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WITHAMINIMIN, A WITHANOLIDE FROM *PHYSALIS MINIMA*

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Key Word Index—*Physalis minima*; Solanaceae; withanolides; withaminimin; steroidal lactones.

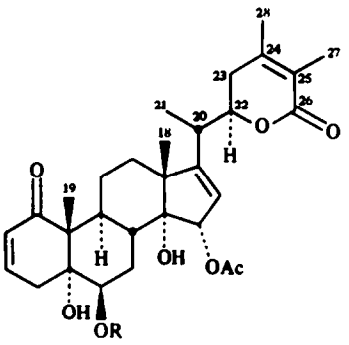
Abstract—The structure of withaminimin, a new ergostane-type steroid from *Physalis minima*, was established by spectral analysis (^{1}H and ^{13}C NMR, MS) and chemical transformations, as (20*S*, 22*R*)-15α-acetoxy-5α,6β,14α-trihydroxy-1-oxowitha-2,16,24-trienolide. An unusual MH_2O^+ quasi-molecular ion was observed in the chemical ionization mass spectrum of the natural product.

INTRODUCTION

C_{28}-Steroidal lactones based on the ergostane framework are exclusive to the Solanaceae. Of the different genera of this family that are known to yield such compounds, *Physalis* is special for elaborating complex structural variants of simpler withanolides [1, 2]. *Physalis*-derived C_{28}-steroidal lactones include withaphysalins [3], physalins [4], ixocarpalactones [5] and perulactones [6, 7], in addition to well known withanolides. While withanolides are the major constituents of *Physalis peruviana* [8, 9], no withanolide and only withaphysalins and physalins have so far been isolated from *Physalis minima* [3, 10], an annual herb growing wild in India, Sri Lanka and tropical regions of Africa. Systematic fractionation of the alcoholic extract of the plant material yielded in our hands a new withanolide for which the name withaminimin has been given. The functionalities present in the D ring of withaminimin make it a likely precursor for 13,14-secosteroids native in this plant [3, 10]. Withaminimin has also been found to be present in a poorer yield in *Physalis minima* Linn. var. *indica* C. B. Clarke [11].

RESULTS AND DISCUSSION

The ^{1}H NMR and ^{13}C NMR spectra of withaminimin (**1a**) (see Tables 1 and 2) were consistent with a withanolide structure, in which 1-keto-Δ^2 and 5α,6β-dihydroxy functionalities were clearly present, as well as a typical α,β-unsaturated δ-lactone in the side-chain [1, 2, 12]. A negative Cotton effect at 332 nm and a positive Cotton effect at 252 nm confirmed A/B *trans*-fused rings and a 22*R*-configuration. The problem remained of locating a trisubstituted double bond, an oxymethine and an oxygenated non-protonated carbon; connected to one of these oxygens was an acetate group (carbons at δ170.63 and 21.36, protons at 2.01). This was accomplished by selective decoupling experiments in the ^{1}H NMR spectrum. In the most revealing of these, irradiation at the frequency of the H-20 multiplet at δ2.53 not only collapsed the 21-methyl signal into a singlet and the H-22 (*ddd*) into a *dd* (12 and 4 Hz), but also sharpened the doublet at δ5.60. This suggested an allylic H-20 and therefore a 16,17 double bond. The olefinic H-16 was in turn adjacent to the oxymethine; the strong deshielding of H-15 indicated that it belonged to a CHOAc unit. Finally, the sharp doublet for H-15 revealed the presence of a tertiary alcohol at position C-14. Inspection of models showed that the



1a R = H
1b R = Ac

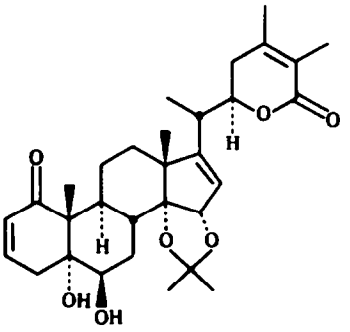


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Table 1. ^{1}H NMR data for withaminimin (1a), withaminimin acetate (1b) and withaminimin acetonide (2)

