Coagulansins A and B, New Withanolides from *Withania coagulans* DUNAL

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Coagulansins A (**1**) and B (**2**), two new withanolides, were isolated from the $CHCl_3$-soluble fraction of *Withania coagulans* DUNAL, along with two known withanolides, coagulin R and withanolide P. Their structures were assigned by means of ^{1}H- and ^{13}C-NMR spectra, DEPT, and ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC experiments.

Introduction. – The genus *Withania* belongs to the family Solanaceae which comprises many species. *Withania coagulans* DUNAL, which grows widely in Pakistan and India, is used as a folk medicine [1], and fruits of the plant have milk-coagulating properties [2]. Different parts of this plant possess a variety of biological activities [1]. A compound isolated from the aqueous extract of fruits of *W. coagulans* exerts hepatoprotective and anti-inflammatory activity [3]. Antifungal and antibacterial properties have also been demonstrated in the EtOH extract of the whole plant and leaves, respectively [4][5]. Previously, a number of withanolides have been reported from this species [6–13]. The ethnopharmacological and chemotaxonomic importance of the genus *Withania* prompted us to carry out further studies on *W. coagulans*. As a result, we isolated two new withanolides, named coagulansins A (**1**) and B (**2**), along with coagulin R (=(3β,17α,22*R*)-14,20-epoxy-3,17,22-trihydroxy-1-oxoergosta-5,24-dien-26-oic acid δ-lactone) [8] and withanolide P (=(17α,22*R*)-14,17,22-trihydroxy-1-oxoergosta-2,5,24-trien-26-oic acid δ-lactone) [14].

Result and Discussion. – The MeOH extract of the whole plant was divided into fractions soluble in hexane, $CHCl_3$, AcOEt, BuOH, and H_2O. Column chromatography of the $CHCl_3$-soluble fraction provided two new withanolides which were named coagulansins A (**1**) and B (**2**), along with coagulin R and withanolide P (*Fig. 1*).

Coagulansin A (**1**) was obtained as a white amorphous powder. The HR-FAB-MS (positive-ion mode) provided a $[M+H]^+$ peak at *m*/*z* 487.2687, indicating the molecular formula $C_{28}H_{39}O_7$. In the EI-MS, the peaks at *m*/*z* 141 and 345 resulting from the cleavage of the C(20)–C(22) bond suggested the presence of a hydroxylated α,β-unsaturated δ-lactone ring. Another peak at *m*/*z* 185, resulting from the cleavage of the C(17)–C(20) bond, indicated the presence of an OH group at C(20) [15]. The UV showed an absorption at λ_{max} 219 nm. The hypsochromic shift compared to the characteristic absorption (225 nm) of the usual dimethyl-substituted α,β-unsaturated δ-lactone ring of the withanolides indicated the presence of an α-CH_2OH group [16]. The

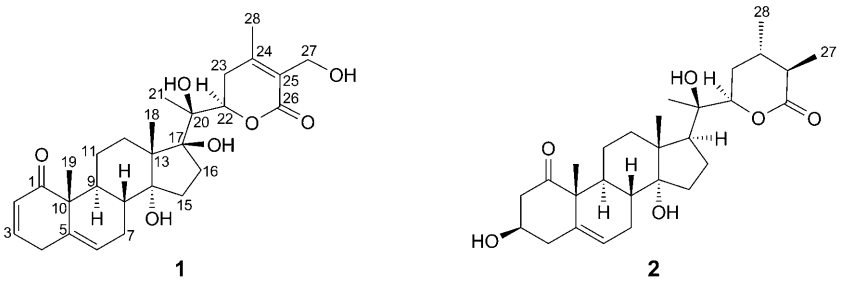

Fig. 1. *Coagulansins A* (**1**) *and B* (**2**), *isolated from* Withania coagulans DUNAL

