Two New Ergostane-Type Steroidal Lactones from *Withania coagulans*

Atta-ur-Rahman,* M. Iqbal Choudhary,* Samina Qureshi, Waseem Gul, and Muhammad Yousaf

International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

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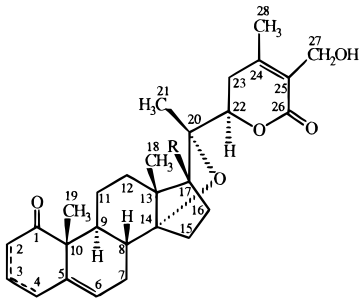
Two new withanolides (steroidal lactones) named coagulin F [27-hydroxy-14,20-epoxy-1-oxo-(22*R*)-witha-3,5,24-trienolide] (**1**) and coagulin G [17β,27-dihydroxy-14,20-epoxy-1-oxo-(22*R*)-witha-2,5,24-trienolide] (**2**) were isolated from the whole plant of *Withania coagulans*, and their structures were deduced by spectral analysis.

Withania coagulans Dunal. (Solanaceae) is a small, evergreen shrub that is reputed to have been used as a remedy for dyspepsia, flatulent colic, and other intestinal diseases. The fruit is diuretic and has coagulating properties.[1,2] These pharmacological activities have been attributed to withanolides that are present in this plant. In continuation of our work on the withanolidal constituents of *W. coagulans*,[3−5] we report here the structure elucidation of coagulin F (**1**) and coagulin G (**2**). The identity of the new compounds was determined by using a combination of spectroscopic data including 2D-NMR techniques and chemical transformations.



1 R= H, Δ^3
2 R= OH, Δ^2
3 R= OH, Δ^3

The IR spectrum of coagulin F ($C_{28}H_{36}O_5$) (**1**) indicated the presence of a hydroxyl group (3400 cm^{-1}), a six-membered cyclic ketone (1700 cm^{-1}), and an α,β-unsaturated δ-lactone (1715 cm^{-1}).[6] The UV spectrum of **1** exhibited absorption at 230 nm characteristic of an α,β-unsaturated δ-lactone chromophore.[7] Positive FABMS showed a $[M + H]^+$ peak at *m/z* 453. The EI mass spectra of **1** showed the M^+ at *m/z* 452.2493 analyzing for $C_{28}H_{36}O_5$ (calcd 452.2562), which possessed 11 degrees of unsaturation. Seven of these were accounted for by the pentacyclic α,β-unsaturated steriodal lactone skeleton, two were due to additional double bonds, and one was on account of the ketonic carbonyl. The remaining unsaturation site can be accommodated as a α-oriented cyclic ether.[3] The peak at *m/z* 141.0654 ($C_7H_9O_3$) resulting from cleavage of the C-20/C-22 bond indicated the presence of a six-membered lactone substituent at C-20.[8]

The ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **1** closely resembled that of the reported withanolide coagulin (**3**) and indicated the presence of a 1-oxo-3,5-diene system in rings A and B of the steroidal skeleton and an α,β-unsaturated δ-lactone in the side chain.[3] The four methyl groups resonated as singlets at δ 0.93, 1.15, 1.28 and 2.01. Two mutually coupled olefinic signals resonating at δ 5.61 (multiplet) and δ 6.02 (dd, $J_{4,3}$ = 10.2 Hz, $J_{4,2}$ = 2.2 Hz) were assigned to C-3 and C-4 vinylic protons, respectively. Another downfield olefinic signal resonating at δ 5.66 (dd, $J_{6,7a}$ = 5.2 Hz, $J_{6,7b}$ = 2.1 Hz) showed COSY 45° couplings to two protons of a C-7 methylene group (δ 2.20 and 1.86) and was assigned to the C-6 vinylic proton. The H-22 signal at δ 4.05 (dd, $J_{22,23a}$ = 12.5 Hz, $J_{22,23b}$ = 4.0 Hz) exhibited cross-correlation peaks in the COSY spectrum only with the H-23 signals at δ 2.40 and 2.05. Downfield AB doublets resonating at δ 4.19 and 4.12 ($J_{27,27'}$ = 11.5 Hz) were due to the C-27 hydroxymethylene protons, which shifted downfield to δ 4.88 and 4.86 in its monoacetate. The configuration of C-22 was assumed to be *R* on biogenetic grounds as found in related withanolides.[9]

The ^{13}C NMR spectra (BB and DEPT) showed the presence of four methyl, eight methylene, seven methine, and nine quaternary carbon signals. A notable feature was the appearance of downfield signals for the quaternary carbons at δ 82.4 and 74.7, which were assigned to the α-oriented epoxy bearing C-14 and C-20, respectively.[3,10] Assignments of all the functional groups were acheived by HMQC and HMBC experiments[11,12] and by comparison with similar withanolides.[3] These spectroscopic data led to structure **1** for coagulin F.

