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Three withanolides from *Withania coagulans*

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Abstract

Three new withanolides, coagulins P, Q and R, were isolated from the whole plant of *Withania coagulans*. Their structures were determined as 20,27-dihydroxy-3β-(*O*-β-D-glucopyranosyl)-1-oxo-(20*S*,22*R*)-witha-5,14,24-trienolide, 1α,20-dihydroxy-3β-(*O*-β-D-glucopyranosyl)-(20*S*,22*R*)-witha-5,24-dienolide and 3β,17β-dihydroxy-14,20-epoxy-1-oxo-(22*R*)-witha-5,24-dienolide by a combination of 1D- and 2D-NMR and mass spectroscopic studies.

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

1. Introduction

Withania coagulans Dunal, belonging to the family Solanaceae, is well known in the indigenous system of medicine for the treatment of ulcers, rheumatism, dropsy, consumption and sensile debility (Chadha, 1976). Our recent work on this plant has resulted in the isolation of a number of new withanolides (Atta-ur-Rahman, Choudhary, Yousaf, Gul & Qureshi, 1998a; Atta-ur-Rahman et al., 1998b). This paper describes the isolation of three new withanolides (steroidal lactones) designated as coagulins P, Q and R (**1**–**3**).

2. Results and discussion

Coagulin P (**1**) showed UV absorption at 210 nm characteristic of an α,β-unsaturated δ-lactone chromophore. The IR spectrum showed absorptions at 3482, 1714, 1697 and 1652 cm^{-1} assignable to hydroxy, α,β-unsaturated δ-lactone, six-membered cyclic ketone and C=C functionalities, respectively. Acetylation of **1** provided a penta-acetate derivative which still showed hydroxyl absorption at 3440 cm^{-1} in its IR spectrum, confirming the presence of a tertiary hydroxyl group in **1**. The HRFAB-MS of **1** afforded the $[M + H]^+$ peak at m/z 633.3258 corresponding to the formula $C_{34}H_{49}O_{11}$ (calcd. 633.3261). Further peaks were observed at 615 and 471 due to the loss of water and hexose moieties, respectively, from the M^+. The ^{1}H-NMR spectrum of **1** showed close resemblance to the ^{1}H-NMR spectrum of coagulin O (**4**) (Atta-ur-Rahman et al., 1998a). However, a hydroxyl group was present at C-27 with an olefinic double bond between C-14/C-15 in compound **1**. Four 3H singlets at δ 1.23, 1.29, 1.44 and 1.86 were assigned to the C-18, C-19, C-21 and C-28 methyl groups, respectively. The presence of AB doublets at δ 4.64, 4.77 ($J_{27a,\,27b} = 12.0$ Hz) implied that C-27 position is substituted with a hydroxyl group. The characteristic double doublet at δ 4.98 ($J_{22\alpha,\,23\beta} = 12.5$ Hz, $J_{22\alpha,\,23\beta} = 3.0$ Hz) was assigned to the C-22 methine proton of the lactone moiety. The multiplicity of the H-22 signal indicated the absence of any proton at vicinal C-20. A one-proton broad doublet at δ 6.02 ($J_{6,\,7a} = 5.0$ Hz) was assigned to the C-6 vinylic proton. A broad singlet at δ 5.58 was assigned to the C-15 olefinic proton (Glotter, Kirson, Abraham & Lavie, 1973). A broad multiplet of 1H at δ 4.21 was assigned to H-3 geminal to the β-pyranose sugar moiety. A one-proton doublet

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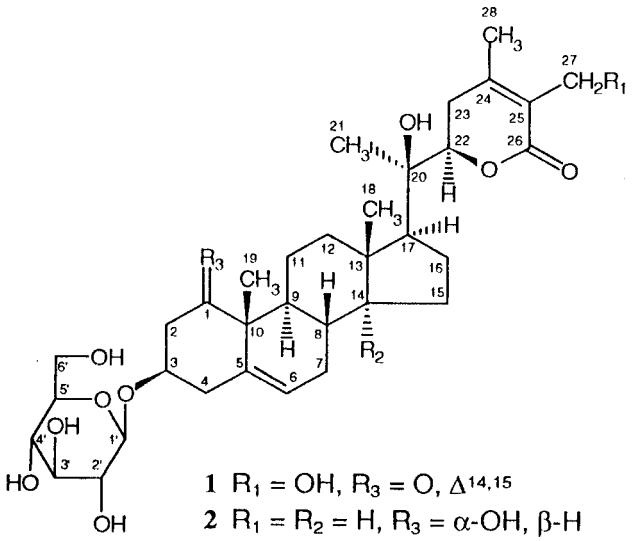
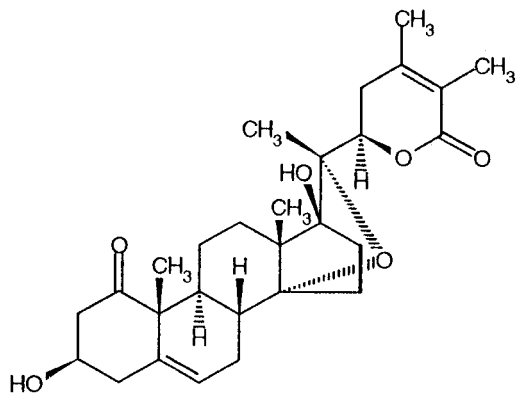


