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Withanolides from *Withania coagulans*

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Abstract

Two withanolides, 20β-hydroxy-1-oxo-(22*R*)-witha-2,5,24-trienolide (**1**) and withacoagulin (**2**), along with a known withanolide, 17β-hydroxy-14α, 20α-epoxy-1-oxo-(22*R*)-witha-3,5,24-trienolide (**3**) were isolated from *Withania coagulans*. Their structures were elucidated with the help of different spectroscopic techniques.

Keywords: Solanaceae; *Withania coagulans*; Withanolides; Withacoagulin

1. Introduction

Withania coagulans Dunal belongs to family Solanaceae. *Withania* is a small genus of shrubs, which are distributed in the East of the Mediterranean region and extend to South Asia. The berries of the shrub are used for milk coagulation. This property is attributed to the pulp and husk of the berry, which is known to contain an enzyme. The main components of berries are esterases, free amino acids, fatty oils, essential oils and withanolides. Withanolides are steroidal lactones with an ergostane skeleton (Glotter, 1991). The fruits of the plant are sweet and are reported to be sedative, emetic, alterative and diuretic. They are useful in chronic complaints of liver. The fruits are also used in dyspepsia, flatulent colic and other intestinal infections. They are employed for the treatment of asthma, biliousness and stranguary. In some parts of the sub-continent, the berries are used as a blood purifier. The twigs are chewed for cleaning teeth and the smoke of the plant is inhaled for relief in toothache (Kiritika and Basu, 1981). Our extensive studies on *Withania* species resulted in isolation of several new withanolides (Atta-ur-Rahman et al., 1993, 1998), in continuation of our work on *Withania coagulans* we report herein the isolation of two new withanolides, 20β-hydroxy-1-oxo-(22*R*)-witha-2, 5, 24-trienolide (**1**), withacoagulin (**2**) and a known withanolide (**3**) which was isolated for the first time from this plant (Ahmad et al., 1998).

2. Results and discussion

The UV spectrum (MeOH) of **1** ($C_{28}H_{38}O_4$) showed a strong absorption at 218 nm, which is characteristic of α,β-unsaturated carbonyl and α,β-unsaturated lactone chromophores (Lavie et al., 1965). The IR spectrum ($CHCl_3$) displayed bands at 3426, 1712 and 1684 cm^{-1} indicative of hydroxyl, six-membered cyclic ketone and α,β-unsaturated lactone functionalities, respectively [Pavia et al., 1979].

The ^1H NMR spectrum of **1** ($CDCl_3$, 500 MHz) showed signals for five tertiary methyls at δ 0.89, 1.21, 1.28, 1.86, 1.93. The appearance of the C-21 methyl as a singlet indicated that the neighboring C-20 had no proton. The lowfield shift of C-21 methyl (δ 1.28) suggested that an oxygen function may be present on C-20. The downfield chemical shifts of the C-27 and C-28 methyl singlets (δ 1.86 and 1.93, respectively) indicated that they both substituted on a double bond. Three downfield signals at δ 5.85 (*dd*, $J_{2,3}=9.8$ Hz, $J_{2,4}=3.0$ Hz), 6.74 (*m*) and 5.58 (*br d*, $J_{6,7}=6.0$ Hz) represented three vinylic protons H-2, H-3 and H-6, respectively. A downfield methine double doublet at δ 4.20 ($J_{22,23\alpha}=13.2$ Hz, $J_{22,23\beta}=3.4$ Hz) was assigned to C-22 methine proton of the lactone moiety.

In the COSY 45° spectrum of **1**, the C-3 methine proton showed couplings with the C-2 methine and C-4

