

New Withanolides from *Withania coagulans*

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Three new withanolides, coagulins M, N, and O were isolated from the whole plant of *Withania coagulans*. Their structures were established as (14*R*,17*R*,20*ξ*,22*R*)-5*α*,6*β*,27-trihydroxy-14,20-epoxy-1-oxo-witha-24-enolide (1), (14*R*,17*S*,20*ξ*,22*R*)-15*α*,17-dihydroxy-14,20-epoxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxo-witha-5,24-dienolide (2) and (14*R*,20*ξ*,22*R*)-14,20-dihydroxy-3*β*-(*O*-*β*-D-glucopyranosyl)-1-oxo-witha-5,24-dienolide (3) respectively, by extensive spectroscopic studies.

Key words *Withania coagulans*; Solanaceae; withanolide; steroidal lactone

Withanolides are polyoxygenated, ergostane-derived C₂₈ steroidal lactones, which have been isolated from several genera of Solanaceae and have also been found in soft corals.^{1,2)} Many of these compounds are known to exhibit a variety of pharmacological activities, such as antitumor, antibacterial, antifungal, antifeedant, anti-inflammatory, cytotoxic, insecticidal, hepatoprotective and immunosuppressive activities.²⁾ During a search for the bioactive constituents of solanaceous plants, we studied the species *Withania somnifera* DUNAL.³⁾ and *Withania coagulans* DUNAL. The crude extract of the latter was found to be active against a number of pathogenic fungi.⁴⁾ As part of our studies on the constituents of *W. coagulans*,⁴⁾ we report here the isolation and structural elucidation of three new withanolides which we have named coagulins M, N and O (1–3).

Results and Discussion

Column chromatography followed by preparative TLC of the ethanolic extract of *W. coagulans*, gave coagulins M–O (1–3). Coagulin M (1), C₂₈H₄₀O₇ displayed a UV absorption maximum at 214 nm characteristic of an α,β -unsaturated δ -lactone chromophore.⁵⁾ IR absorption bands at 3440, 1720 and 1698 cm⁻¹ were assigned to hydroxyl, α,β -unsaturated δ -lactone and cyclohexanone groups, respectively.⁶⁾ The absence of the 2-en-1-one system²⁾ in 1 was clear from the ¹H-NMR spectrum, which did not show an olefin signal and included three methyl singlets at δ 0.93 (Me-18), 0.97 (Me-19) and 1.13 (Me-21). The presence of an α,β -unsaturated δ -lactone ring substituted by a –CH₂OH and CH₃ was indicated by a vinylic methyl singlet at δ 2.00 (Me-28), two AB doublets at δ 4.10, 4.18 (2H, $J_{27a,27b}$ = 11.5 Hz, H-27) and a downfield double doublet at δ 4.03 (1H, $J_{22\alpha,23\alpha}$ = 12.7 Hz, $J_{22\alpha,23\beta}$ = 3.4 Hz, H-22). The multiplicity of H-22 indicated the absence of proton attached to vicinal C-20.²⁾ The presence of the side chain δ -lactone was further confirmed by the mass spectrum which showed a fragment ion at m/z 141.0654 of composition C₇H₉O₃ which could result from cleavage of the C-20/C-22 bond.²⁾ A broad singlet at δ 3.13 in the ¹H-NMR spectrum and signals corresponding to a quaternary carbon atom and to a methine group both bearing a hydroxyl function, at δ 74.1 and 71.1, respectively, in the ¹³C-NMR spectrum, suggested the existence of 5*α*, 6*β* dihydroxy functionality as found in the known withametelin C.⁷⁾ Since an axial 6*β*-OH can shield the C-19 protons, the upfield position of the Me-19 resonance (δ 0.97) in 1 suggested the β -orientation of 6-OH.⁸⁾ Acetylation of 1 afforded a diacetate deriva-

