) epoxy group in viscosalactone A (Structure 2). Acetylation of 2 with acetic anhydride in pyridine yielded a crystalline diacetate 4, mp. 142-144°C. Upon hydrogenation over Pd/C in methanol, the diacetate 4 afforded 27-deoxyviscosalactone A acetate (5). The ^{13}C NMR data of 4 and 5 are in agreement with their respective structures (Table 1).

The ^{13}C NMR spectral analysis of viscosalactone B (3) indicated the presence of a hydroxyl group at the C(3) position. This conclusion was confirmed by comparing the ^{13}C NMR spectrum of viscosalactone B with that of 6 (Table 1). Hydrogenation of 3 over Pd/C in methanol for 2 hours yielded a compound which was identical (^1H and ^{13}C NMR spectra, same retention time on HPLC using reverse phase C_{18}-column) with 27-deoxy-3-hydroxy-2, 3 dihydrowithaferin A (6). The latter was prepared from withaferin A. Michael addition of benzyl alcohol to withaferin A in the presence of catalytic amounts of DBU gave 7, which was hydrogenated over Pd/C in methanol to afford compound 6. Treatment of 3 with acetic anhydride and pyridine at room temperature yielded an unexpected product, withaferin A diacetate (8). Facile elimination of the C(3) hydroxyl group during acetylation of viscosalactone B suggests that the C(3) hydroxyl group is present in a β-axial configuration. Attempts to prepare viscosalactone B directly from withaferin A met with failure.

It is of interest that the presence of the hydroxyl and epoxy groups at C(2) and C(3) is encountered for the first time in this class of natural products. Most of the seventy-five known withanolides[5,6] possess an α,β-unsaturated ketone group in ring A as is present in withaferin A. Viscosalactone A has shown cytotoxicity at the 5.3 μg/ml level against the NCI 90 KB cell line.

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1. This plant was collected on Jekyll Island, Georgia during the summers of 1977, 1978 and 1979. A voucher specimen of each collection has been deposited in University of Georgia Herbarium.
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