IR spectrum revealed the presence of OH groups (3414 cm^{-1}), a six-membered conjugated cyclic ketone (1685 cm^{-1}), and an α,β-unsaturated δ-lactone ring (1705 cm^{-1}). The ^{1}H-NMR spectrum (*Table*) included the downfield signals of rings *A* and *B* which were similar to a steroidal 2,5-dien-1-one skeleton, on the basis of their chemical shifts and well-known splitting pattern at δ(H) 5.88 (*dd*, $J = 2.2$, 10.0 Hz, 1 H), 6.76 (*dd*, $J = 4.9$, 10.0 Hz, 1 H), and 5.60 (*d*, $J = 5.4$ Hz, 1 H) [17][18]. The Me *s* at δ(H) 1.06 and 1.23 were assigned to Me(18) and Me(19), while comparatively deshielded signals at δ(H) 1.30 and 2.02 could be assigned to Me(21) and to an olefinic Me group, *i.e.*, Me(28) of the δ-lactone moiety. The absence of a second olefinic Me group and the appearance of two *AB d* at δ(H) 4.30 and 4.38 (*d*, $J = 12.5$ Hz, 1 H each) suggested the presence of a CH_2OH group at either C(24) or C(25). Its location at C(25) was confirmed by HMBC experiments (*Fig. 2*) which revealed a ^2J correlation of the CH_2O H-atoms at δ(H) 4.30 and 4.38 to C(25) and ^3J correlation to C(24) and C(26). The CHO H-atom resonating at δ(H) 4.60 showed a one-bond heteronuclear connectivity to a C-atom at δ(C) 81.0 (C(22)) in the HMQC spectrum, and ^2J couplings to C(23) at δ(C) 32.9 and C(20) at δ(C) 77.0 and ^3J couplings to C(17) at δ(C) 88.6 and C(26) at δ(C) 166.1 in the HMBC, confirming its placement at C(22). It was assigned the α-orientation (22*R*) in analogy to commonly occurring withanolides. This was confirmed by its multiplicity in the ^{1}H-NMR spectrum since H–C(22) resonates as a broad *s* with $W_{1/2} \approx 5$ Hz in the case of (22*S*)-configuration but as a *dd* with two coupling constants characteristic for axial/axial and axial/equatorial interactions with H–C(23) in the case of (22*R*)-configuration [19]. The *dd* signal of H–C(22) of **1** revealed the (22*R*)-configuration. The occurrence of Me(21) as *s* and the multiplicity of H–C(22) confirmed the presence of an OH group at C(20). One of the remaining OH groups was placed at C(14) because of its downfield shift in the ^{13}C-NMR. This assumption was confirmed by HMBC experiments showing a ^2J correlation of Me(18) at δ(H) 1.06 to C(13) at δ(C) 51.0 and ^3J correlations to C(12) at δ(C) 27.0, C(14) at δ(C) 84.0, and C(17) at δ(C) 88.6. It has been observed that a 14β-OH group does not cause shielding of C(12) [20], while a 14α-OH group shields C(7), C(9), and C(12) and deshields C(8) [21]. Thus, OH–C(14) was assigned the α-orientation. The remaining OH group was assigned to C(17) as δ(C) 88.6 showed ^3J correlations to H–C(22) at δ(H) 4.60 and Me(21) at δ(H) 1.30. The β-configuration of OH–C(17) could be deduced from the characteristic pyridine-induced downfield shift for Me(18) as has been observed with other 17β-hydroxy-substituted withanolides [22][23]. Based on these evidences, the

structure of (14α,17*S*,20*S*,22*R*)-14,17,20,27-tetrahydroxy-1-oxowitha-2,5,24-trienolide was assigned to coagulansin A (**1**).

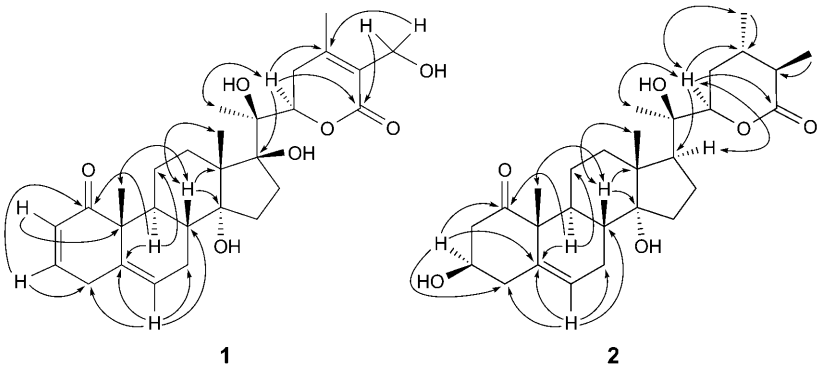

Fig. 2. *Key HMBC* (H → C) *and NOESY* (H ↔ H) *correlations of compounds* **1** *and* **2**