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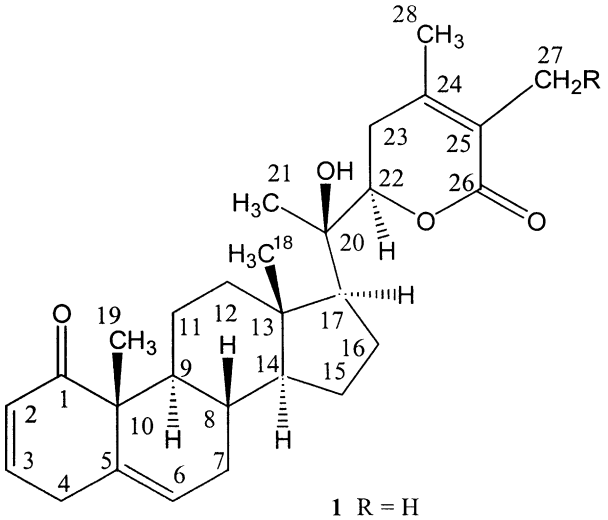
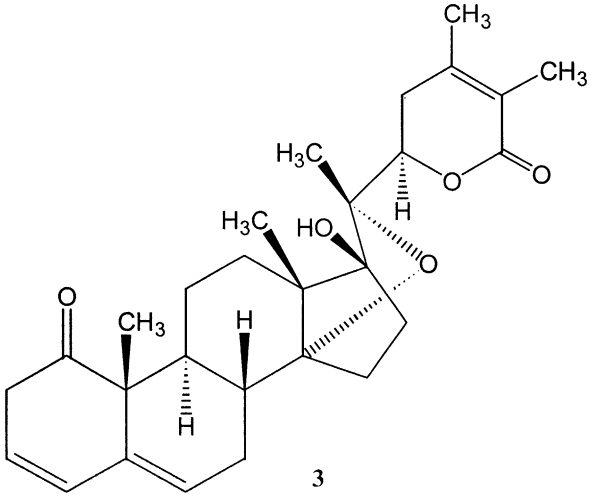


1 R = H

2 R = OH, Δ^{14}



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methylene protons, whereas the C-6 methine proton showed couplings with the C-7 methylene protons. The C-22 methine proton showed vicinal coupling with the C-23 methylene protons.

The broad-band decoupled ^{13}C NMR and DEPT spectra ($CDCl_3$, 125 MHz) indicated the presence of 28 carbon resonances including five methyl, seven methylene, eight methine and eight quaternary carbons. The lowfield signals at δ 204.5 and 166.2 were due to ketone and lactone carbonyl carbons, respectively. The signals at δ 145.2, 127.9 and 124.7 were assigned to three vinylic carbons (C-3, C-2 and C-6, respectively) in rings A and B, while the peaks at δ 149.0, 135.9 and 122.0 were attributed to the quaternary vinylic carbons (C-24, C-5 and C-25, respectively). The peak at δ 81.0 was assigned to the oxygen-bearing methine (C-22), while the peak appearing at δ 75.2 was assigned to hydroxy-bearing quaternary carbon (C-20). The signals appearing at δ 21.7, 20.5, 18.9, 13.6 and 12.7 were assigned to the C-28, C-21, C-19, C-18 and C-27 methyls, respectively. The chemical shift assignments for carbons of **1** are presented in Table 1.

In the HMBC (Heteronuclear Multiple Bond Connectivity) spectrum of **1**, C-2 proton (δ 5.85) exhibited correlations with the carbon resonating at δ 50.1 (C-10) and 33.4 (C-4). Similarly, the C-3 proton (δ 6.74) showed correlations with the downfield carbons resonating at δ 204.5 (C-1) and 135.9 (C-5). The HMBC spectrum also displayed heteronuclear shift correlations between the C-6 proton (δ 5.58) and quaternary C-5 (δ 135.9) as well as with C-10 (δ 50.1), while the C-9 proton (δ 1.60) showed heteronuclear interactions with C-11 (δ 21.9) and C-12 (δ 23.4). The C-27 and C-28 methyl protons (δ 1.86 and 1.93, respectively) exhibited couplings with C-24 (δ 149.0) and C-25 (δ 122.0), respectively. The C-27 methyl protons also displayed interaction with the lactone carbonyl (C-26) resonating at δ 166.2, while the C-28 methyl protons exhibited interaction with C-23 (δ 30.6).

