FIVE NEW WITHANOLIDES FROM *WITHANIA COAGULANS*

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Abstract — Phytochemical studies on the whole plant of *Withania coagulans* have resulted in the isolation of five new withanolides, namely coagulins H, I, J, K and L (**1-5**). Their structures have been established as (17*S*,20*S*,22*R*)-5α,6β,14α,15α,17,20-hexahydroxy-1-oxowitha-2,24-dienolide (**1**), (14*R*,17*S*,20*S*,22*R*)-5α,6β,17-trihydroxy-14,20-epoxy-1-oxowitha-2,24-dienolide (**2**), (14*R*,17*R*,20*R*,22*R*)-3β,27-dihydroxy-14,20-epoxy-1-oxowitha-5,24-dienolide (**3**), (14*R*,17*R*,20*R*,22*R*)-14,20-epoxy-3β-(*O*-β-D-glucopyranosyl)-1-oxowitha-5,24-dienolide (**4**) and (14*R*,17*S*,20*S*,22*R*)-14,17,20-trihydroxy-3β-(*O*-β-D-glucopyranosyl)-1-oxowitha-5,24-dienolide (**5**), respectively on the basis of spectroscopic techniques.

The withanolides are a group of ergostanolides, chemically characterized by a lactone-containing side chain which is made up of nine carbons, and includes either a γ- or a δ-lactone at C-17 and different oxygen substituents, mainly in the A, B and E rings.[1] They are not restricted to Solanaceaeous plants[2] only, and reports of their isolation from marine organisms (soft coral)[3] and from members of Taccaceae[4,5] and Leguminosae[6] suggested that they are much more widely distributed. Withanolides are interesting because many of them show antitumor, antibacterial, antifungal, antiinflammatory, cytotoxic, hepatoprotective and immunosupressive activities.[7] *Withania coagulans* Dunal., which grows widely in India and Pakistan, is used in the indigenous system of medicine[8] and fruits of the plant have milk coagulating properties.[9] We have previously reported a number of C_{28} steroidal lactones from the ethanolic

extract of *W.* coagulans.[10-11] In continuing our studies on the constituents of this plant, we report here the isol ation and structure elucidation of five new withanolides which we have named as coagulins H, I, J, K and L (**1-5**).

Coagulin H (**1**), $C_{28}H_{40}O_9$, isolated as an amorphous powder, showed the pseudo-molecular ion peak at *m/z* 521 $[M+H]^+$ in the positive fast atom bombardment mass spectrum (FABMS). The IR spectrum of **1** indicated the presence of hydroxyl (3480 cm^{-1}), α,β-unsaturated ketone (1690 cm^{-1}) and α,β-unsaturated δ-lactone groupings (1718 cm^{-1}).[12] The UV spectrum showed an absorption maximum at 224 nm characteristic of conjugated enone and conjugated δ-lactone chromophores commonly present in withanolides.[13] In the MS spectrum of **1**, the fragment ions at *m/z* 169.0845 ($C_9H_{13}O_3$) and 152.0835 ($C_9H_{12}O_2$) (base peak), which could arise by cleavage of the C-17/C-20 bond, suggested the presence of a 20-hydroxywithanolide.[14] The peak at *m/z* 125.0603 ($C_7H_9O_2$) formed by fission across the C-20/C-22 bond indicated that **1** contains an unsubstituted α,β-unsaturated δ-lactone moiety.[7]

The ^{1}H-NMR spectrum of **1** showed three singlets at δ 1.20, 1.17 and 1.24 which were assigned to the C-18, C-19 and C-21 methyl groups, respectively. The chemical shift of the deshielded C-21 methyl singlet and the appearance of the C-22 methine proton signal as a double doublet at δ 4.67 (J = 12.5 and 3.5 Hz) also supported the presence of an OH group at C-20. The two vinylic methyl singlets at δ 1.74 and 1.87 were assigned to the methyl groups attached to the conjugated lactone group in the side chain. The α- and β-olefinic hydrogens of the conjugated enone in ring A appeared at δ 5.62 (dd, J = 10.0 and 2.6 Hz) and 6.55 (ddd, J = 10.0, 5.2 and 2.6 Hz), respectively. The multiplicities of the allylic methylene signals at δ 3.08 (dd, J = 19.0 and 5.2 Hz) and 1.90 (dt, J = 19.0 and 2.6 Hz) indicated that C-4 is unsubstituted and that the vicinal C-5 bears no hydrogen atom. Thus, the hydroxyl group can be placed at C-5. The stereochemistry at various asymmetric centers was assigned on the basis of chemical shift comparisons with related withanolides.[14-17] The 6-H and 15-H geminal to OH groups were observed at δ 3.40 (br s) and 3.70 (d, J = 6.5 Hz), which shifted downfield to δ 4.75 and 4.90, respectively, in its diacetate derivative (**1a**). The α-orientation of the C-5 hydroxyl group was inferred from the ^{13}C-NMR chemical shift of C-19 (δ 15.0) which was similar to that found in withanolide S[15] and withaminimin[16] but different to that found in withaperuvin[17] which had a β-C-5 hydroxyl group and in which C-19 consequently resonated at δ 9.7. The ^{1}H-NMR spectrum of **1** was recorded in pyridine-d_5 since pyridine is known to influence the chemical shifts of neighboring protons of the OH substituent.[18] The shift differences observed are useful in locating positions of the hydroxyl and neighboring groups. The configuration of the OH-17-(β) group could be deduced by the chemical shifts of the Me-18, Me-21 and H-22 and by the pyridine-induced chemical shifts values. It has been observed that the OH-17β strongly deshields the signal of the Me-21 and Me-18 whereas an OH-17α induces a sizable downfield shift only in the Me-21 signal.[14] The chemical shift values