H	1a	1b	2
2	5.87 (*dd*, 10, 2.5)	5.92 (*dd*, 10, 2.5)	5.90 (*dd*, 10.5, 3)
3	6.58 (*ddd*, 10, 5, 2)	6.56 (*ddd*, 10, 5.5, 2.5)	6.63 (*ddd*, 10.5, 5.5, 2.5)
4α			2.10 (*dd*, 20, 5.5)
4β	3.19 (*dt*, 20, 2.5)		3.34 (*dt*, 20, 3)
6	3.60 (*br t*, 2)	5.17 (*br*, $W_{1/2}$ = 5)	3.78 (*br*, $W_{1/2}$ = 7)
7α			1.84 (*td*, 14, 3.5)
7β			2.00 (*dt*, 14, 3)
8			2.30 (*ddd*, 14, 11.5, 3)
9			2.13 (*m*)
11–12			1.5–1.65 (*m*, 4H)
15	5.33 (*d*, 2.5)	5.51 (*d*, 2)	5.15 (*d*, 2)
16	5.60 (*br d*, 2.5)	5.58 (*d*, 2)	5.70 (*d*, 2)
18	1.16 (*s*, 3H)	1.14 (*s*, 3H)	1.15 (*s*, 3H)
19	1.21 (*s*, 3H)	1.25 (*s*, 3H)	1.40 (*s*, 3H)
20	2.53 (*m*)		2.44 (quintet, 7)
21	1.12 (*d*, 7, 3H)	1.23 (*d*, 7, 3H)	1.08 (*d*, 7, 3H)
22	4.34 (*ddd*, 12, 7, 4)	4.34 (*dt*, 13, 4.5)	4.29 (*ddd*, 12.5, 6.5, 3.5)
23α	2.31 (*dd*, 17, 4)		2.26 (*dd*, 17.5, 3.5)
23β	2.42 (*dd*, 17, 12)		2.48 (*dd*, 17.5, 12.5)
27	1.87 (*br s*, 3H)	1.87 (*br s*, 3H)	1.87 (*br s*, 3H)
28	1.98 (*br s*, 3H)	1.93 (*br s*, 3H)	1.93 (*br s*, 3H)
6-OAc		2.12 (*s*, 3H)	
15-OAc	2.01 (*s*, 3H)	2.03 (*s*, 3H)	
			1.45 (*s*, 3H)
			1.41 (*s*, 3H)

Table 2. ^{13}C NMR data for withaminimin (1a) and its acetonide (2)

C	1a	2	C	1a	2
1	204.06	204.16	18	16.80	20.69
2	128.71	128.46	19	15.10	14.02
3	141.33	141.61	20	36.11	35.15
4	36.03	35.67	21	17.21	17.61
5	77.22	77.51	22	78.47	79.16
6	74.26	74.75	23	32.35	32.58
7	26.47	30.54	24	150.25	148.33
8	35.43	31.04	25	121.44	122.19
9	35.43	35.15	26	167.52	166.23
10	52.20*	54.25†	27	12.37	12.44
11	23.18	20.88	28	20.64	20.41
12	38.82	33.87	$\underline{C}H_3CO$	21.36	
13	52.23*	54.23†	$CH_3\underline{C}O$	170.63	
14	82.28	93.77	$O_{\underline{C}}{}^{\underline{Me}}$		27.97
15	83.36	84.46	$O^{\underline{C}}{}_{\underline{Me}}$		29.28
16	120.37	126.18	$O_2\underline{C}(Me)_2$		110.43
17	161.30	154.00			

*†Signals with the same superscript may be interchanged.

relatively small (2.5 Hz) coupling constant between hydrogens at C-15 and C-16 is consistent only with an α-acetate moiety, leaving a quasi-axial β-proton at C-15.

The ^{13}C NMR data presented in Table 2 confirmed structure **1a** for withaminimin. Indeed, the carbons of rings A and B are virtually identical to those of other withanolides with this substitution pattern [12]; the 14α-OH group deshields C-8 (a β-effect) and shields C-7 and C-9 (γ-effects), as expected [12]. Withaminimin is, therefore, (20*S*,22*R*)-15α-acetoxy-5α,6β,14α-trihydroxy-1-oxowitha-2,16,24-trienolide. The natural product can be converted to the 6-acetate **1b** by treatment with acetic anhydride–pyridine (see Experimental).