Coagulansin B (**2**) was isolated as an amorphous solid. The molecular formula $C_{28}H_{43}O_6$ was established by HR-FAB-MS showing the $[M+H]^+$ peak at *m*/*z* 475.3050. In the UV spectrum, **2** did not show the usual absorptions for the unsaturated ketone of ring *A* and the unsaturated δ-lactone of the side chain; however, the IR showed their C=O groups, the frequencies suggesting them to be saturated. The reduced δ-lactone ring was also inferred by the EI-MS which exhibited peaks at *m*/*z* 127 and 347 ($[M-127]^+$) resulting from the cleavage of the C(20)–C(22) bond. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) showed the presence of a secondary OH group (δ(H) 3.88–3.99 (*m*, 1 H); δ(C) 69.8), three *s* Me groups (δ(H) 1.12, 1.27, and 1.38 (3 *s*); δ(C) 19.0), 17.6, and 18.5), two *d* Me groups (δ(H) 1.25 (*d*, *J* = 6.8 Hz) and 1.14 (*d*, *J* = 6.8 Hz); δ(C) 14.5 and 20.1), a nonconjugated C=O group (δ(C) 211.2), a nonconjugated δ-lactone group (δ(C) 176.5), a tertiary OH group (δ(C) 79.2), and a characteristic CH group (δ(H) 4.21 (*dd*, *J* = 3.5, 13.0 Hz); δ(C) 80.7). Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of 3β-hydroxy-2,3-dihydrowithanolide H (=(3β,22*R*)-3,14,20,22,27-pentahydroxy-1-oxoergosta-5,24-dien-26-oic acid δ-lactone) [24] confirmed the presence of a C=O group at C(1) and of an OH group at C(3). The ^{13}C-NMR spectrum showed two sp^2-C-atoms in addition to the two C=O groups, suggesting the presence of a C=C bond located at C(5) by comparison of the NMR data with those of related steroids [8][25]. The three tertiary Me groups could be assigned to Me(18), Me(19), and Me(21), respectively. The positions of the two secondary Me groups of the reduced lactone were in accordance with 24,25-dihydro-27-deoxywithaferin A (=(4β,5β,6β,22*R*)-5,6-epoxy-4,22-dihydroxy-1-oxoergost-2-en-26-oic acid δ-lactone) [26]. The identity of ^{13}C-NMR chemical shift of the δ-lactone moiety with those of philadelphicalactone A (=(4β,5β,6β,22*R*,25*R*)-5,6-epoxy-4,17,20,22-tetrahydroxy-1-oxoergost-2-en-26-oic acid δ-lactone) [27] indicated that the two are of the same structure, including the configuration as that of philadelphicalactone A. The remaining tertiary OH group was assigned to C(14) based on the similarity to compound **1**. All the positions of substituents were confirmed through ^{1}H,^{1}H-COSY, NOESY, HMQC, and

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[a])	δ(C)[b])
C(1)	–	204.7	–	211.2
H–C(2) or CH_2(2)	5.88 (*dd*, *J* = 2.2, 10.0)	127.6	2.56 (br. *d*, *J* = 14.7), 2.87 (br. *d*, *J* = 14.7)	47.4
H–C(3)	6.76 (*dd*, *J* = 4.9, 10.0)	145.7	3.88–3.93 (*m*)	69.8
CH_2(4)	2.75 (*dd*, *J* = 4.0, 20.8), 3.24 (br. *d*, *J* = 20.8)	33.3	2.35–2.39 (*m*), 2.48–2.52 (*m*)	35.8
C(5)	–	135.1	–	135.0
H–C(6)	5.60 (*d*, *J* = 5.4)	124.7	5.52 (br. *d*, *J* = 5.6)	125.9
CH_2(7)	1.60–1.68 (*m*), 2.20–2.27 (*m*)	24.7	1.67–1.74 (*m*), 2.02–2.08 (*m*)	27.6
H–C(8)	1.78–1.83 (*m*)	36.6	1.80–1.86 (*m*)	38.2
H–C(9)	2.10–2.17 (*m*)	35.8	1.98–2.02 (*m*)	37.1
C(10)	–	50.7	–	51.8
CH_2(11)	1.50–1.57 (*m*), 2.20–2.25 (*m*)	21.8	1.53–1.58 (*m*), 2.05–2.12 (*m*)	22.1
CH_2(12)	1.40–1.46 (*m*), 1.60–1.66 (*m*)	27.0	1.51–1.57 (*m*), 1.69–1.75 (*m*)	30.4
C(13)	–	51.0	–	48.9
C(14)	–	84.0	–	84.2
CH_2(15)	1.30–1.37 (*m*), 1.60–1.66 (*m*)	32.0	1.32–1.36 (*m*), 1.58–1.64 (*m*)	25.5
CH_2(16)	1.50–1.55 (*m*), 2.40–2.45 (*m*)	33.0	1.40–1.48 (*m*), 1.60–1.66 (*m*)	39.0
C(17)	–	88.6	1.86–1.91 (*m*)	55.8
Me(18)	1.06 (*s*)	19.6	1.12 (*s*)	19.0
Me(19)	1.23 (*s*)	18.5	1.27 (*s*)	17.6
C(20)	–	77.0	–	79.2
Me(21)	1.30 (*s*)	18.8	1.38 (*s*)	18.5
H–C(22)	4.60 (*dd*, *J* = 3.8, 12.3)	81.0	4.21 (*dd*, *J* = 3.5, 13.0)	80.7
CH_2(23)	2.32–2.39 (*m*), 2.50–2.56 (*m*)	32.9	1.58–1.62 (*m*), 2.09–2.15 (*m*)	31.7
C(24) or H–C(24)	–	156.0	1.76–1.82 (*m*)	30.5
C(25) or H–C(25)	–	124.5	2.19–2.24 (*m*)	40.1
C(26)	–	166.1	–	176.5
CH_2(27) or Me(27)	4.38 (*d*, *J* = 12.5), 4.30 (*d*, *J* = 12.5)	56.2	1.25 (*d*, *J* = 6.8)	14.5
Me(28)	2.02 (*s*)	19.9	1.14 (*d*, *J* = 6.8)	20.1