tive (1a). The downfield shift of the C-6H to δ 4.60 in 1a, also indicated the secondary hydroxyl group to be at the 6*β*-position.⁹⁾ The stereochemistry of the A to B ring fusion was determined from the ¹³C-NMR chemical shift of the C-19 methyl group. In 5,6-dihydroxy or 4,5,6-trihydroxy withanolides the ¹³C-NMR chemical shift of the C-19 methyl group indicates whether the C-19 methyl and hydroxyl group at C-5 are *gauche* (*cis* fused A/B rings) or not (*trans* A/B fused rings). In the former the chemical shift of C-19 appears at around δ 10¹⁰⁾ but in the latter, it appears at around δ 15.^{9,11)} The characteristic difference of chemical shift thus readily enables one to determine the stereochemistry of the hydroxyl group at C-5. In the case of 1, the chemical shift of C-19 was at δ 14.7, which corresponded to the latter case, i.e. to a *trans* fused pattern of rings. The presence of an α -oriented ether linkage between C-14 (δ 83.0) and C-20 (δ 74.2) was inferred from the mass spectrum (an extra double bond equivalent), ¹³C-NMR spectroscopic studies and by comparison with known withanolides.^{4,12)} The orientation of the C-17 methine proton was inferred to be $\beta(R)$ based on the fact that the alternative arrangement was not possible when an α -oriented ether bridge exists between C-14/C-20.¹²⁾ It has been found that when H-22 has an *S*-configuration, it resonates as a broad singlet with $W_{1/2}$ \approx 5 Hz, while in the *R*-configuration it resonates as a double doublet with two characteristic coupling constants due to axial-axial and axial-equatorial interactions with H-23.¹³⁾ In the case of compounds 1–3, H-22 resonated as a double doublet, which corresponds to the *R*-configuration.

The ¹H–¹H correlation spectroscopy (COSY) 45° spectrum of 1 indicated the presence of two important spin systems in the molecule. The H-6 methine (δ 3.13) showed COSY connectivity with methylenic H₂-7 (δ 1.65, 1.56). The H-22 methine (δ 4.03) displayed strong cross-peaks with methylenic H₂-23 (δ 2.41, 2.28). The homonuclear Hartmann–Hahn spectroscopy (HOHAHA) spectrum (100 ms) displayed long-range homonuclear interactions between H-6 methine and the H-8 (δ 2.06) and H-9 (δ 1.50) methines. Long-range interactions between H-22 methine and H₃-28 methyl were also observed.

The ¹³C-NMR spectrum of coagulin M exhibited 28 signals which, according to the distortionless enhancement by polarization transfer (DEPT) spectrum, corresponded to four methyl, ten methylene, five methine and nine quaternary carbons. Lowfield signals at δ 217.4, 165.1, 154.1, and 125.5 were assigned to saturated ketonic, and α,β -unsaturated δ -

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lactonic carbonyl carbons, and to the olefinic carbons C-24 and C-25 respectively. Methyl signals at δ 14.3, 14.7, 16.9 and 19.8 were ascribed to C-18, C-19, C-21 and C-28, respectively. The hydroxyl-bearing carbons C-5, C-6 and C-27 resonated at δ 74.1 (C), 71.1 (CH) and 54.5 (CH₂), respectively.

Assignments for all functional groups were achieved by heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments.¹⁴⁾ In the HMQC spectrum, the secondary hydroxyl-bearing carbon at δ 71.1 (C-6) was coupled with the proton at δ 3.13, while the carbon atom at δ 33.1 (C-7) showed interactions with the geminal methylene protons at δ 1.65 and 1.56 (H₂-7). The carbon at δ 81.2 (C-22) interacted with the proton at δ 4.03. C-17 (δ 48.7) displayed one-bond heteronuclear interaction with the proton at δ 2.19. On the other hand, the methyl groups at δ 0.93 (18-Me), 0.97 (19-Me), 1.13 (21-Me) and 2.00 (28-Me), had one-bond interactions with the carbon atoms at δ 14.3, 14.7, 16.9 and 19.8, respectively. In the HMBC spectrum the 19-Me (δ 0.97) showed long-range couplings (3J) with C-1 (δ 217.4), C-5 (δ 74.1), and C-9 (δ 39.2). The hydroxyl-bearing C-6 methine proton displayed correlations (2J) with C-5 (δ 74.1), C-7 (δ 33.1) and 3J with C-10 (δ 51.7). Similarly, the epoxy-bearing C-14 quaternary carbon (δ 83.1) showed 3J couplings with the 17-methine and 18-methyl protons. Likewise H₃-21 showed 2J and 3J couplings with C-20 (δ 74.2) and C-17 (δ 48.7) respectively. This spectroscopic evidence thus led to structure **1** for coagulin M.

