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NEW WITHANOLIDES FROM *WITHANIA* SPP.ATTA-UR-RAHMAN,* SAMINA ABBAS, DUR-E-SHAHWAR,
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ABSTRACT.—Two new withanolides named coagulin [17 β ,27-dihydroxy-14,20-epoxide-1-oxo-(22*R*)-witha-3,5,24-trienolide] [1] and withasomidienone [27-hydroxy-3-oxo-(22*R*)-witha-1,4,24-trienolide] [2] have been isolated from *Withania coagulance* and *Withania somnifera*, respectively. The structures have been determined on the basis of spectroscopic studies.

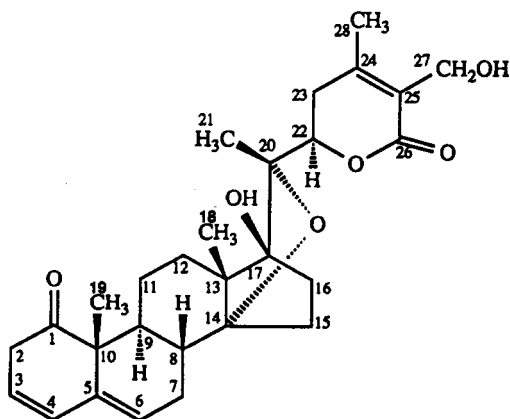
Withania coagulance Dun. (Solanaceae) is widely distributed throughout the drier parts of the South Asian subcontinent (1). The fruit of the plant is commonly used in Sind (Pakistan), North India, and Afghanistan instead of rennet to coagulate milk (2). It is also used as an emetic and in smaller doses as a remedy for dyspepsia arising from chronic liver diseases (3).

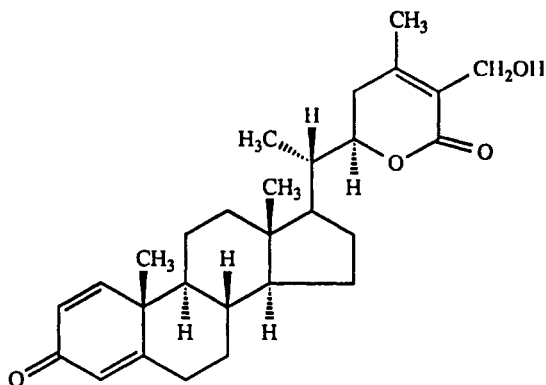
Withania somnifera Dun., a perennial plant, is widely distributed along the shores of the Mediterranean sea, as well as in India, South Africa, Pakistan and other countries (4). Various therapeutic properties have been attributed to this plant and it has been used in the indigenous system of medicine for the treatment of ulcers, rheumatism, cough, dropsy, consumption, and sensile debility (5).

A number of withanolides have been isolated previously from *Withania* spp. Some of these compounds have shown interesting biological activities. For instance, withaferin A has shown antibacterial properties against acid-fast bacilli and Gram-positive microorganisms (6–8). 3 β -Hydroxy-2,3-dihydrowithanolide F has hepatoprotective activity against CCl₄-induced hepatotoxicity in adult albino rats (9). We now describe the isolation and structure elucidation of two new withanolides, coagulin [1] and withasomidienone [2].

RESULTS AND DISCUSSION

Coagulin [1], C₂₈H₃₆O₆, isolated from *W. coagulance*, showed a uv spectrum with an absorption at 226 nm characteristic of an α,β -unsaturated lactone chromophore (10). The ir spectrum displayed bands at 3350, 1705, and 1690 cm⁻¹ indicative of a hydroxyl, a six-membered cyclic ketone, and an α,β -unsaturated lactone, respectively. Positive





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fabms showed the molecular ion to be at m/z 469 (m/z 468.2559 as determined by hreims) corresponding to the molecular formula $C_{28}H_{36}O_6$, indicating eleven degrees of unsaturation.