[a]) Recorded at 400 MHz. [b]) Recorded at 100 MHz.

HMBC experiments. Thus, the structure of coagulansin B (**2**) was assigned as (3β,14α,20*S*,22*R*)-3,14,20-trihydroxy-1-oxowith-5-enolide.

Experimental Part

General. TLC: silica gel plates (SiO_2; *Si 60 F_{254}*; *E. Merck*). Column chromatography (CC): SiO_2 (230–400 mesh; *E. Merck*). Optical rotations: *Jasco-DIP-360* digital polarimeter. UV Spectra: *Hitachi-*

UV-3200 spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-302-A* spectrophotometer; in $CHCl_3$; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker* instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-, FAB-, and HR-FAB-MS: *Jeol-JMS-HX-110* and *JMS-DA-500* mass spectrometers; in *m*/*z* (rel. %).

Plant Material. The whole plant of *W. coagulans* DUNAL (Solanaceae) was collected from Karachi (Pakistan) in April 2008. The plant material was identified by a plant taxonomist, Prof. *Surraiya Khatoon*, Department of Botany, University of Karachi. A voucher specimen was deposited with the herbarium (KUH# 46528) of the University of Karachi.

Extraction and Isolation. The whole plant of *W. coagulans* was shade-dried, ground, and extracted with MeOH (3×50 l). The combined MeOH extract (550 g) was divided into hexane-, $CHCl_3$-, AcOEt-, BuOH-, and H_2O-soluble fractions. The $CHCl_3$-soluble fraction (75 g) was subjected to CC (hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH in increasing order of polarity): *Fractions A–D*. *Fr. B*, obtained with $CHCl_3$/MeOH 9.8 : 0.2, was subjected to CC (SiO_2, $CHCl_3$/MeOH 9.5 : 0.5): coagulansin B (**2**; 12 mg) and coagulin R (18 mg) from top and tail fractions, resp. *Fr. C*, obtained with $CHCl_3$/MeOH 9.5 : 0.5, was subjected to CC (SiO_2, $CHCl_3$/MeOH 9.3 : 0.7), and the resulting binary mixture was finally resolved by prep. TLC ($CHCl_3$/MeOH 9.0 : 1.0): coagulansin A (**1**; 14 mg) and withanolide P (15 mg).

Coagulansin A (= *(14α,17*S*,20*S*,22*R*)-14,17,20,27-Tetrahydroxy-1-oxowitha-2,5,24-trienolide = (17α,22*R*)-14,17,20,22,27-Pentahydroxy-1-oxoergosta-2,5,24-trien-26-oic Acid δ-Lactone*; **1**): Amorphous powder. M.p. 179–181°. $[\alpha]_D^{25} = +105$ ($c = 0.03$, $CHCl_3$). UV ($CHCl_3$): 219 (4.34). IR (KBr): 3414, 2924, 2855, 1705, 1685, 1664, 1601. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 468 (12), 432 (18), 415 (15), 345 (9), 185 (25), 141 (40), 125 (100). HR-FAB-MS (pos.): 487.2687 ($[M+H]^+$, $C_{28}H_{39}O_7^+$; calc. 487.2696).

Coagulansin B (= *(3β,14α,20*S*,22*R*)-3,14,20-Trihydroxy-1-oxowith-5-enolide = (3β,22*R*)-3,14,20,22-Pentahydroxy-1-oxoergost-5-en-26-oic Acid δ-Lactone*; **2**): Amorphous powder. M.p. 168–169°. $[\alpha]_D^{25} = +65$ ($c = 0.02$, $CHCl_3$). UV ($CHCl_3$): 225 (4.20). IR (KBr): 3430, 1705, 1685, 1650. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 456 (12), 438 (15), 347 (18), 127 (100), 110 (75). HR-FAB-MS (pos.): 475.3050 ($[M+H]^+$,$C_{28}H_{43}O_6^+$; calc. 475.3060).

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