1 R_1 = OH, R_3 = O, $\Delta^{14,15}$
2 R_1 = R_2 = H, R_3 = α-OH, β-H
4 R_1 = H, R_2 = OH, R_3 = O
5 R_1 = R_2 = H, R_3 = α-OAc, β-H



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at δ 4.94 ($J_{1',2'}$ = 7.5 Hz) was assigned to the anomeric proton of the sugar moiety. This data indicated a 3β-*O*-β-D-glucopyranosyl system in the molecule. The stereochemistry at various asymmetric centers of **1** was assigned by comparing with coagulin O (**4**). All the assignments were made on the basis of COSY-45°, HOHAHA, HMQC and HMBC experiments (Atta-ur-Rahman, 1989). These spectroscopic data led to the structure **1** for coagulin P.

The IR, UV, ^{1}H- and ^{13}C-NMR data of coagulin Q (**2**) showed that it had some resemblance to the structure of **1**. The molecular formula ($C_{34}H_{52}O_{10}$) indicated the presence of two fewer double bond equivalents compared to **1**. Similarly, the ^{13}C-NMR spectrum of **2** lacked C-1 carbonyl, C-14/C-15 olefinic and a C-27 hydroxymethylene signals instead it has a secondary hydroxyl group at C-1 (δ 71.0, CH). The α-stereochemistry of the OH group at C-1 was assigned on the basis of chemical shift comparison with physalolactone B 3β-*O*-β-D-glucopyranoside (**5**) isolated from *Physalis peruviana* (Kirson, Glotter, Ray, Ali, Gottlieb & Sahai, 1983). These observations led to structure **2** for coagulin Q.

The IR, UV, ^{1}H- and ^{13}C-NMR data of coagulin R (**3**), $C_{28}H_{38}O_6$ revealed the presence of 1-keto-3-hydroxyl substitution in ring A (Ramaiah, Lavie, Budhiraja, Sudhir & Garge, 1984). The mass spectrum of **3** showed fragment ions at *m/z* 125.0684 ($C_7H_9O_2$) and 345.2075 ($C_{21}H_{29}O_4$) resulting from cleavage of the C-20/C-22 bond, indicating the presence of a six-membered lactone substituent at C-20 of the steroidal skeleton. The chemical shift and splitting pattern of signal of the H-3 (δ 3.85, $W_{1/2}$ = 5 Hz) indicated 3β-hydroxy function (Ramaiah et al., 1984). These studies led to the structure **3** for coagulin R, the glycoside of which has been isolated from *P. peruviana* (Ahmed, Malik, Afza & Yasmin, 1999).

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. The IR spectra were recorded on a JASCO 302-A spectrophotometer. The UV spectra were recorded on a Hitachi U 3200 spectrophotometer. The EI, FAB and HREI-MS were recorded on JMS HX 110 mass spectrometer with JMS-DA 500 data system. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker spectrometers operating at 500, 400 and 300 MHz.

3.2. Chromatographic conditions

CC: silica gel, 230–400 mesh. TLC: precoated silica gel GF-254 chromatoplates (20 × 20 cm, 0.2 mm thick) (E. Merck). Visualization of the TLC plates was achieved at 254 and 366 nm and Dragendorff's spray reagent was used for detection.

3.3. 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 the Karachi University.

3.4. Extraction and isolation

The dried plant (25 kg) was extracted with EtOH (60 l) at room temperature for two weeks and the resulting extract was concentrated to a gum. This gum (1 kg) was partitioned between *n*-hexane and MeOH. The defatted MeOH extract was evaporated and dissolved in H_2O. The aqueous extract was successively extracted with $CHCl_3$ and EtOAc. The EtOAc fraction (10 g) was subjected to column chromatography on silica gel. Elution of the column with $CHCl_3$ and then with $CHCl_3$–MeOH yielded several fractions. A fraction (0.9 g) obtained on elution with $CHCl_3$–MeOH (97 : 3) was found to contain three compounds (**1**–**3**). These were purified by TLC (silica gel) using $CHCl_3$–MeOH (95 : 5) as the solvent system.