The 1H -nmr spectrum (400 MHz, $CDCl_3 + CD_3OD$) of **1** showed close resemblance with isowithanolide F (11) and indicated the presence of 3,5-diene-1-oxo system in rings A and B of the steroidal skeleton and an α,β -unsaturated lactone in the side chain. The 1H -nmr spectrum included signals for four quaternary methyls at δ 1.35 (Me-18), 1.02 (Me-19), 1.30 (Me-21), and 2.0 (Me-28). Appearance of the Me-21 protons as a singlet indicated the presence of an oxygen function on vicinal C-20. A multiplet resonating at δ 5.60 was due to the vinylic H-3. A broad doublet of doublets centered at δ 5.90 ($J_{4,3} = 9.6$ Hz, $J_{4,2} = 2.01$ Hz) was assigned to the vinylic H-4. Another vinylic signal resonating at δ 5.55 as a doublet of doublets ($J_{6,7\alpha} = 5.1$ Hz, $J_{6,7\beta} = 2.8$ Hz) was assigned to the H-6 of the conjugated diene. Two doublet of doublets resonating at δ 2.10 and 1.90 were assigned to the C-15 methylene protons vicinal to C-14, 2.8 Hz) which was linked with C-20 by an ether linkage. A downfield methine double doublet at δ 4.60 ($J_{22\alpha,23\alpha} = 12.5$ Hz, $J_{22,23\beta} = 3.9$ Hz) was assigned to the C-22 methine proton of the lactone moiety. Downfield AB doublets resonating at δ 4.17 and 4.11 ($J_{27,27'} = 12.0$ Hz) were due to the C-27 hydroxymethylene protons. The presence of an ether linkage between C-14 and C-20 was inferred from ms and ^{13}C -nmr studies. Peaks at m/z 169 and 327 in the mass spectrum indicated the presence of only three oxygen functions in the rings A, B, C, and D (cyclopentanophenanthrene) and two oxygens as a lactone at C-20. Excluding the ketonic oxygen of ring A, oxygen of primaryhydroxyl group and two lactonic oxygens, only two oxygens remained to be incorporated in the skeleton, whereas the ^{13}C -nmr spectrum exhibited signals for three oxygen-bearing carbons in ring D and in the C-17 side chain. These results indicated the presence of an ether linkage either between C-14/C-17 or C-14/C-20. The Drieding model of **1** (Figure 1) confirmed that an ether linkage is only possible between C-14 and C-20 (joining of C-14 with C-17 would afford a 4-membered cyclic ether within a 5-membered ring which would be too strained to exist). These observations supported a tetracyclic steroidal skeleton with a lactone substituent as found in other withanolides (4,12).

The homonuclear COSY 45° spectrum of **1** indicated the presence of two important spin systems in the molecule. H-3 (δ 5.60) showed the COSY 45° connectivity with H-2 α (δ 2.40) and H-2 β (δ 3.10), as well as the H-4 (δ 5.90), whereas the methine H-4 showed strong cross peaks with the vinylic H-3 and allylic H-2. H-22 (δ 4.60) showed strong cross peaks with methylenic H-23 (δ 2.40 and 1.60). The configuration at C-22 was assumed to be *R* on biogenetic grounds as found in all other withanolides (4,8,13).

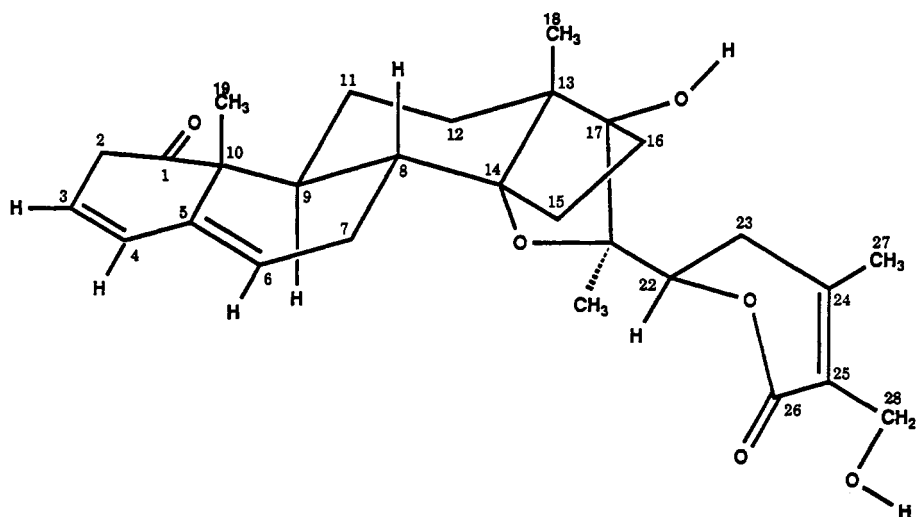


FIGURE 1. Perspective view of coagulin [1].