The EIMS of **2** afforded the M^+ at *m/z* 468.2538 corresponding to the molecular formula $C_{28}H_{36}O_6$ (calcd 468.2511). The IR spectrum showed absorptions for hydroxyl, ketonic carbonyl, and α,β-unsaturated δ-lactone groups, respectively, at 3350, 1695, and 1712 cm^{-1}. The presence of α,β-unsaturated δ-lactone was also inferred from the UV absorption at 226 nm.[7] The ^{1}H NMR spectrum of **2** resembles that of **1** with major differences being the presence of a downfield multiplet at δ 6.75, a double doublet at δ 5.87 ($J_{2,3}$ = 10.2 Hz, $J_{2,4\beta}$ = 2.3 Hz), and another double doublet centered at δ 5.60 ($J_{6,7a}$ = 5.1 Hz, $J_{6,7b}$ = 1.9 Hz), which indicated the presence of a 2,5-dien-1-one system in rings A and B of withanolide.[5] The ^{13}C NMR spectrum showed a downfield signal at δ 204.1 that was due to the α,β-unsaturated carbonyl carbon.[13] An additional downfield signal at δ 79.1 was assigned to an oxygen-bearing C-17

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carbon[3] that was observed at δ 48.9 in **1**. The stereochemistry at C-17 was inferred to be β (*S*) based on the fact that the alternative arrangement (*R*) was not possible when an α-oriented ether bridge exists between C-14/C-20.[10] On the basis these spectral observations, structure **2** was deduced for coagulin F.

The crude extract of *W. coagulans* showed antifungal activity,[4] but coagulins F and G did not show significant antifungal activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer. UV spectra were recorded on a Hitachi U 3200 spectrophotometer. EI, FAB, and HREI MS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H NMR spectra were recorded on Bruker spectrometers operating at 500 and 400 MHz and ^{13}C NMR spectra at 125 and 100 MHz. The chemical shift values are reported in ppm (δ) and the coupling constants (J) in Hz. Standard pulse sequences were used for COSY, DEPT, HMQC, and HMBC experiments.

Chromatographic Conditions. Column chromatography: silica gel, 230−400 mesh. TLC: precoated silica G-25, UV_{254} plates. Visualization of the TLC plates was achieved at 254 and 366 nm, and Dragendorff's spray reagent was used for detection.

Plant Material. The whole plant of *W. coagulans* Dunal. (Solanaceae) was collected from the suburban areas of Karachi (Pakistan) in April 1991. The plant material was identified by Mr. Tahir Ali, plant taxonomist, Department of Botany, University of Karachi. A voucher specimen was deposited in the herbarium (KUH-46528) of Karachi University.

Extraction and Isolation. The dried plant (25 kg) was extracted with EtOH at room temperature for 2 weeks, and the resulting extract was concentrated to a gum. This gum (1.0 kg) was partitioned between *n*-hexane and MeOH. The defatted MeOH extract was evaporated and dissolved in H_2O. The aqueous extract was extracted with $CHCl_3$ at different pH values (pH 9−10 and pH 2−3), the pH being adjusted by the addition of NH_4OH and AcOH solutions. The fraction (pH 9−10) was subjected to column chromatography on silica gel. Elution with $CHCl_3$ and then with $CHCl_3$−MeOH yielded several fractions. A fraction obtained on elution with $CHCl_3$ was found to contain two compounds (**1** and **2**). These were purified by TLC (silica gel) using $CHCl_3$−MeOH (99.8:0.2) as the solvent system.

27-Hydroxy-14,20-epoxy-1-oxo-22(*R*)-witha-3,5,24-trienolide (coagulin F) (1): greenish solid; yield 1.5 × 10^{-4}%; R_f = 0.45; $[\alpha]^{25}_D$ +112° ($CHCl_3$, c = 0.26); UV λ_{max} (MeOH) 230 nm (ϵ 18 600); IR ν_{max} ($CHCl_3$) 3400, 1715, 1700 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.02 (1H, dd, J = 10.2, 2.2 Hz, H-4), 5.66 (1H, dd, J = 5.2, 2.1 Hz, H-6), 5.61 (1H, m, H-3), 4.19, 4.12 (2H, AB d, $J_{27,27'}$ = 11.5 Hz, H-27), 4.05 (1H, dd, J = 12.5, 4.0 Hz, H-22), 2.01 (3H, s, H-28), 1.28 (3H, s, H-19), 1.15 (3H, s, H-21), 0.93 (3H, s, H-18); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 210.1 (C-1), 165.0 (C-26), 154.0 (C-24), 140.4 (C-5), 128.7 (C-4), 127.4 (C-3), 125.5 (C-25), 121.6 (C-6), 82.4 (C-14), 81.3 (C-22), 74.7 (C-20), 54.5 (C-27), 48.9 (C-17), 48.8 (C-10), 47.1 (C-13), 39.4 (C-2), 33.6 (C-8), 33.2 (C-9), 31.8 (C-23), 31.5 (C-7), 30.2 (C-16), 29.7 (C-15), 25.2 (C-12), 21.9 (C-11), 20.7 (C-28), 19.8 (C-21), 19.7 (C-18), 16.3 (C-19); FABMS [M + H]$^+$ m/z 453; EIMS m/z (rel int) [M$^+$] 452 (7), 311 (9), 185 (20), 141 (15), 124 (99), 96 (100).