Coagulin N (**2**), C₃₄H₄₈O₁₂ was isolated as an amorphous solid. The presence of hydroxyl, α,β -unsaturated lactone and ketonic carbonyl was inferred from the IR absorption bands at 3480, 1716 and 1702 cm⁻¹, respectively. The UV absorption at 216 nm indicated the presence of an α,β -unsaturated δ -lactone.⁶⁾ This was also evident from the electron impact (EI) mass spectrum fragment ion at m/z 125.0602 (C₇H₉O₂, base peak), which was indicative of a side chain where cleavage could occur between C-20 and C-22.²⁾ In the ¹H-NMR spectrum of **2**, a broad doublet at δ 5.60 ($J=5.1$ Hz) which showed 1J coupling to the carbon at δ 126.6 in the HMQC spectrum, and the presence in the ¹³C-NMR spectrum of a signal at δ 134.1 (C), were attributable to a trisubstituted double bond. A broad multiplet at δ 4.19 exhibited one-bond heteronuclear connectivity to the carbon at δ 75.3 in the HMQC spectrum, and was assigned to 3 α -H.¹⁵⁾ Characterization of the hexose sugar commenced with the assignment of the glycoside protons in the ¹H-NMR spectrum of **2** which was supported by analysis of the corresponding ¹³C-NMR and HMQC data. The chemical shift (¹H-NMR: δ 4.92; ¹³C-NMR: δ 102.7) and multiplicity (doublet, $J=7.7$ Hz) allowed identification of the H-1' anomeric proton. Analysis of the HOHAHA and HMBC spectra of **2** corroborated the identification of the sugar unit and the location of its attachment to the aglycone. Starting from the anomeric proton resonating at δ 4.92 in the HOHAHA spectrum (100 ms) of **2**, a spin system for a glucose unit was traced. In the HMBC spectrum the anomeric proton showed long-range correlations 3J with C-3 (δ 75.3) of the aglycone, while H-3 (δ 4.19) of the aglycone exhibited 3J with the anomeric carbon (δ 102.7). The relative configuration of glucose was determined by the analysis of vicinal coupling constants for the sugar proton