The broad-band decoupled ^{13}C -nmr spectrum (125 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$, Table 1) and DEPT spectra (polarization pulse angles $\theta = 45^\circ$, 90° , and 135°) indicated the presence of 28 carbon resonances, in agreement with the molecular formula, $\text{C}_{28}\text{H}_{36}\text{O}_6$. The DEPT experiment showed that there were four methyl, eight methylene, and six methine carbons. The remaining ten quaternary carbon signals appeared in the broad-band spectrum. The lowfield signals at δ 211.5 and 166.3 were due to the ketone and lactone carbonyls, respectively. The three vinylic methine carbons of ring A and B resonated at δ 127.2 (CH), 129.2 (CH), 121.3 (CH) due to C-3, C-4, and C-6, respectively, while the quaternary C-5 vinylic carbon appeared at δ 140.5. The C-14 and C-20 linked through an ether linkage resonated at δ 88.9 and 80.9, respectively. The chemical shift assignments for various carbons of compound **1** are presented in Table 1. These assignments were made on the basis of hetero-COSY experiments (14) and by comparing the data with reported withanolides having similar skeletons (12).

The one-bond $^1\text{H}/^{13}\text{C}$ chemical shift correlations of **1** were determined by an HMQC (Heteronuclear Multiple Quantum Coherence) experiment (14). The carbon at δ 39.7 (C-2) was coupled with the geminal protons at δ 2.40 (H-2 α) and 3.10 (H-2 β), while the carbon at δ 32.6 (C-23) showed interactions with δ 1.60 and 2.40 (H-23 α and H-23 β). The carbon at δ 25.0 (C-15) was linked with the protons at δ 1.90 and 2.10 (H-15 α and H-15 β). The downfield methine protons at δ 5.60 (H-3), 5.90 (H-4), 5.55 (H-6), and 4.60 (H-22) showed one-bond heteronuclear interactions with the carbons at δ 127.2 (C-3), 129.2 (C-4), 121.3 (C-6), and 81.0 (C-22), respectively.

The long-range $^{13}\text{C}/^1\text{H}$ correlations determined by an HMBC (Heteronuclear Multiple Bond Connectivity) (15) experiment showed that H-4 (δ 5.90) has long-range coupling with C-2 (δ 39.7). The C-13 (δ 51.3) and C-14 (δ 88.9) carbons showed long-range correlations with the Me-21 protons (δ 1.30). Similarly long-range correlation between C-20 (δ 80.9) and the methine H-22 was also observed. The methylene protons resonating at δ 1.09 and 3.10 (H-16 α and H-16 β) showed long-range correlations with C-13 (δ 51.3) and C-14 (δ 88.9).

The hreims of **1** showed the molecular ion peak at m/z 468.2559 indicative of the molecular formula $\text{C}_{28}\text{H}_{36}\text{O}_6$. The ion at m/z 141.0654 of composition $\text{C}_7\text{H}_9\text{O}_3$ resulted by the cleavage of the C-20/C-22 bond and further confirmed the presence of six-