In an attempt to further substantiate the proposed structure, withaminimin was treated with 8 N H_2SO_4 in acetone (see Experimental), conditions which usually result in elimination of the 14-OH group as water [8, 13]. The product, however, proved to be the acetonide, **2** (two extra methyl groups and a dioxygenated sp^3-hybridized carbon at 110.4, see Tables 1 and 2). The formation of **2** is only consistent with a *cis*-relationship between the C-14 and C-15 oxyfunctions, and is accompanied by relatively minor conformational changes, as reflected in the NMR data shown in Tables 1 and 2.

The chemical ionization mass spectrum (CI-MS) of withaminimin (reagent gas: isobutane) showed the expected MH^+ quasi-molecular ion at *m/z* 529; in addition, a weak $MC_3H_7^+$ at *m/z* 571 is also seen, confirming that the molecular weight of **1a** is 528. Some fragmentation of the MH^+ ion was also present (Table 3); the ion lost, in sequence, a molecule of acetic acid and three molecules of water. Two low-mass fragments were also noteworthy: *m/z* 125, from the side-chain lactone moiety (see e.g. refs [14, 15]), and *m/z* 61 (protonated acetic acid). All this refers to a mass spectral examination in which **1a** was dissolved in $CHCl_3$, some of the solution was transferred to a sample vial, and the solvent was dried with a flow of N_2. Interestingly, if a crystal of the natural product was introduced directly into the sample vial, the resulting mass

Table 3. CI-MS data* for withaminimin (1a) and its acetonide (2)

Ion	1a†	1a‡	2
$[MH_2O]^+ = A^+$		546 (0.29)	544 (3.12)
$MH^+ = B^+$	529 (2.14)	529 (0.02)	
$[B-H_2O]^+$		511 (2.37)	
$[A-H_2O-Me]^+$			511 (9.29)
$[B-2H_2O]^+$		493 (0.02)	
$[A-AcOH]^+$		486 (0.29)	
$[B-AcOH]^+$	469 (34.11)	469 (38.35)	
$[A-H_2O-Me-CH_2CO]^+$			469 (42.81)
$[A-AcOH-H_2O]^+$		468 (4.91)	
$[B-AcOH-H_2O]^+$	451 (100.00)	451 (100.00)	
$[A-2H_2O-Me-CH_2CO]^+$			451 (100.00)
$[B-AcOH-2H_2O]^+$	433 (46.41)	433 (37.54)	
$[A-3H_2O-Me-CH_2CO]^+$			433 (69.95)
$[B-AcOH-3H_2O]^+$	415 (8.11)	415 (7.39)	
$[A-4H_2O-Me-CH_2CO]^+$			415 (9.09)
$[C_7H_9O_2]^+$	125 (3.37)	125 (5.69)	125 (0.69)
$AcOH_2^+$	61 (87.15)	61 (16.40)	

*The data is presented as *m/z* (rel. int.).
†From $CHCl_3$ solution (see text).
‡From crystals (see text).

spectrum was not identical to the one described above (Table 3). While the MH^+ peak at *m/z* 529 was present, it was considerably less abundant. There was, however, a new peak at *m/z* 546, which can only be attributed to a MH_2O^+ quasi-molecular ion. This species then lost, in sequence, a molecule of acetic acid and one of water to give new peaks at *m/z* 486 and 468, respectively. The fragmentation of MH^+ was the same as in the first spectrum, but in addition, a new pathway seemed to be present, involving an initial loss of two molecules of water (to *m/z* 511 and 493). From the latter ion, loss of ketene (42 mass units) would branch back to the original scheme.

For acetonide **2**, the chemical ionization mass spectrum (Table 3) was idependent of the sample insertion technique. In both cases, MH^+ was not observed; instead, the MH_2O^+ peak at *m/z* 544 was present. The fragmentation is characteristic for acetonides [16], for which molecular ions are usually absent, but $[M-Me]^+$ (at *m/z* 511) and $(M-Me-CH_2O)^+$ (at *m/z* 469) ions dominate. The latter species went on to lose, in sequence, three water molecules.