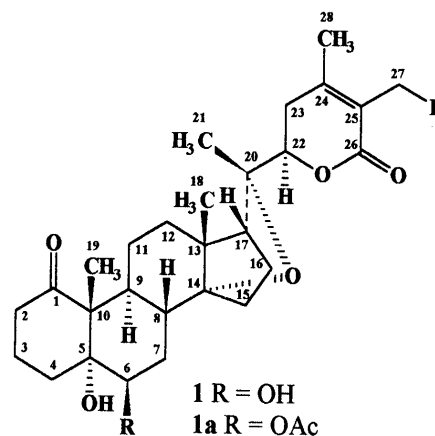


Chart 1

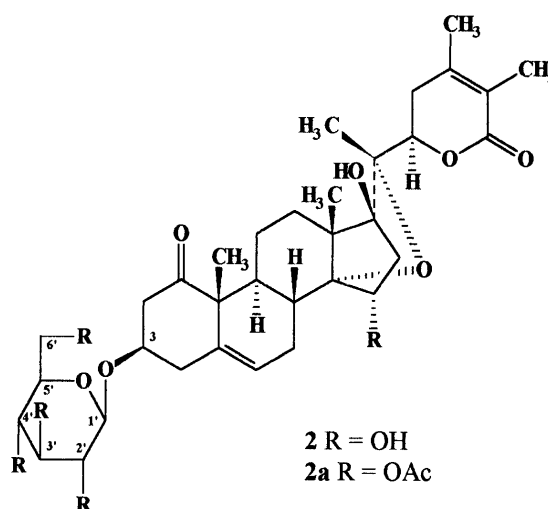


Chart 2

signals through consideration of the well-known dependence of J (coupling constant) in pyranoses.¹⁶⁾ Thus, the large, ($J_{1',2'}=7.7$ Hz) coupling constant indicated a diaxial relationship between H-1' and H-2' and a β -pyranose form for the glucose which was also identified through paper chromatography (PC) and through the retention time of its tetramethylsilyl (TMS) ether by gas chromatography (GC).¹⁷⁾ A doublet at δ 4.45 ($J=6.6$ Hz) which shifted downfield to δ 4.80 in its pentaacetate derivative (**2a**), indicated the presence of a secondary OH-15 α group.¹⁸⁾ This proton showed one-bond heteronuclear coupling to the carbon at δ 76.1 in the HMQC spectrum and long-range two- and three-bond couplings to C-14 (δ 89.1) and C-17 (δ 82.8), respectively, in the HMBC spectrum. It also exhibited COSY 45° vicinal connectivity to methylenic 16-Ha (δ 3.48, dd, $J=15.5$, 6.7 Hz), which in turn showed strong geminal couplings to 16-Hb (δ 2.24, d, $J=15$ Hz). Furthermore, the 15 α -acetate group of **2a** did not show any interaction with 18-H₃ in the nuclear Overhauser effect (NOE) difference spectrum. This supported further the α -orientation of the 15-OH group. Based on these spectral observations, structure **2** was deduced for coagulin N.

The IR, UV, ¹H-NMR and ¹³C-NMR of coagulin O (**3**) showed that it had close resemblance to **2** with distinct similarities in the substitution pattern of rings A, B, C and E. The molecular formula (C₃₄H₅₀O₁₁) indicated one fewer double

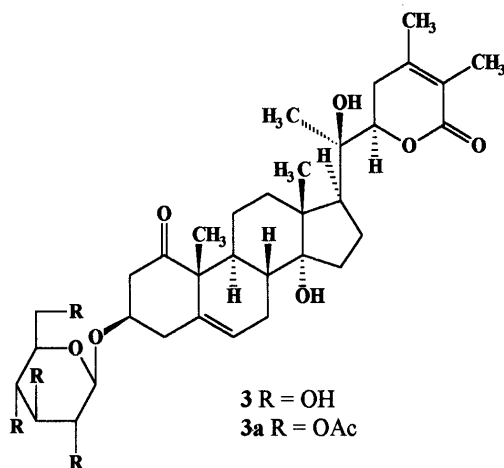


Chart 3

bond equivalent compared to **2**. Together with the ^{13}C -NMR data, this indicated that **3** had tertiary hydroxyl groups at C-14 (δ 83.8) and C-20 (δ 78.3) instead of an C-14/C-20 ether bridge, as present in **2**. The ^1H - and ^{13}C -NMR spectra also implied that **3** did not contain C-15 α secondary hydroxyl and C-17 β tertiary hydroxyl groups, as compared to **2**. A multiplet at δ 1.82, showing one-bond connectivity to the carbon at δ 56.1 in the HMQC spectrum of **3**, was assigned to the C-17 α methine proton. The lactone containing C-17 side chain was assumed to be β by comparison with similarly substituted withanolides.¹⁵⁾

Experimental

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 HR-MS were recorded on a JMS HX 110 with a data system and on a JMS-DA 500 mass spectrometer. The ^1H - and ^{13}C -NMR spectra were recorded on Bruker spectrometers operating at 500, 400 and 300 MHz. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. Standard pulse sequences were used for COSY, DEPT, HMQC and HMBC experiments.¹⁴⁾

Chromatographic Conditions Column chromatography (CC): silica gel, 230–400 mesh. TLC: Precoated Silica gel GF-254 chromatoplates (20 \times 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.

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 Procedures The dried plant (25 kg) was extracted with EtOH (80 l) at room temperature for two 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 (600 g) was evaporated and dissolved in distilled H₂O. The aqueous extract was extracted with CHCl₃ at different pH values (pH 9–10, pH 2–3), the pH being adjusted by the addition of NH₄OH and AcOH solutions. The pH 9–10 extracted fraction (100 g) was subjected to column chromatography on silica gel. Elution with CHCl₃ and then with CHCl₃-MeOH yielded several fractions. A fraction (0.80 g) obtained on elution with CHCl₃-MeOH (95:5) was found to contain the three compounds (**1**–**3**). These were purified by TLC (silica gel) using CHCl₃-MeOH (90:10) as the solvent system.