of the Me-18 at δ 1.55 and Me-21 at δ 1.72 signals of **1** were in agreement with a OH-17β group. The chemical shift differences (δ in DMSO-d_6 – δ in C_5D_5N) for Me-18 (– 0.35 ppm) and Me-21 (– 0.48 ppm) signals suggested the β-orientation of the OH-17 group in **1**. The Me-19 protons resonated at δ 1.63 and the chemical shift difference for C-19 protons (– 0.46 ppm) supported the β-stereochemistry for the OH group at C-6. Similarly 14α-OH group is known to shield C-9 and C-12 through a γ effect as compared to 14β-OH substituted withanolides.[19] The C-9 and C-12 carbons resonated at δ 33.1 and 34.3 respectively in **1**, which supported the α-orientation of the OH group at C-14. The orientation of the secondary OH group at C-15 was assumed to be α based on the fact that the coupling pattern (δ 3.70, d, J = 6.5 Hz) for 15-H in the ^{1}H-NMR spectrum of **1** was similar to the pattern observed in another withanolide.[19] Furthermore the 15α-acetate group of the diacetate of **1** did not show any interaction with 18-H_3 in the NOE difference spectrum which further supported the α-orientation of the 15-OH. The configuration at C-22 was assumed to be R on biogenetic grounds as found in all related withanolides.[1] Furthermore, the ^{1}H-NMR spectrum of **1** displayed four signals at δ 6.60 (s), 5.53 (s), 4.53 (d, J = 3.3 Hz) and 4.05 (s) which collapsed on shaking the DMSO-d_6 solution with deuterium oxide, and were therefore assigned to the hydroxyl groups.[20]

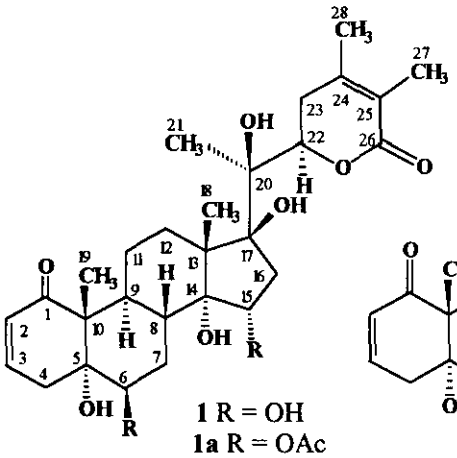
The COSY 45° spectrum of **1** indicated the presence of four important spin systems in the molecule. H-2 (δ 5.62) and H-3 (δ 6.55) showed COSY 45° connectivities with each other and with H-4α (δ 1.90) and β (δ 3.08). The H-6 methine (δ 3.40) showed cross-peaks with methylenic H_2-7 (δ 1.95, 1.40). H-15 (δ 3.70) exhibited strong-cross peaks with methylenic H_2-16 (δ 2.70, 1.53) while H-22 (δ 4.67) showed cross-peaks with methylenic H_2-23 (δ 2.40, 2.05).

The ^{1}H-^{1}H long-range connectivities were determined by recording a series of HOHAHA spectra with variable delays (100, 60, 20 msec). H-2 (δ 5.62) showed interactions with allylic methylene H-4α (δ 1.90) and β (δ 3.08). H-6 showed long-range couplings with H-8 (δ 2.40) and H-9 (δ 2.32) methines, and H_2-11 methylene (δ 2.20).