TABLE 1. ^{13}C -nmr Chemical Shifts for **1** and **2**.^a

Carbon	Compound	
	1	2
C-1	211.5 (C)	155.8 (CH)
C-2	39.7 (CH ₂)	127.5 (CH)
C-3	127.2 (CH)	186.4 (C)
C-4	129.2 (CH)	123.9 (CH)
C-5	140.5 (C)	166.9 (C) ^b
C-6	121.3 (CH)	39.4 (CH ₂)
C-7	34.0 (CH ₂)	27.2 (CH ₂)
C-8	35.1 (CH)	38.8 (CH)
C-9	33.0 (CH)	35.5 (CH)
C-10	52.4 (C)	43.6 (C) ^c
C-11	21.0 (CH ₂)	22.6 (CH ₂)
C-12	26.4 (CH ₂)	33.8 (CH ₂)
C-13	51.3 (C)	43.0 (C) ^c
C-14	88.9 (C) ^b	56.2 (CH)
C-15	25.0 (CH ₂)	29.8 (CH ₂)
C-16	32.7 (CH ₂)	24.4 (CH ₂)
C-17	85.7 (C) ^b	51.9 (CH)
C-18	20.0 (Me)	11.5 (Me)
C-19	18.5 (Me)	13.3 (Me)
C-20	80.9 (C)	51.8 (CH)
C-21	20.2 (Me)	8.0 (Me)
C-22	81.0 (CH)	78.7 (CH)
C-23	32.6 (CH ₂)	32.5 (CH ₂)
C-24	156.3 (C)	152.8 (C)
C-25	124.5 (C)	125.7 (C)
C-26	166.3 (C)	169.1 (C) ^b
C-27	56.1 (CH ₂)	58.0 (CH ₂)
C-28	20.1 (Me)	20.0 (Me)

^aMultiplicity assignments based on DEPT experiment.^{b,c}Assignments are interchangeable.

membered lactone substituents at C-20 of the steroidal skeleton, while the fragment ion at m/z 327.1972 (C₂₁H₂₇O₃) represented the remaining half of the molecule. The ion at m/z 169.0930 (C₉H₁₃O₃) resulted from cleavage between C-17 and C-20. The fragment at m/z 135.0806 (C₉H₁₁O) may arise by cleavage of rings between rings B and C. The overall mass fragmentation pattern of **1** was characteristic of withanolides (16). The stereochemistry at various asymmetric centers was assigned on the basis of chemical shift comparisons with known withanolides and from biogenetic considerations. The Drieding model of compound **1** allowed only an α configuration for the C-14/C-20 epoxy linkage, while the C-17 hydroxy was proposed to be α -oriented based on ^{13}C -nmr chemical shift comparison with a known withanolide (11). Thus spectroscopic evidence led to the elucidation of structure **1** for this new withanolide.

Withasomidienone [**2**], C₂₈H₃₈O₄ (m/z 438.2768), was isolated from *W. somnifera* and exhibited uv absorption at 234 nm, indicating the presence of a conjugated cyclohexadienone system (17). The ir spectrum displayed absorptions at 3550 (O-H), 1650 (α,β -unsaturated ketone), and 1615 (C=C) cm⁻¹ (18).

The ^1H -nmr spectrum of **2** revealed the presence of three tertiary methyls by 3H singlets at δ 0.77, 1.22, and 2.03. A doublet resonating at δ 1.03 ($J_{21,20}$ = 6.6 Hz) was assigned to the Me-21 secondary protons. An AB doublet at δ 4.35 and 4.37 ($J_{27,27'}$ = 12.6 Hz) was due to the C-27 hydroxymethylene protons. A downfield broad doublet at δ 4.39

($J=13.2$ Hz) was assigned to the methine H-22 of a lactone moiety. The presence of a conjugated cyclohexadienone system inferred from the ir (1650 cm^{-1}) was further confirmed by the appearance of three downfield signals in the ^1H -nmr spectrum. A doublet at δ 7.05 ($J_{1,2}=10.1$ Hz) and a double doublet at δ 6.23 ($J_{1,2}=10.1$ Hz, $J_{2,4}=1.9$ Hz) were assigned to the vinylic H-1 and H-2. Another broad singlet at δ 6.06 was due to the vinylic H-4. These observations supported a C-3 ketone function in conjugation with the C-2/C-1 and C-4/C-5 double bonds.

The COSY 45° spectrum of **2** revealed many important homonuclear connectivities. For instance, the Me-21 doublet (δ 1.03) showed a strong cross-peak with a one-proton multiplet at δ 2.50, which was therefore assigned a methine proton (H-20). H-20 was further coupled to the downfield H-22 methine (δ 4.39) in the COSY 45° spectrum. The methine H-22 (δ 4.39) was in turn coupled with the methylene H₂-23 resonating at δ 2.00. Coupling between the vinylic H-2 and H-4 was also observed in the spectrum.