Acetylation of 1. A solution of **1** (10 mg) in pyridine (1 mL) was treated with Ac_2O (1 mL) and left overnight at room temperature. The reagents were removed in vacuo, and the residue was crystallized from Me_2CO−hexane as fine needles: $[\alpha]^{25}_D$ +106° ($CHCl_3$, c = 0.3); IR ν_{max} ($CHCl_3$) 1720, 1710, 1680, 1240, 1225 cm^{-1}; UV λ_{max} (MeOH) 228 (ϵ 18 400); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (1H, dd, J = 10.2, 2.1 Hz, H-4), 5.66 (br d, J = 6 Hz, H-6), 5.60 (1H, m, H-3), 4.88, 4.86 (2H, AB d, $J_{27,27'}$ = 12 Hz, H-27), 4.20 (1H, dd, J = 13, 3.5 Hz, H-22), 2.07 (3H, s, COCH_3-27), 2.04 (3H, s, H-28), 1.61 (3H, s, H-19), 1.38 (3H, s, H-21), 1.24 (3H, s, H-18); FABMS [M + H]$^+$ m/z 495; EIMS m/z (rel int) [M$^+$] 494 (7), 416 (10), 210 (45), 124 (100).

17β,27-Dihydroxy-14,20-epoxy-1-oxo-22(*R*)-witha-3,5,24-trienolide (coagulin G) (2): white powder: yield 1.6 × 10^{-5}%; R_f = 0.33; $[\alpha]^{25}_D$ +60° ($CHCl_3$, c = 0.3); UV λ_{max} (MeOH) 226 nm (ϵ 18 000); IR ν_{max} ($CHCl_3$) 3350, 1712, 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.75 (1H, m, H-3), 5.87 (1H, dd, J = 10.2, 2.3 Hz, H-2), 5.60 (1H, dd, J = 5.1, 1.9 Hz, H-6), 4.64(1H, dd, J = 12.5, 3.5 Hz, H-22), 4.38, 4.34 (2H, AB d, $J_{27,27'}$ = 12.5 Hz, H-27), 2.03 (3H, s, H-28), 1.32 (3H, s, H-21), 1.24 (3H, s, H-18), 1.07 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.1 (C-1), 166.3 (C-26), 155.0 (C-24), 145.1 (C-3), 136.5 (C-5), 128.0 (C-2), 124.5 (C-25), 124.4 (C-6), 88.0 (C-14), 81.4 (C-22), 79.1 (C-17), 76.4 (C-20), 57.5 (C-27), 50.7 (C-13), 50.0 (C-10), 36.2 (C-9), 33.6 (C-8), 36.1 (C-8), 33.2 (C-4), 32.9 (C-7), 31.8 (C-16), 29.6 (C-23), 29.5 (C-15), 26.6 (C-12), 22.9 (C-11), 20.5 (C-28), 19.4 (C-21), 18.9 (C-18), 14.0 (C-19); FABMS [M + H]$^+$ m/z 469; EIMS m/z (rel int) [M$^+$] 468 (6), 327 (8), 141 (25), 124 (100).

Acetylation of 2. Acetylation of **2** was carried out (as described for **1**) to obtain the monoacetate: $[\alpha]^{25}_D$ +46° ($CHCl_3$, c = 0.35); IR ν_{max} ($CHCl_3$) 3400, 1730, 1710, 1690 cm^{-1}; UV λ_{max} (MeOH) 226 (ϵ 18 500); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.80 (1H, m, H-3), 5.88 (1H, dd, J = 10.2, 2.4 Hz, H-2), 5.71 (br d, J = 6.2 Hz, H-6), 4.90, 4.88 (2H, AB d, $J_{27,27'}$ = 12.1 Hz, H-27), 4.68 (1H, dd, J = 12.4, 4.0 Hz, H-22), 2.10 (3H, s, COCH_3-27), 2.08 (3H, s, H-28), 1.36 (3H, s, H-21), 1.25 (3H, s, H-18), 1.08 (3H, s, H-19); FABMS [M + H]$^+$ m/z 511; EIMS m/z (rel int) [M$^+$] 510 (3), 492 (10), 432 (12), 228 (40), 96 (100).

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