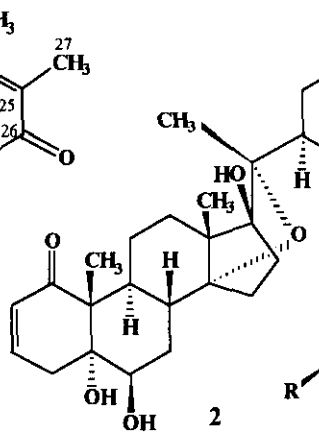
The ^{13}C-NMR spectra of **1** indicated that there were five methyl, six methylene, seven methine, and ten quaternary carbons in the molecule.[21] Lowfield signals at δ 204.3 and 166.0 were assigned to α,β-unsaturated ketonic and lactonic carbonyl carbons, respectively. The remaining four downfield carbon resonances at δ 127.7 (CH) , 142.1 (CH), 150.8 (C) and 120.1 (C) were assigned to the olefinic C-2, C-3, C-24 and C-25, respectively. Methyl signals at δ 19.4, 15.0, 20.2, 12.1 and 20.5 were ascribed to C-18, C-19, C-21, C-27 and C-28, respectively. The hydroxyl-bearing carbons C-5, C-6, C-14, C-15, C-17 and C-20 resonated at δ 78.0 (C), 73.6 (CH), 83.0 (C), 74.5 (CH), 87.9 (C) and 76.2 (C), respectively. The ^{1}H- and ^{13}C-NMR spectra of **1** were consistent with a withanolide skeleton, in which 1-keto-Δ^2 and 5α,6β-dihydroxy functionalities were clearly present, along with an α,β-unsaturated δ-lactone in the side chain.[22]

The one-bond $^{1}H/^{13}C$ correlations in **1** were determined by HMQC experiment.[23] The C-2 methine (δ 127.7) showed a cross-peak with H-2 at δ 5.62. Similarly the vinylic C-3 (δ 142.1) correlated with the proton resonating at δ 6.55 (H-3). The C-4 methylene resonating at δ 35.3 was coupled with the H_2-4 (δ 3.08, 1.90). The downfield methine C-6 (δ 73.6) and C-15 (δ 74.5) showed one-bond heteronuclear interactions with H-6 (δ 3.40) and H-15 (δ 3.70), respectively. The C-22 methine (δ 81.3) was found to be connected with the proton that resonated at δ 4.67 (H-22). The C-23 methylene carbon at δ 32.4 displayed cross-peaks with protons at δ 2.40 (H_2-23).

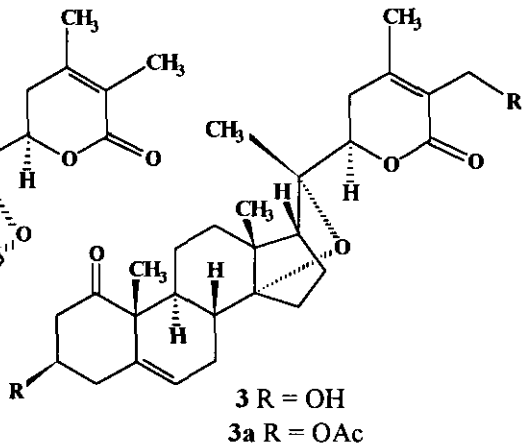
The long-range $^{13}C/^{1}H$ correlations were determined by an HMBC experiment[24] and used to connect different structural fragments as well as to confirm the above chemical shift assignments. For instance, starting from the ketonic carbonyl at δ 204.3 three-bond (^{3}J) correlations could be observed with H-3 and H_3-19. The oxygenated quaternary carbon C-5 resonating at δ 78.0 was connected by ^{2}J to H_2-4 and H-6 and by ^{3}J to H-3. H-15 showed ^{2}J coupling to C-14 which, in turn, showed ^{3}J couplings to H_3-18. Likewise, H_3-21 showed ^{2}J and ^{3}J couplings to C-20 and C-17, respectively. The spectroscopic evidence thus led to structure **1** for this new withanolide.



1 R = OH
1a R = OAc



2



3 R = OH
3a R = OAc

The IR, UV, ^{1}H-NMR and ^{13}C-NMR data of coagulin I (**2**) showed that it had some resemblances with **1** in substitution pattern of the rings A, B and C. The presence of an ether linkage between C-14 and C-20 was inferred from the MS and ^{13}C-NMR spectroscopic studies. The molecular formula $C_{28}H_{38}O_7$, was obtained from the high resolution FABMS measurement, indicating ten degrees of unsaturation. Four of these were accounted for by the tetracyclic steroidal skeleton, two by double bonds, two by the δ-lactone and one by the C-1 ketonic carbonyl. The remaining unsaturation site can therefore only be accommodated by the existence of a cyclic ether.[25] The molecular ion was not present in the LREIMS and the base peak at *m/z* 125.0601 ($C_7H_9O_2$) was due to a δ-lactone ring without a hydroxyl group (C-27-OH).[7] The ion