Coagulin M (1): Amorphous powder, 40 mg, yield $1.6 \times 10^{-4}\%$, $R_f=0.85$, $[\alpha]_D^{+64}$ ($c=0.46$, CHCl₃). IR (CHCl₃) cm^{-1} : 3440, 1720, 1698. UV λ_{max} (MeOH) nm (log ϵ): 214 (3.99). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 489.2840 (Calcd for C₂₈H₄₁O₇: 489.2841). EI-MS m/z (rel. int. %): 328 (11), 310 (19), 287 (100), 259 (35), 239 (19), 171 (15), 141 (40). ^1H -NMR (300 MHz, DMSO-*d*₆) δ : 0.93 (3H, s, H-18), 0.97 (3H, s, H-19), 1.13 (3H, s, H-21),

2.00 (3H, s, H-28), 3.13 (1H, brs, H-6), 4.03 (1H, dd, $J_{22\alpha,23\alpha}=12.7$ Hz, $J_{22\alpha,23\beta}=3.4$ Hz, H-22), 4.10, 4.18 (2H, AB d, $J_{27a,27b}=11.5$ Hz, H-27). ^{13}C -NMR (75 MHz, DMSO-*d*₆) δ : 14.3 (C-18), 14.7 (C-19), 16.9 (C-21), 19.8 (C-28), 20.3 (C-11), 20.7 (C-15), 30.2 (C-23), 30.9 (C-8), 31.4 (C-3), 31.5 (C-16), 31.6 (C-9), 33.1 (C-7), 39.2 (C-2), 39.8 (C-12), 47.2 (C-13), 48.7 (C-17), 51.7 (C-10), 54.5 (C-27), 71.1 (C-6), 74.1 (C-5), 74.2 (C-20), 81.2 (C-22), 83.0 (C-14), 125.5 (C-25), 154.1 (C-24), 165.1 (C-26), 217.4 (C-1).

Acetylation of Coagulin M (1) A solution of **1** (15 mg) in pyridine (1 ml) was treated with Ac₂O (1 ml) and left overnight at room temperature. The reagents were removed *in vacuo* and the residue was purified on a preparative TLC plate (silica gel) and characterized as a diacetate derivative (**1a**). $[\alpha]_D^{+78}$ ($c=0.40$, CHCl₃). IR (CHCl₃) cm^{-1} : 3420, 1735, 1718, 1700. UV λ_{max} (MeOH) nm (log ϵ): 216 (3.95). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 573.3045 (Calcd for C₃₂H₄₅O₉: 573.3051). EI-MS m/z (rel. int. %) 572 (2), 512 (5), 494 (10), 436 (3), 311 (30), 283 (55), 184 (65), 124 (100). ^1H -NMR (500 MHz, CDCl₃) δ : 1.05 (3H, s, H-18), 1.08 (3H, s, H-19), 1.25 (3H, s, H-21), 2.03 (3H, s, H-28), 2.05 (3H, s, COCH₃), 2.06 (3H, s, COCH₃), 4.22 (1H, dd, $J_{22\alpha,23\alpha}=13.0$ Hz, $J_{22\alpha,23\beta}=3.0$ Hz, H-22), 4.60 (1H, brs, H-6), 4.83, 4.87 (2H, AB d, $J_{27a,27b}=12.0$ Hz, H-27).

Coagulin N (2): Amorphous solid, 35 mg, yield $1.4 \times 10^{-4}\%$, $R_f=0.35$, $[\alpha]_D^{+106}$ ($c=0.46$, MeOH). IR (KBr) cm^{-1} : 3480, 1716, 1702 cm^{-1} . UV λ_{max} (MeOH) nm (log ϵ): 216 (4.02). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 649.3228 (Calcd for C₃₄H₄₉O₁₂: 649.321). EI-MS m/z (rel. int. %) 450 (7), 325 (14), 307 (16), 211 (21), 169 (55), 152 (65), 125 (100). ^1H -NMR (500 MHz, C₅D₅N) δ : 1.24 (3H, s, H-19), 1.71 (3H, s, H-27), 1.76 (3H, s, H-21), 1.86 (3H, s, H-18), 1.90 (3H, s, H-28), 2.24 (1H, d, $J=15.0$ Hz, H-16), 3.48 (1H, dd, $J=15.5$, 6.7 Hz, H-16), 3.95 (1H, t, $J=8.0$ Hz, H-2'), 3.88 (1H, m, H-5'), 4.17 (1H, m, H-4'), 4.19 (1H, m, H-3), 4.21 (1H, m, H-3'), 4.30 (1H, dd, $J=11.5$, 6.5 Hz, H-6'), 4.45 (1H, d, $J=6.6$ Hz, H-15), 4.47 (1H, m, H'-6'), 4.92 (1H, d, $J=7.7$ Hz, H-1'), 5.26 (1H, dd, $J_{22\alpha,23\alpha}=13.1$ Hz, $J_{22\alpha,23\beta}=3.0$ Hz, H-22), 5.60 (1H, d, $J=5.1$ Hz, H-6). ^{13}C -NMR (125 MHz, C₅D₅N) δ : 12.4 (C-27), 18.3 (C-19), 19.8 (C-18), 20.0 (C-21), 20.7 (C-28), 22.5 (C-11), 25.9 (C-12), 32.5 (C-23), 32.7 (C-8), 35.0 (C-7), 36.1 (C-9), 38.3 (C-4), 46.3 (C-2), 47.9 (C-16), 53.3 (C-13), 54.4 (C-10), 62.5 (C-6'), 71.4 (C-4'), 74.9 (C-2'), 75.3 (C-3), 76.1 (C-15), 78.2 (C-3'), 78.3 (C-5'), 79.2 (C-20), 81.7 (C-22), 82.8 (C-17), 89.1 (C-14), 102.7 (C-1'), 121.3 (C-25), 126.6 (C-6), 134.1 (C-5), 150.8 (C-24), 166.8 (C-26), 211.2 (C-1).