The ^1H - ^1H long-range connectivities were determined by recording a series of HOHAHA spectra with variable delays (100, 60, 20 msec). H-1 (δ 7.05) showed long-range interaction with vinylic H-4 (δ 6.06), while H-4 exhibited couplings with the vinylic H-2 (δ 6.23) as well as with the allylic H-6 (δ 2.28) methylene protons of ring B. The Me-21 protons (δ 1.03) displayed a cross-peak with H-22 (δ 4.39) and H-23 (δ 2.00), whereas the methine H-20 (δ 2.50) also showed cross-peaks with H₂-23. On the other hand the methylene H₂-23 displayed long-range interactions with H-20, H₃-21, and H₃-28. Hydroxymethylene H₂-27 also exhibited homoallylic couplings with the allylic H₃-28.

The ^{13}C -nmr spectra (100 MHz, CDCl_3 , DEPT) indicated that there were four methyls, eight methylenes, nine methines, and seven quaternary carbons in the molecule (14), which further confirmed that there were a total of 28 carbons associated with 37 hydrogens. Lowfield signals at δ 169.1 and 186.4 were assigned to α,β -unsaturated lactonic and ketonic carbonyls, respectively. The remaining four downfield carbon resonances at δ 123.9 (CH), 127.5 (CH), 155.8 (CH), and 166.9 (C) were assigned to the olefinic C-4, C-2, C-1, and C-5, respectively. Methyl signals at δ 8.0, 11.5, 13.3, and 20.0 were ascribed to C-21, C-18, C-19, and C-28, respectively. The chemical shift assignments for the various carbons of compound **2** are presented in Table 1.

The one-bond $^1\text{H}/^{13}\text{C}$ correlations for **2** were determined on the basis of HMQC experiments. The C-2 methine (δ 127.5) showed a cross-peak with H-2 at δ 6.23. Similarly the vinylic C-1 (δ 155.8) was directly correlated with the proton that resonated at δ 7.05 (H-1). C-20 (δ 51.8) and C-22 (δ 78.7) were found to be connected with the protons that resonated at δ 2.50 (H-20) and 4.39 (H-22), respectively. The C-23 methylene carbon at δ 32.5 displayed cross peaks with protons at δ 2.0 (H₂-23). The other downfield methylene C-27 (δ 58.0) had one-bond correlations with the protons at δ 4.35 and 4.37 (H-27 α and H-27 β). The long-range $^1\text{H}/^{13}\text{C}$ interactions of **2** were determined by HMBC experiments. The H-1 (δ 7.05) showed couplings with C-2, C-3, and C-10, while H-2 (δ 6.23) exhibited long-range interactions with C-4 and C-10. The couplings of H-4 (δ 6.05) with C-2 and C-10, also appeared in the HMBC spectrum. The signal at δ 4.39 (H-22) showed coupling with carbons C-21 and C-25. The Me-21 protons (δ 1.03) showed crosspeaks with the C-20 and C-22 carbons in the HMBC spectrum. Structure **2** was assigned on the basis of above-mentioned spectroscopic observations to this new withanolide named withasomidienone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on JASCO DIP-360. Ir spectra were recorded on JASCO 302-A spectrophotometer. Uv spectra were recorded on a Hitachi U 3200 spectrophotometer. Eims, fabms, and hreims were recorded on JMS H X 110 with data system DA 5000

and on MAT 112S mass spectrometers. The ^1H -nmr and ^{13}C -nmr spectra were recorded on Bruker AM 400 MHz and 500 MHz nmr spectrometers.

PLANT MATERIAL.—*W. coagulance* and *W. somnifera* (whole plants) were collected from the suburban areas of Karachi (Pakistan) in 1991. Voucher specimens KUH 46258 and 46259, respectively, were deposited in the herbarium of Department of Botany, University of Karachi.