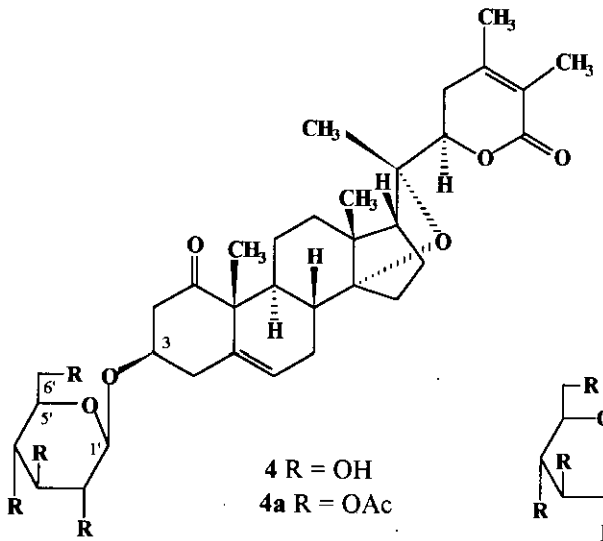
fragment at *m/z* 152.0835 of composition $C_9H_{12}O_3$ may arise by the cleavage of the C-17/C-20 bond indicating the presence of an oxygen function at C-20. The ions at *m/z* 125.0601 ($C_7H_9O_2$) and 152.0835 ($C_9H_{12}O_2$) indicated the presence of only two oxygen functionalities in the lactone side chain while five oxygens were present in the cyclopentanophenanthrene part (rings A, B, C and D). Excluding the ketonic oxygen of the ring A, oxygens of a secondary and two tertiary hydroxyl groups and two lactonic oxygens, only one oxygen remained to be incorporated in the skeleton, whereas the ^{13}C-NMR spectra exhibited signals for two oxygen-bearing quaternary carbons (δ 82.5 and 78.0) in the ring D and in the C-17 side chain. These results indicated the presence of an ether linkage between C-14/C-20.[10] The stereochemistry of the OH group at C-17 was inferred to be β (*S*) since an α-oriented ether bridge exists between C-14/C-20. Based on these spectral observations structure (**2**) was deduced for coagulin I which was obtained earlier by partial derivatization of withanolide E by I. Kirson *et al.* [25] but has not been isolated from natural sources.

The IR, UV, ^{1}H-NMR and ^{13}C-NMR data of coagulin J, $C_{28}H_{38}O_6$ (**3**) revealed the presence of 1-keto-3-hydroxyl substitution in ring A. The mass spectrum of **3** showed a fragment ion at *m/z* 141.0654 ($C_7H_9O_3$) indicated the presence of the primary 27-hydroxyl group in ring E. Further evidence for the presence of 27-hydroxyl was deduced by the analysis of the ^{1}H-NMR and ^{13}C-NMR spectra of **3** and its diacetate (**3a**). The chemical shift and splitting pattern of the signal of C-3 proton (δ 3.58, 1H, seven lines) indicated 3-β-OH function.[26] The stereochemistry at C-17 was inferred to be *R* with α-oriented C-17 side chain based on the the fact that the alternative arrangement *S* was not possible when α-oriented ether bridge exist between C14/C20.[25] The structure of **3** was assigned by correlation of its ^{1}H- and ^{13}C-NMR data with analogous withanolide.[26]