3.5. Coagulin P (*1*)

Amorphous powder (35.3 mg), yield 5.4×10^{-5}%; $R_f = 0.42$; $[\alpha]_D$ 45 (c = 0.31, MeOH); IR ν_{max} (KBr): 3482, 1714, 1697, 1652 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 210 (4.11); HRFAB-MS: found, $[M + H]^+$ m/z 633.3258; (calcd. for $C_{34}H_{49}O_{11}$, 633.3261); EI-MS m/z (rel. int. %) 470 (2), 452 (5), 434 (61), 169 (97), 141 (96), 124 (100). ^{1}H-NMR (500 MHz, C_5D_5N): δ 6.02 (1H, *d*, $J_{6,\,7a} = 5.0$ Hz, H-6), 5.58 (1H, br *s*, H-15), 4.98 (1H, *dd*, $J_{22\alpha,\,23\beta} = 12.5$ Hz, $J_{22\alpha,\,23\beta} = 3.0$ Hz, H-22), 4.94 (1H, *d*, $J_{1',\,2'} = 7.5$ Hz, H-1′), 4.77, 4.64 (2H, AB *doublets*, $J_{27a,\,27b} = 12.0$ Hz, H-27) 4.21 (1H, *m*, H-3), 1.86 (3H, *s*, H-28), 1.44 (3H, *s*, H-21), 1.29 (3H, *s*, H-19), 1.23 (3H, *s*, H-18).

3.6. Acetylation of coagulin P (*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 purified on a preparative chromatoplate using $CHCl_3$ as a solvent and characterized as a pentaacetate derivative of **1**. (6.4 mg, yield 80%); $[\alpha]_D$ 48 (c = 0.46, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3440, 1736, 1717, 1703 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 212 (4.01); HRFAB-MS: found, $[M + H]^+$ m/z 843.3782; calcd. for $C_{44}H_{59}O_{16}$, 843.3784); EI-MS m/z (rel. int. %): 752 (3), 452 (12), 434 (16), 124 (100). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.65 (1H, *d*, $J_{6,\,7a} = 5.6$ Hz, H-6), 5.19 (1H, br *s*, H-15), 5.16 (1H, *t*, $J_{3',\,4'} = 9.5$ Hz, H-3′), 5.01 (1H, *t*, $J_{4',\,5'} = 9.9$ Hz, H-4′), 4.92 (1H, *dd*, $J_{2',\,3'} = 9.6$ Hz, $J_{2',\,1'} = 8.0$ Hz, H-2′), 4.88, 4.85 (2H, AB *doublets*, $J_{27a,\,27b} = 12.1$ Hz, H-27) 4.55 (1H, *d*, $J_{1',\,2'} = 7.9$ Hz, H-1′), 4.26 (1H, *dd*, $J_{22\alpha,\,23\beta} = 13.2$ Hz, $J_{22\alpha,\,23\beta} = 3.4$ Hz, H-22), 4.22 (1H, *dd*, $J_{6'a,\,6'b} = 12.2$ Hz, $J_{6'a,\,5'} = 5.3$ Hz, Ha-6′), 4.07 (1H, *dd*, $J_{6'b,\,6'a} = 12.2$ Hz, $J_{6'b,\,5'} = 2.4$ Hz, Hb-6′), 3.69 (1H, *m*, H-3), 3.65 (1H, *m*, H-5′), 2.07 (3H, *s*, H-28), 2.06, 2.04, 2.03, 1.99, 1.97 (3H, *s*, $5 \times COCH_3$), 1.31 (3H, *s*, H-21), 1.28 (3H, *s*, H-19), 1.12 (3H, *s*, H-18). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 209.3 (C-1), 170.7, 170.5, 170.1, 169.3, 169.1 ($5 \times COCH_3$), 164.1 (C-26), 156.3 (C-24), 153.0 (C-14), 133.7 (C-5), 126.3 (C-6), 122.1 (C-25), 118.3 (C-15), 100.2 (C-1′), 81.1 (C-22), 77.1 (C-3), 74.6 (C-20), 72.8 (C-3′), 71.9 (C-5′), 71.5 (C-2′), 68.5 (C-4′), 62.1 (C-6′), 57.9 (C-27), 57.3 (C-17), 52.7 (C-10), 48.0 (C-13), 46.5 (C-2), 41.9 (C-8), 38.4 (C-4), 36.6 (C-9), 32.0 (C-23), 30.4 (C-16), 29.2 (C-12), 23.2 (C-7), 21.9 (C-11), 20.8, 20.7, 20.6, 20.6, 20.5 ($5 \times COCH_3$), 20.5 (C-21), 20.5 (C-28), 19.2 (C-19), 18.7 (C-18).