Acetylation of Coagulin N (2) Acetylation was carried out (as described for **1**) to obtain the pentaacetate derivative (**2a**). $[\alpha]_D^{+96}$ ($c=0.40$, CHCl₃). IR (CHCl₃) cm^{-1} : 3450, 1738, 1717, 1705, 1240, 1225. UV λ_{max} (MeOH) nm (log ϵ): 214 (4.19). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 859.3721 (Calcd for C₄₄H₅₉O₁₇: 859.3735). EI-MS m/z (rel. int. %) 528 (0.5), 468 (6), 450 (5), 432 (2), 309 (11), 200 (23), 169 (37), 152 (33), 125 (50), 115 (100). ^1H -NMR (500 MHz, CDCl₃) δ : 1.28 (3H, s, H-19), 1.29 (3H, s, H-27), 1.38 (3H, s, H-21), 1.85 (3H, s, H-18), 1.91 (3H, s, H-28), 1.97, 1.99 (3H, s, COCH₃), 2.02 (6H, s, 2 \times COCH₃), 2.06 (3H, s, COCH₃), 2.74 (1H, d, $J=7.5$ Hz, H-16 α), 3.15 (1H, dd, $J=16.5$, 6.5 Hz, H-16 β), 3.67 (1H, ddd, $J=9.5$, 5.0, 2.6 Hz, H-5'), 3.74 (1H, m, H-3), 4.06 (1H, dd, $J=12.5$, 2.5 Hz, H-6'), 4.22 (1H, dd, $J=12.0$, 5.0 Hz, H'-6'), 4.57 (1H, d, $J=8.0$ Hz, H-1'), 4.80 (1H, d, $J=7.0$ Hz, H-15), 4.87 (1H, dd, $J_{22\alpha,23\alpha}=12.5$ Hz, $J_{22\alpha,23\beta}=3.0$ Hz, H-22), 4.92 (1H, dd, $J=9.5$, 8 Hz, H-2'), 5.01 (1H, t, $J=10.0$ Hz, H-4'), 5.16 (1H, t, $J=9.5$ Hz, H-3'), 5.60 (1H, d, $J=5.0$ Hz, H-6).

Acid Hydrolysis of Coagulin N (2) Compound **2** (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 H₂O, then extracted with EtOAc and the aqueous phase was concentrated and glucose was identified by PC [Schleicher & Schuell 2043b, *n*-BuOH-HOAc-H₂O (4:1:5) and H₂O-saturated C₆H₅OH, detection with aniline-phthalic acid]. It was further confirmed by comparing the retention time of its TMS ether with a standard sample by GC.¹⁷⁾