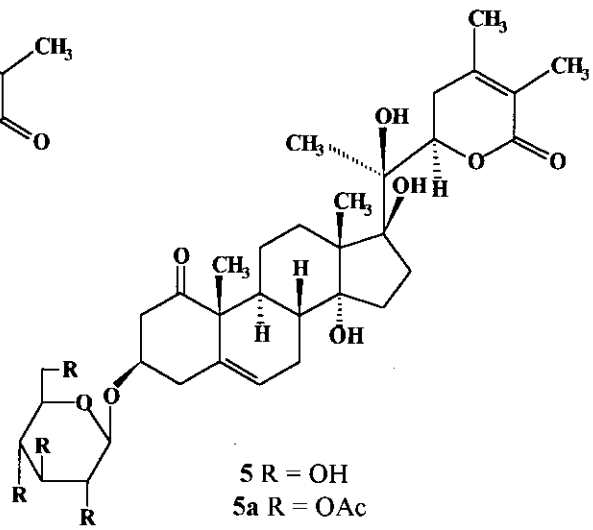
The IR, UV, ^{1}H-NMR and ^{13}C-NMR data of coagulin K, $C_{34}H_{48}O_{10}$; *m/z* 616.3644 (**4**), indicated similarities in substitution pattern of the rings A, B, C and D with **3**, the difference being the presence of a glucose moiety at C-3 and a methyl group at C-27. In the mass spectrum of **4** the peak at *m/z* 125.0602 ($C_7H_9O_2$) can result by the cleavage of the C-20/C-22 bond and indicated the presence of a six-membered δ-lactone ring.[7] The ^{1}H-NMR spectrum of its tetraacetate (**4a**) revealed the existence of a 2,3,4,6-tetra-*O*-acetyl-*O*-D-glucopyranosyl moiety [δ 4.55 (d, J = 7.9 Hz, H-1′), δ 4.93 (dd, J = 9.6, 7.9 Hz, H-2′), δ 5.17 (t, J = 9.6 Hz, H-3′), δ 5.02 (t, J = 9.6 Hz, H-4′), δ 3.66 (ddd, J = 9.6, 5.0, 2.6 Hz, H-5′), δ 4.08 (dd, J = 12.2, 2.6 Hz, H-6′) and δ 4.22 (dd, J = 12.2, 5.0 Hz, H′-6′)]. Identification of the sugar unit and the location of its attachment were made by the combined use of HOHAHA and HMBC techniques.[24] Starting from the anomeric proton signal at δ 4.55 in the HOHAHA spectrum, a spin system of tetraacetylglucose unit was traced. The coupling constant $J_{1,2}$ = 7.9 Hz of the anomeric proton showed the tetraacetylglucose moiety to have the β-D-conformation. The anomeric proton (δ 4.55) showed long-

range correlations (^3J) with C-3 (δ 77.1) of the aglycone while H-3 showed long-range couplings (^3J) with the anomeric carbon (δ 100.2) in the HMBC spectrum.

Coagulin L, $C_{34}H_{50}O_{12}$; *m/z* 650.3288 (**5**) was obtained as an amorphous solid. The molecular formula was deduced from the positive HRFABMS and implied one fewer degree of unsaturation than **4**. The ^{1}H-NMR spectrum of **5** closely resembled to that of **4**, however, the broad-band decoupled ^{13}C-NMR spectrum of **5** showed downfield signals at δ 81.8 (C), 87.9 (C) and 78.9 (C), and were assigned to the hydroxyl bearing C-14, C-17 and C-20 carbons respectively. The stereochemistry of these OH groups was assigned on the basis of chemical shift comparison with related withanolide.[27] The spectroscopic data led to structure (**5**) for coagulin L, the aglycone of which was reported earlier by V. V. Velde *et al.* from *Withania coagulans*.[27]



4 R = OH
4a R = OAc



5 R = OH
5a R = OAc

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 HREIMS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H- 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.[24]

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.

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 (60 L) at rt 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 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 32% NH_4OH and AcOH solutions. The fraction (pH 9-10) (100 g) was subjected to column chromatography on silica gel. Elution with $CHCl_3$ and then with $CHCl_3$-MeOH yielded several fractions. A fraction (0.95 g) obtained on elution with $CHCl_3$-MeOH (95 : 5) was found to contain five compounds (**1-5**). These were purified by TLC (silica gel) using $CHCl_3$-MeOH (90 : 10) as the solvent system.

Coagulin H (1): Amorphous powder; 20 mg, yield 8×10^{-5} %; $R_f = 0.48$; $[\alpha]_D$ +98° (c = 0.45, MeOH); IR ν_{max} (KBr) 3480, 1718, 1690 cm^{-1}; UV λ_{max} (MeOH) 224 (log ε 4.25); HRFABMS $[M+H]^+$ *m/z* 521.2730 (Calcd for $C_{28}H_{41}O_9$: 521.2739); LREIMS *m/z* (rel. int. %) 466 (3), 359 (2), 332 (4), 278 (12), 169 (20), 152 (100), 125 (69). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.60 (1H, s, O*H*), 6.55 (1H, ddd, J = 10.0, 5.2, 2.6 Hz, H-3), 5.62 (1H, dd, J = 10.0, 2.6 Hz, H-2), 5.53 (1H, s, O*H*), 4.67 (1H, dd, J = 12.5, 3.5 Hz, H-22), 4.53 (1H, d, J = 3.3 Hz, O*H*), 4.05 (1H, s, O*H*), 3.70 (1H, d, J = 6.5, H-15), 3.40 (1H, br s, H-6), 3.08 (1H, dd, J = 19.0, 5.2 Hz, H-4β), 1.90 (1H, dt, J = 19.0, 2.6 Hz, H-4α), 1.87 (3H, s, H-28), 1.74 (3H, s, H-27), 1.24 (3H, s, H-21), 1.20 (3H, s, H-18), 1.17 (3H, s, H-19). ^{1}H-NMR (C_5D_5N, 400 MHz) δ: 6.65 (1H, ddd, J = 10.3, 5.4, 2.6 Hz, H-3), 6.12 (1H, dd, J = 10.3, 2.6 Hz, H-2), 5.20 (1H, dd, J = 12.6, 3.7 Hz, H-22), 4.40 (1H, d, J = 7.0, H-15), 4.15 (1H, br s, H-6), 1.88 (3H, s, H-28), 1.87 (3H, s, H-27), 1.72 (3H, s, H-21), 1.63 (3H, s, H-19), 1.55 (3H, s, H-18). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 204.3 (C-1), 166.0 (C-26), 150.8 (C-24), 142.1 (C-3), 127.7 (C-2), 120.1 (C-25), 87.9 (C-17), 83.0 (C-14), 81.3 (C-22), 78.0 (C-5), 76.2 (C-20), 74.5 (C-15), 73.6 (C-6), 53.5 (C-13), 51.4 (C-10), 46.1 (C-16), 35.3 (C-4), 34.3 (C-12), 33.1 (C-9), 32.4 (C-23), 29.8 (C-8), 27.9 (C-7), 22.1 (C-11), 20.5 (C-28), 20.2 (C-21), 19.4 (C-18), 15.0 (C-19), 12.1 (C-27).