Compound **1** has the formula $C_{28}H_{38}O_4$ as indicated by the M^+ at m/z 438.2752 (calc. 438.2770) in the HREI MS spectrum. The base peak m/z 126 ($C_7H_{10}O_2$) resulted from the cleavage of the C-20/C-22 bond and the remaining half of the molecule also appeared as an ion at m/z 313.2032 ($C_{21}H_{29}O_2$). Another important ion at m/z 169.0939 ($C_9H_{13}O_3$) resulted from the cleavage of C-17/C-20 bond and indicated the presence of an oxygen function at C-20.

The α stereochemistry at C-22 was assigned on biogenetic grounds and by chemical shift comparisons (Lavie et al., 1970). These spectroscopic evidences led to the structure **1** for this withanolide. This compound has been previously synthesized by Velde and Lavie (1981) and this is the first report of its isolation as a natural product. Spectral comparison with the reported data (UV, IR, ^1H NMR etc.) further confirmed the identity of the compound.

The ^1H and ^{13}C NMR spectra of compound **2** ($C_{28}H_{36}O_5$) showed its resemblance with compound **1** with additional signals for a double bond and presence of a CH_2OH group, instead it lacked a signal for CH_3. The ^1H NMR exhibited an olefinic proton at δ 5.18 which in the HMQC (Heteronuclear Multiple Quantum Coherence) spectrum showed a direct connectivity with C-15 (δ 118.0). The COSY 45° spectrum of **2** showed vicinal couplings of the C-15 methine proton (δ 5.18) with the C-16 methylene protons (δ 2.0, 2.1). The C-17 methine proton appeared at δ 1.90 as a multiplet and showed couplings with the C-16 methylene protons. The lack of further coupling of C-15 methine proton with any other proton indicated that the other end of the double bond is fully substituted. In HMBC spectrum, C-15 methine proton (δ 5.18) showed heteronuclear connectivities with C-13 (δ 47.9), C-17 (δ 57.3) and C-16 (δ 42.0). The C-17 (δ 1.90) methine proton exhibited HMBC interactions with the C-13, C-14 (δ 152.4) and

Table 1
$^{1}H/^{13}C$ connectivities of **1**, **2** and **3** in $CDCl_3$

C. No.	^{13}C NMR (δ)	^{1}H NMR (δ) coupling constants J_{HH} (Hz)	^{13}C NMR (δ)	^{1}H NMR (δ) Coupling Constants J_{HH} (Hz)	^{13}C NMR (δ)	^{1}H NMR (δ) coupling constants J_{HH} (Hz)
	1		**2**		**3**	
1	204.5	–	203.6	–	211.0	–
2	127.9	5.85 (*dd*, $J_{2,3}=9.8$, $J_{2,4\alpha}=3.0$)	127.9	5.83 (*dd*, $J_{2,3}=9.9$, $J_{2,4\alpha}=2.4$)	39.6	3.31 (*dd*, $J_{2\alpha,2\beta}=21.1$, $J_{2\beta,4}=2.7$) 2.55 (*dd*, $J_{2\alpha,2\beta}=21.1$, $J_{2\alpha,3}=4.8$)
3	145.2	6.74 *m*	145.2	6.74 (*dd* $J_{3,2}=9.9$, $J_{3,4\alpha}=3.1$)	129.3	6.05 (*dd*, $J_{3,4}=9.5$, $J_{3,2\alpha}=4.8$)
4	33.4	3.28 (*dd*, $J_{4\alpha,4\beta}=21.6$, $J_{2,4\ \alpha}=3.0$) 2.79 (*dd*, $J_{4\alpha,4\beta}=21.6$, $J_{3,4\beta}=4.8$)	33.4	3.28 (*ddd*, $J_{4\alpha,4\beta}=21.2$, $J_{4\alpha,2}=2.4$, $J_{4\alpha,3}=3.1$) 2.85 (*dd*, $J_{4\alpha,4\beta}=21.2$, $J_{4\beta,3}=4.9$)	127.3	5.71 *m*
5	135.9	–	135.4	–	136.6	–
6	124.7	5.58 (*br d* $J=6.0$)	124.3	5.58 (*d*, $J=6.0$)	121.0	5.56 *m*
7	31.6	1.98 *m*, 1.90 *m*	30.0	2.75 *m* 2.15 *m*	33.9	2.15 *m* 1.99 *m*
8	39.7	1.52 *m*	31.9	1.55 *m*	36.6	1.49 *m*
9	40.1	1.60 *m*	42.4	1.65 *m*	36.2	1.61 *m*
10	50.1	–	50.1	–	52.3	–
11	21.9	1.50 *m*	28.8	1.50 *m*	22.1	1.32 *m*
12	23.4	1.69 *m*	26.3	1.35 *m*	26.4	1.38 *m*
13	49.6	–	47.9	–	54.3	–
14	54.7	3.65 *m*	152.4	–	88.0	–
15	29.6	1.09–1.25 *m*	118.0	5.18 *br.s*	30.8	1.21 *m*
16	42.9	2.01 *m*	42.0	2.0–2.1 *m*	33.6	2.12 *m*
17	56.6	1.58 *m*	57.3	1.90 *m*	78.5	–
18	13.6	0.89 *s*	18.7	1.13 *s*	17.3	0.92 *s*
19	18.9	1.21 *s*	18.8	1.25 *s*	20.3	1.15 *s*
20	75.2	–	74.6	–	84.0	–
21	20.5	1.28 *s*	20.0	1.31 *s*	17.7	1.25 *s*
22	81.0	4.20 (*dd*, $J_{22\alpha,23\alpha}=13.2$, $J_{22\alpha,23\beta}=3.4$)	81.7	4.28 (*dd*, $J_{22\alpha,23\alpha}=13.3$, $J_{22\alpha,23\beta}=3.5$)	81.4	4.61 (*dd*, $J_{22\alpha,23\alpha}=12.7$, $J_{22\alpha,23\beta}=3.9$)
23	30.6	2.38 *m* 2.10 *m*	31.7	2.51 *m* 2.18 *m*	33.7	2.49 *m* 2.51 *m*
24	149.0	–	153.4	–	150.5	–
25	122.0	–	125.9	–	121.0	–
26	166.2	–	165.7	–	165.7	–
27	12.7	1.86 *s*	57.4	4.38, 4.33 (AB *d*, $J=12.4$)	12.3	1.75 *s*
28	21.7	1.93 *s*	20.5	2.03 *s*	20.0	1.88 *s*