3.7. Acid hydrolysis of coagulin P (*1*)

Compound (**1**) (10 mg) was refluxed for 4 h with 1 M methanolic HCl (5 ml). The solution was concentrated under reduced pressure and diluted with 5 ml of H_2O. It was extracted with EtOAc and the aqueous phase was concentrated, and methyl glucoside was identified by paper chromatography.

3.8. Coagulin Q (*2*)

Amorphous powder (28.5 mg), yield 8.3×10^{-5}%; $R_f = 0.43$; $[\alpha]_D$ 30 (c = 0.31, MeOH); IR ν_{max} (KBr): 3482, 1709, 1648 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 223 (4.06); HRFAB-MS: found, $[M + H]^+$ m/z 621.3638; calcd. for $C_{34}H_{53}O_{10}$, m/z 621.3624); EI-MS m/z (rel. int. %): 584 (1), 482 (2), 440 (4), 422 (29), 404 (10), 253 (19), 125 (100). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 5.37 (1H, *d*, $J_{6,\,7a} = 5.3$ Hz, H-6), 4.19 (1H, *d*, $J_{1',\,2'} = 7.7$ Hz, H-1′), 4.05 (1H, *dd*, $J_{22\alpha,\,23\beta} = 12.8$ Hz, $J_{22\alpha,\,23\beta} = 3.2$ Hz, H-22), 3.92 (1H, *m*, H-3), 3.70 (1H, *t*, $W_{1/2} = 5.0$ Hz, H-1), 1.90 (3H, *s*, H-28), 1.74 (3H, *s*, H-27), 1.12 (3H, *s*, H-21), 0.88 (3H, *s*, H-19), 0.76 (3H, *s*, H-18). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 165.7 (C-26), 150.3 (C-24), 138.6 (C-5), 122.8 (C-6), 120.0 (C-25), 100.8 (C-1′), 80.9 (C-22), 76.7 (C-3′), 76.6 (C-5′), 74.0 (C-20), 73.4 (C-2′), 72.2 (C-3), 71.0 (C-1), 70.0 (C-4′), 61.1 (C-6′), 56.0 (C-14), 54.0 (C-17), 52.4 (C-10), 54.1 (C-13), 40.5 (C-9), 39.3 (C-4), 37.8 (C-2), 36.5 (C-12), 31.4 (C-7), 31.3 (C-23), 31.2 (C-8), 30.0 (C-16), 23.6 (C-15), 20.0 (C-28), 19.6 (C-11), 19.5 (C-21), 19.1 (C-19), 13.5 (C-18), 12.1 (C-27).

3.9. Acid hydrolysis of coagulin Q (*2*)

Compound **2** (5 mg) was refluxed for 4 h with 1 M

methanolic HCl (3 ml). Analogous work-up (as described for **1**) yielded methyl glucoside.

3.10. Coagulin R (**3**)

Amorphous powder (25.3 mg), yield 8.3×10^{-5}%; $R_f = 0.62$; $[\alpha]_D$ 35 (c = 0.37, MeOH); IR ν_{max} ($CHCl_3$): 3451, 1703, 1684, 1621 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 225 (3.87); HREI-MS: found, $[M + H]^+$ m/z 470.2677; calcd. for $C_{28}H_{38}O_6$, 470.2658; EI-MS: m/z (rel. int. %) 470 (20), 452 (9), 434 (11), 345 (26), 301 (68), 283 (76), 237 (26), 125 (100). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.55 (1H, d, $J_{6,\,7a} = 5.3$ Hz, H-6), 4.80 (1H, dd, $J_{22\alpha,\,23\beta} = 12.5$ Hz, $J_{22\alpha,\,23\beta} =$ 3.7 Hz, H-22), 3.85 (1H, m, H-3), 1.86 (3H, s, H-28), 1.79 (3H, s, H-27), 1.32 (3H, s, H-21), 1.19 (3H, s, H-19), 1.02 (3H, s, H-18). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ 210.3 (C-1), 167.5 (C-26), 151.8 (C-24), 134.7 (C-5), 125.2 (C-6), 120.5 (C-25), 87.0 (C-14), 82.0 (C-20), 81.0 (C-22), 78.2 (C-17), 67.9 (C-3), 54.1 (C-13), 52.1 (C-10), 47.1 (C-2), 40.1 (C-4), 36.1 (C-16), 35.5 (C-8), 35.2 (C-9), 34.0 (C-12), 31.7 (C-23), 29.8 (C-15), 25.1 (C-7), 21.5 (C-11), 19.8 (C-28), 19.7 (C-19), 18.2 (C-21), 17.8 (C-18), 11.1 (C-27).

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