Acetylation of coagulin H (1): A solution of **1** (10 mg) in pyridine (1 mL) was treated with Ac_2O (1 mL) and left overnight at rt. The reagents were removed *in vacuo* and the residue was purified on a preparative chromatoplate using $CHCl_3$ as a solvent and characterized as compound (**1a**). 7.5 mg; $[\alpha]_D$ +78° (c = 0.46,

$CHCl_3$); IR ν_{max} ($CHCl_3$) 1720, 1710, 1690, 1240, 1225 cm^{-1}; UV λ_{max} (MeOH) 220 (log ε 4.23); HRFABMS $[M+H]^+$ *m/z* 605.2765 (Calcd for $C_{32}H_{45}O_{11}$: 605.275); LREIMS *m/z* (rel. int. %) 526 (8), 448 (11), 402 (9), 358 (12), 170 (55), 152 (99), 125 (100). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 6.54 (1H, ddd, J = 10.4, 5.4, 2.5 Hz, H-3), 5.87 (1H, dd, J = 10.4, 2.5 Hz, H-2), 4.90 (1H, d, J = 5.6 Hz, H-15), 4.77 (1H, dd, J = 12.4, 3.4 Hz, H-22), 4.75 (1H, s, H-6), 2.08, 1.99 (2 × 3H, s, $COCH_3$), 1.92 (3H, s, H-28), 1.87 (3H, s, H-27), 1.40 (3H, s, H-21), 1.33 (3H, s, H-18), 1.24 (3H, s, H-19).

Coagulin I (2): Amorphous solid; 15 mg, yield 6 × 10^{-5} %; R_f = 0.56; $[\alpha]_D$ +14° (c = 0.3, MeOH); IR ν_{max} ($CHCl_3$) 3460, 1715, 1692 cm^{-1}; UV λ_{max} (MeOH) 226 (log ε 4.24); HRFABMS $[M+H]^+$ *m/z* 487.2676 (Calcd for $C_{28}H_{39}O_7$: 487.2682); LREIMS *m/z* (rel. int. %) 317 (3) , 241 (7) , 171 (18), 152 (22), 125 (50), 60 (100). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.71 (1H, s, O*H*), 6.55 (1H, ddd, J = 10.2, 5.3, 2.5 Hz, H-3), 5.62 (1H, dd, J = 10.2, 2.5 Hz, H-2), 5.57 (1H, s, O*H*), 4.66 (1H, dd, J = 12.5, 3.6 Hz, H-22), 4.56 (1H, s, O*H*), 3.33 (1H, br s, H-6), 1.87 (3H, s, H-28), 1.74 (3H, s, H-27), 1.22 (3H, s, H-21), 1.14 (3H, s, H-19), 1.00 (3H, s, H-18). ^{1}H-NMR (C_5D_5N, 400 MHz) δ: 6.64 (1H, ddd, J = 10.2, 5.3, 2.6 Hz, H-3), 6.10 (1H, dd, J = 10.2, 2.6 Hz, H-2), 5.10 (1H, dd, J = 12.5, 3.4 Hz, H-22), 4.12 (1H, br s, H-6), 1.88 (3H, s, H-28), 1.86 (3H, s, H-27), 1.65 (3H, s, H-19), 1.54 (3H, s, H-21), 1.28 (3H, s, H-18). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 204.1 (C-1), 165.9 (C-26), 150.6 (C-24), 142.0 (C-3), 127.6 (C-2), 120.1 (C-25), 87.5 (C-17), 82.5 (C-14), 81.4 (C-22), 78.0 (C-20), 76.2 (C-5), 73.3 (C-6), 54.2 (C-13), 51.3 (C-10), 35.8 (C-4), 35.5 (C-9), 34.4 (C-16), 33.1 (C-8), 31.9 (C-23), 30.7 (C-15), 28.9 (C-12), 28.8 (C-7), 22.2 (C-11), 20.7 (C-21), 20.2 (C-28), 19.3 (C-18), 15.0 (C-19), 12.1 (C-27).