C-16 (δ 42.0), thus confirming the position of the double bond between C-14/C-15. On the other hand, AB doublets appeared at δ 4.38 and 4.33 ($J=12.4$ Hz) for the C-27 hydroxymethylenic protons which exhibited direct $^{1}H/^{13}C$ coupling with C-27 (δ 57.4). These protons also showed couplings in the HMBC spectrum with C-28 (δ 20.5). The C-28 methyl protons (δ 2.03) showed connectivities with C-24 olefinic (δ 153.4) and C-25 (δ 122.0), while hydroxymethylenic C-27 (δ 4.38 and 4.33) exhibited long-range heteronuclear connectivity with the olefinic C-25 (δ 125.9). The C-27 methylene protons also displayed interaction with C-26 (δ 165.7). The $^{1}H/^{13}C$ connectivities and chemical shifts of protons and carbons are presented in Table 1.

The HREI MS of **2** showed the M^+ at m/z 452.2621 corresponding to the molecular formula $C_{28}H_{36}O_5$ (calc. 452.2562). The ion at m/z 141.0845 ($C_7H_9O_3$) resulted from the cleavage of the C-20/C-22 bond, whereas the base peak at m/z 124.0595 resulted from the loss of a H_2O molecule from the ion at m/z 142.0631. Two other important fragments at m/z 185.1030 ($C_9H_{13}O_4$) and 267.1851 ($C_{19}H_{23}O$) resulted from the cleavage of the C-17/C-20 bond. Another important fragment at m/z 109.0762 (C_7H_9O) was due to the cleavage of ring A along with the C-19 methyl group and also indicated the presence of a carbonyl group in ring A. The mass fragmentation pattern was characteristic of withanolides. These spectral evidences led to structure **2** for withacoagulin (Neogi et al., 1987).

Withanolide **3** was identified as 17β-hydroxy-14α,20α-epoxy-1-oxo-(22*R*)-witha-3,5,24-trienolide by comparing the data in hand with those reported earlier. This compound was earlier isolated from the ethanolic extracts of *Physallis peruviana* (Ahmad et al., 1998). However this is the first report of its isolation from this plant.