EXTRACTION AND ISOLATION OF COAGULIN [1].—The EtOH extract of the whole plant of *W. coagulance* (25 kg) was concentrated to a gum (1.8 kg). This gum was dissolved in MeOH and defatted with petroleum ether. The defatted MeOH extract was again evaporated and dissolved in H_2O . The aqueous extract was extracted with CHCl_3 at different pH values, the pH being adjusted by addition of HOAc or NH_4OH . The acidic fraction (pH 2.5) was loaded on a Si gel column (2.8 gm) and eluted first with hexane and then with hexane/ CHCl_3 mixtures. Several fractions were obtained. The fraction collected with hexane/ CHCl_3 was again subjected to cc on a short Si gel column (0.5 gm). The fraction (20.8 mg) collected with CHCl_3 /MeOH from this column was further purified by tlc to yield coagulin [1] (4.9 mg): white crystals; $[\alpha]_D^{25} - 11^\circ$ ($c=0.0062$, $\text{CHCl}_3 + \text{MeOH}$); uv λ max (MeOH) 226 nm; ir ν max (CHCl_3) 3350, 1705, 1690 cm^{-1} ; fabms m/z 469; eims m/z (rel. int. %) $[\text{M}]^+$ 468 (7), 168 (35), 125 (100); ^1H nmr (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) δ 1.02 (3H, s, Me-19), 1.30 (3H, s, Me-21), 1.35 (3H, s, Me-18), 2.01 (3H, s, Me-28), 1.90 (1H, dd, H-15a), 2.10 (1H, dd, H-15b), 4.11 and 4.17 (2H, AB, d, $J_{27,27'} = 12.0$ Hz; H-27), 4.60 (1H, dd, $J_{22\alpha,23\alpha} = 12.5$ Hz, $J_{22\alpha,23\beta} = 3.9$ Hz, H-22 α), 5.55 (1H, d, $J_{6,7\alpha} = 5.1$ Hz, $J_{6,7\beta} = 2.8$ Hz, H-6), 5.60 (1H, m, H-3), 5.90 (1H, bd, $J_{4,3} = 9.6$ Hz, $J_{4,2} = 2.0$ Hz, H-4); ^{13}C nmr ($\text{CD}_3\text{OD} + \text{CDCl}_3$, 125 MHz) see Table 1.

EXTRACTION AND ISOLATION OF WITHASOMIDIENONE [2].—MeOH extracts of the whole plant of *W. somnifera* (100 kg) were concentrated to a gum (2.7 kg). The gum was then dissolved in MeOH and defatted with petroleum ether. The defatted MeOH extract was again evaporated and dissolved in H_2O . The aqueous extract was extracted with CHCl_3 at different pH values. The fraction obtained at pH 7 was loaded on a Si gel column and eluted first with CHCl_3 and then with CHCl_3 /MeOH mixtures. The fraction obtained in CHCl_3 -MeOH (95:5) was again chromatographed by cc, eluting with petroleum ether (40–60 $^\circ$), and petroleum ether/ CHCl_3 . The fraction collected on elution with CHCl_3 -petroleum ether (80:20) was subjected to vlc using petroleum ether and CHCl_3 . The fraction collected on elution with CHCl_3 -petroleum ether (40:60) was purified by tlc to afford withasomidienone [2] (24 mg): $[\alpha]_D^{25} + 13.2^\circ$ ($c=0.0753$, MeOH); uv λ max (MeOH) 204, 234, 290 nm; ir ν max (CHCl_3) 1615, 1650, 1681, 2900, 3550 cm^{-1} ; eims m/z (rel. int. %) $[\text{M}]^+$ 438 (98), 317 (63), 253 (5), 145 (29), 122 (33), 83 (100); ^1H nmr (400 MHz, CDCl_3) 0.77 (3H, s, Me-18), 1.03 (3H, d, $J_{21,20} = 6.6$ Hz, Me-21), 1.22 (3H, s, Me-19), 2.03 (3H, s, Me-28), 2.28 (2H, m, H-6), 2.44 (2H, m, H-7), 4.35 and 4.37 (2H, AB d, $J_{27,27'} = 12.6$ Hz, H-27), 4.39 (1H, br d, $J = 13.2$ Hz, H-22), 6.06 (1H, br s, H-4), 6.23 (1H, dd, $J_{2,1} = 10.1$ Hz, $J_{2,4} = 1.9$ Hz, H-2), 7.05 (1H, d, $J_{1,2} = 10.1$ Hz, H-1); ^{13}C nmr (CDCl_3 , 100 MHz) see Table 1.

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