Coagulin J (3): Amorphous powder; 30 mg, yield 1.2 × 10^{-4} %; R_f = 0.65; $[\alpha]_D$ +45° (c = 0.35, MeOH); IR ν_{max} ($CHCl_3$) 3540, 1720, 1705 cm^{-1}; UV λ_{max} (MeOH) 214 (log ε 3.99); HRFABMS $[M+H]^+$ *m/z* 471.2730 (Calcd for $C_{28}H_{39}O_6$: 471.2736); LREIMS *m/z* (rel. int. %) 311 (11), 295 (48), 225 (52), 207 (66), 157 (100), 141 (28). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.56 (1H, br d, J = 5.1 Hz, H-6), 4.17, 4.10 (2H, AB d, J = 11.5 Hz, H-27), 4.02 (1H, dd, J = 12.5, 3.3 Hz, H-22), 3.58 (1H, m, H-3), 2.00 (3H, s, H-28), 1.16 (3H, s, H-19), 1.13 (3H, s, H-21), 0.90 (3H, s, H-18). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 211.0 (C-1), 165.2 (C-26), 154.1 (C-24), 135.5 (C-5), 125.5 (C-25), 124.8 (C-6), 82.5 (C-14), 81.2 (C-22), 74.7 (C-20), 67.7 (C-3), 54.5 (C-27), 51.9 (C-10), 48.4 (C-17), 47.9 (C-2), 47.0 (C-13), 40.9 (C-4), 35.1 (C-8), 33.7 (C-9), 31.9 (C-23), 31.7 (C-12), 31.5 (C-15), 25.1 (C-7), 20.9 (C-11), 20.8 (C-21), 20.2 (C-16), 19.9 (C-28), 18.3 (C-19), 16.9 (C-18).

Acetylation of coagulin J (3): Acetylation was carried out (as described for **1**) to obtain the diacetate (**3a**). $[\alpha]_D$ +115° (c = 0.46, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1725, 1718, 1695, 1230, 1220 cm^{-1}; UV λ_{max} (MeOH) 216 (log ε 4.17); HRFABMS $[M+H]^+$ *m/z* 555.2775 (Calcd for $C_{32}H_{43}O_8$: 555.2791); LREIMS *m/z* (rel. int. %) 494 (2), 434 (12), 311 (42), 267 (21), 171 (45), 155 (45), 124 (83), 60 (100). ^{1}H-NMR

($CDCl_3$, 400 MHz) δ: 5.67 (1H, s, H-6), 4.92 (1H, m, H-3), 4.89 and 4.84 (2H, AB d, J = 11.5 Hz, H-27), 4.24 (1H, dd, J = 13.2, 3.6 Hz, H-22), 2.07, 2.05 (2 × 3H, s, $COCH_3$), 2.02 (3H, s, H-28), 1.29 (3H, s, H-19), 1.28 (3H, s, H-21), 1.05 (3H, s, H-18).

Coagulin K (4): Amorphous powder; 25 mg, yield 1 × 10^{-4} %; R_f = 0.35; $[\alpha]_D$ +106° (c = 0.46, MeOH); IR ν_{max} (KBr) 3450, 1715, 1702 cm^{-1}; UV λ_{max} (MeOH) 220 (log ε 4.02); HRFABMS $[M+H]^+$ m/z 617.3718 (Calcd for $C_{34}H_{49}O_{10}$: 617.3722); LREIMS m/z (rel. int. %) 436 (16), 418 (6), 393 (25), 311 (73), 269 (21), 169 (87), 152 (56), 125 (96), 73 (100). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.72 (1H, d, J = 6.1 Hz, H-6), 4.40 (1H, dd, J = 11.5, 3.5 Hz, H-22), 4.38 (1H, d, J = 7.7 Hz, H-1′), 3.88 (1H, m, H-3), 1.98 (3H, s, H-28), 1.85 (3H, s, H-27), 1.40 (3H, s, H-21), 1.31 (3H, s, H-19), 1.28 (3H, s, H-18).