3. Experimental

Thin layer chromatography was carried out using precoated silica gel sheets (Merck 60 F_{254}). Column chromatography was performed using silica gel (230–400 mesh). Dragendorff's spray reagent and UV (254 nm) light was used for the detection of compounds on TLC. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. IR spectra were recorded on a Jasco 302-A spectrophotometer. A Jasco DIP-360 digital

polarimeter was used to determine optical rotation values. EI MS were recorded on a Varian MAT 311A mass spectrometer. The ^{1}H NMR spectra were recorded on Bruker AM 500 MHz spectrometer, while ^{13}C NMR spectra was recorded at 125 MHz on the same instrument.

3.1. Plant material

The whole plant (140 kg) of *W. coagulans* Dun. (Solanaceae) was collected from the suburban areas of Karachi city (Pakistan) in September 1996. The plant material was identified by Mr. Tahir Ali, plant taxanomist, Department of Botany, University of Karachi. A voucher specimen (KUH-67122) was deposited in the Herbarium of the University of Karachi.

3.2. Extraction and isolation

The plant material (79 kg) was extracted with MeOH (150 l) at room temperatures for two weeks and the resulting extract was concentrated to a gum (2.5 kg). The gum was dissolved in a mixture of MeOH and H_2O (5: 95). The aqueous extract was extracted with pet. ether, chloroform (pH 3.0, 7.0, 9.0) and with ethyl acetate, respectively. The chloroform fraction (pH 3.0, 920 g) was subjected to column chromatography (silica gel, 70–230 mesh size, 3 kg) which was eluted with pet. ether: chloroform (100–0%). The mixture of different fractions of this column i.e. Fr-8 (12 g) was again subjected to column chromatography and eluted with pet. ether:acetone (90:10). The fractions 10–15 of this column which showed a UV active spot were combined and further purified by thin layer chromatography using pet. ether:ethyl acetate (80:20) as the mobile phase to yield compound **1**. The fractions 25–35 of this column showed UV active spots. When these fractions were combined and purified on preparative TLC plates in a mixture of pet. ether:ethyl acetate (70:30), it yielded compound **2**. The chloroform fraction obtained at pH 9.0 (80 g) was also subjected to column chromatography. The fraction obtained upon elution with pet. ether:ethyl acetate (20:80) showed a UV active spot. This fraction (95 mg) was again loaded on a column packed with silica gel (70–230 mesh size, 11 g) and eluted with a mixture of pet. ether:ethyl acetate (30:70) to yield compound **3**.

3.3. 20β-Hydroxy-1-oxo-(22R)-witha-2,5,24-trienolide (*1*)

$[\alpha]_D^{25}$ 34 ($c = 0.0053$, $CHCl_3$); UV λ_{max} (MeOH) 218 nm; IR υ_{max} cm^{-1} 3426, 1712, 1684, EI MS *m*/*z* (rel. int.,%) 438 (9), 313 (22), 169 (47), 126 (100); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR (CDC_{l3} 125 MHz) δ: see Table 1.

3.4. 20β,27-Dihydroxy-1-oxo-(22R)-witha-2,5,24-tetraenolide (*2*)

$[\alpha]_D^{25}$ 37 ($c = 0.0081$, $CHCl_3$); UV λ_{max} (MeOH) 215 nm; IR υ_{max} cm^{-1} 3583, 1706, 1682; EI MS *m*/*z* (rel. int.,%) 452 (9), 267 (13), 141 (87), 124 (100), 109 (53); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) δ: see Table 1.

3.5. 17β-Hydroxy-14α,20α-epoxy-1-oxo-(22R)-witha-3,5,24-trienolide (*3*)

$[\alpha]_d^{25}$ −11 ($c = 0.0062$, $CHCl_3$–MeOH); UV λ_{max} (MeOH) 226; IR υ_{max} cm^{-1} 3350, 1705, 1690; FAB MS *m*/*z* 453 (M + H); EI MS *m*/*z* (rel. int.,%) 452 (6), 434 (8), 152 (100), 125 (95); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) δ: see Table 1.

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