Coagulin O (3): Amorphous solid, 30 mg, yield $1.2 \times 10^{-4}\%$, $R_f=0.40$, $[\alpha]_D^{+35}$ ($c=0.31$, MeOH). IR (KBr) cm^{-1} : 3470, 1715, 1700. UV λ_{max} (MeOH) nm (log ϵ): 220 (3.96). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 635.341 (Calcd for C₃₄H₅₁O₁₁: 635.3417). EI-MS m/z (rel. int. %) 472 (2), 454 (5), 436 (61), 418 (37), 400 (7), 311 (97), 267 (100), 169 (97), 152 (96), 125 (92). ^1H -NMR (400 MHz, C₅D₅N) δ : 1.17 (3H, s, H-19), 1.43 (3H, s, H-18), 1.52 (3H, s, H-21), 1.77 (3H, s, H-27), 1.82 (1H, m, H-17), 1.86 (3H, s, H-28), 1.92 (1H, m, H-8), 1.96 (1H, m, H-9), 3.85 (1H, m, H-5'), 3.92 (1H, t, $J=8.3$ Hz, H-2'), 4.12 (1H, m, H-4'), 4.16 (1H, m, H-3'), 4.19 (1H, m, H-3), 4.28 (1H, m, H-6'), 4.41 (1H, dd, $J=11.6$, 2.0 Hz, H'-6'), 4.50 (1H, dd, $J_{22\alpha,23\alpha}=13.0$ Hz, $J_{22\alpha,23\beta}=3.4$ Hz, H-22), 4.91 (1H, d, $J=7.7$ Hz, H-1'), 5.57 (1H, d, $J=5.0$ Hz, H-6). ^{13}C -NMR (100 MHz, C₅D₅N) δ : 12.5 (C-27),

18.1 (C-18), 19.0 (C-19), 19.9 (C-28), 21.5 (C-21), 21.9 (C-11), 22.3 (C-15), 27.2 (C-7), 31.9 (C-12), 32.5 (C-23), 36.2 (C-9), 38.2 (C-4), 38.4 (C-8), 41.2 (C-16), 46.5 (C-2), 49.4 (C-13), 53.0 (C-10), 56.1 (C-17), 62.4 (C-6'), 71.3 (C-4'), 74.8 (C-2'), 75.3 (C-3), 78.1 (C-5'), 78.2 (C-3'), 78.3 (C-20), 82.0 (C-22), 83.8 (C-14), 102.6 (C-1'), 121.4 (C-25), 126.6 (C-6), 134.2 (C-5), 149.8 (C-24), 166.3 (C-26), 210.5 (C-1).

Acetylation of Coagulin O (3) Acetylation was carried out (as described for **1**) to obtain the tetraacetate derivative (**3a**). $[\alpha]_D^{+82^\circ}$ ($c=0.40$, CHCl_3). IR (CHCl_3) cm^{-1} : 3450, 1733, 1715, 1705, 1240, 1225 cm^{-1} . UV λ_{max} (MeOH) nm (log ϵ): 217 (4.18). HR-FAB-MS $[\text{M}+\text{H}]^+$ m/z 803.3845, (Calcd for $\text{C}_{42}\text{H}_{59}\text{O}_{15}$: 803.3837). EI-MS m/z (rel. int. %): 766 (3), 436 (12), 418 (16), 331 (16), 311 (32), 268 (29), 169 (100), 152 (15), 125 (81). ^1H -NMR (300 MHz, CDCl_3) δ : 1.25 (6H, s, H-18, H-19), 1.39 (3H, s, H-21), 1.85 (3H, s, H-27), 1.92 (3H, s, H-28), 1.96, 1.98, 2.02, 2.06 (3H, s, $4\times \text{COCH}_3$), 3.62 (1H, m, H-5'), 3.64 (1H, m, H-3'), 4.06 (1H, dd, $J=12.2$, 2.4 Hz, H-6'), 4.20 (1H, dd, $J=12.2$, 5.2 Hz, H'-6'), 4.34 (1H, dd, $J_{22\alpha,23\alpha}=13.2$ Hz, $J_{22\alpha,23\beta}=3.4$ Hz, H-22), 4.54 (1H, d, $J=7.9$ Hz, H-1'), 4.91 (1H, dd, $J=9.5$, 7.9 Hz, H-2'), 5.00 (1H, t, $J=9.6$ Hz, H-4'), 5.15 (1H, t, $J=9.5$ Hz, H-3'), 5.67 (1H, d, $J=5.5$ Hz, H-6).

Acid Hydrolysis of Coagulin O (3) Compound **3** (10 mg) was refluxed for 4 h with 1 M methanolic HCl (3 ml). Analogous work-up (as described for **2**) yielded glucose.

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