We are not aware of any precedents in the withanolide field for the presence of a MH_2O^+ quasi-molecular ion; the explanation might be that most such spectra have been run in the EI mode. Two sources come to mind for this added water moiety: fragmentation from another molecule of steroid, or water of crystallization. The low probability of a bimolecular reaction under chemical ionization conditions and the subtle dependence on the handling of the samples seems to favour the latter possibility.

EXPERIMENTAL

Melting points were taken with a Toshniwal apparatus and are uncorr. IR spectra were recorded on a Perkin–Elmer Infracord 137 spectrophotometer. CD was determined on a Jasco J-40 instrument. NMR spectra were run on Bruker WH-270 (270.0 MHz, ^1H for **1b**) and AM-300 (300.1 MHz for ^1H, 75.5 MHz for ^{13}C) spectrometers; all the data presented in Tables 1 and 2 refer to $CDCl_3$ solns. Chemical shifts are given in ppm downfield from internal TMS and coupling constants are in Hz. The MS were obtained by direct probe using a Finnigan 4020 quadrupole instrument. The operating conditions for chemical shift ionization were as follows: reagent gas: isobutane, ionization potential: 58 eV, emission current: 0.25 mA, electron multiplier: 1.8 kV, source temp.: 260°. TLC used chromatoplates coated with BDH silica gel G (Glaxo Laboratories, India) and CC used BDH silica gel (60–120 mesh) and neutral Al_2O_3.

Plant material. The aerial parts of *Physalis minima* Linn. were purchased from United Chemical & Allied Products, Calcutta, India and a specimen sample is being preserved.

Isolation. The air-dried epigeal parts (5.0 kg) were ground, defatted with petrol (bp 60–80°) and extracted with 95% EtOH in a Soxhlet apparatus. The extract was concd under red. pres. to *ca* 2.5 l., diluted with an equal vol. of H_2O, and extracted first with petrol and then with $CHCl_3$. The $CHCl_3$ layer was washed and dried (Na_2SO_4) to give, on removal of solvent, a greenish residue (*ca* 40 g). This was chromatographed over silica gel and eluted first with C_6H_6 and then with increasing amounts of EtOAc in C_6H_6. C_6H_6–EtOAc (3:1) eluates yielded a solid residue (8 g) which was rechromatographed over EtOAc-washed Al_2O_3. Petrol–EtOAc (1:1) eluates from this column afforded withaminimin (**1a**) (0.65 g) as a homogeneous amorphous powder, mp 208°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1715 and 1680. CD (MeOH): $\Delta\varepsilon_{332}$ −0.08, $\Delta\varepsilon_{252}$ +0.22. EI-MS, *m/z* (rel. int.): 513 (0.3), 495 (0.3), 469 (1.6), 450 (5.1), 355 (4.5), 343 (3.4), 337 (1.6), 325 (14.2), 171 (14.6), 135 (21.2), 125 (100.0).

Withaminimin acetate (**1b**). A mixture of withaminimin (50 mg), Ac_2O (1 ml) and pyridine (0.5 ml) was kept under dry conditions for 12 hr. The usual work-up followed by CC yielded **1b** (45 mg), IR ν_{max}^{KBr} cm^{-1}: 3600, 1725, 1715, 1680, 1260 and 1240.

Withaminimin acetonide (**2**). A soln of withaminimin (0.1 g) in dry Me_2CO (20 ml) was treated with 8 N H_2SO_4 (2 ml) and stirred for 24 hr at room temp. The solvent was removed from the reaction mixture at room temp., and the residue was diluted with

H_2O and quickly extracted with $CHCl_3$. The washed, dried and concd $CHCl_3$ extract, on chromatography over silica gel and crystallization from Me_2CO, yielded fine needles of **2** (0.06 g), mp 179–181°. EI-MS, *m/z* (rel. int.): 511 (66.6), 493 (2.7), 469 (3.0), 451 (30.8), 433 (19.9), 415 (10.8), 397 (4.3), 327 (12.6), 291 (10.8), 263 (16.0), 185 (21.2), 171 (53.2), 153 (29.7), 135 (50.0), 125 (100.0).

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