Acetylation of coagulin K (4): Acetylation was carried out (as described for **1**) to obtain the tetraacetate **4a**. $[\alpha]_D$ +96° (c = 0.40, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1720, 1715, 1705, 1240, 1225 cm^{-1}; UV λ_{max} (MeOH) 214 (log ε 4.19); HRFABMS $[M+H]^+$ m/z 785.3786 (Calcd for $C_{42}H_{57}O_{14}$: 785.3771); LREIMS m/z (rel. int. %) 436 (20), 331 (7), 311 (24), 268 (16), 169 (53), 125 (64), 115 (100). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 5.64 (1H, d, J = 4.8 Hz, H-6), 5.17 (1H, t, J = 9.6 Hz, H-3′), 5.02 (1H, t, J = 9.6 Hz, H-4′), 4.93 (1H, dd, J = 9.6, 7.9, H-2′), 4.55 (1H, d, J = 7.9 Hz, H-1′), 4.22 (1H, dd, J = 12.2, 5.0 Hz, H-6′), 4.18 (1H, dd, J = 13.5, 3.5, H-22), 4.08 (1H, dd, J = 12.2, 2.6 Hz, H′-6′), 3.70 (1H, m, 3-H), 3.66 (1H, ddd, J = 9.6, 5.0, 2.6 Hz, H-5′), 2.08, 2.04, 2.00, 1.98 (4 × 3H, s, $COCH_3$), 1.92 (3H, s, H-28), 1.86 (3H, s, H-27), 1.29 (3H, s, H-19), 1.24 (3H, s, H-21), 1.03 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 209.8 (C-1), 170.6, 170.2, 169.4, 169.2 (4 × $COCH_3$), 166.1 (C-26), 148.9 (C-24), 133.6 (C-5), 126.7 (C-6), 122.0 (C-25), 100.2 (C-1′), 84.7 (C-14), 81.3 (C-22), 77.1 (C-3), 75.2 (C-20), 72.7 (C-3′), 71.9 (C-5′), 71.4 (C-2′), 68.5 (C-4′), 62.0 (C-6′), 52.7 (C-10), 49.4 (C-17), 47.5 (C-13), 46.4 (C-2), 38.4 (C-4), 35.8 (C-8), 34.0 (C-9), 32.2 (C-12), 31.9 (C-15), 31.7 (C-23), 25.4 (C-7), 21.1 ($COCH_3$), 20.9 (C-11), 20.7, 20.6 (2 × $COCH_3$), 20.6 (C-16), 20.5 ($COCH_3$), 20.4 (C-21), 20.1 (C-28), 19.4 (C-19), 17.2 (C-18), 12.4 (C-27).

Acid hydrolysis of coagulin K (4): Compound (**4**) (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 aq. phase was concentrated and methyl glucoside was identified by PC [Schleicher & Schuell 2043b, n-BuOH-HOAc-H_2O (4 : 1 : 5) and H_2O-satd C_6H_5OH, detection with aniline-phthalic acid].

Coagulin L (5): Amorphous solid; 30 mg, yield 1.02 × 10^{-5} %; R_f = 0.40; $[\alpha]_D$ +30° (c = 0.3, MeOH); IR ν_{max} (KBr) 3470, 1715, 1700 cm^{-1}; UV λ_{max} (MeOH) 224 (log ε 4.05); HRFABMS $[M+H]^+$ m/z 651.3280 (Calcd for $C_{34}H_{51}O_{12}$: 651.3366); LREIMS m/z (rel. int. %) 452 (5), 434 (6), 416 (2), 327 (9), 309 (10), 283 (20), 169 (32), 125 (100). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.68 (1H, d, J = 4.4 Hz,

H-6), 4.65 (1H, dd, J = 12.5, 3.4 Hz, H-22), 4.39 (1H, d, J = 7.8 Hz, H-1′), 3.78 (1H, m, H-3), 1.87 (3H, s, H-28), 1.73 (3H, s, H-27), 1.24 (3H, s, H-21), 1.19 (3H, s, H-19), 0.98 (3H, s, H-18).

Acetylation of coagulin L (5): Acetylation was carried out (as described for **1**) to obtain the tetraacetate **5a**. $[\alpha]_D$ +36° (c = 0.35, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 1735, 1715, 1698 cm^{-1}; UV λ_{max} (MeOH) 226 (log ε 4.15); HRFABMS $[M+H]^+$ *m/z* 819.3345 (Calcd for $C_{42}H_{59}O_{16}$: 819.3364); LREIMS *m/z* (rel. int. %) 452 (4) , 434 (3) , 416 (2), 406 (2), 327 (9), 309 (10), 242 (15), 200 (23), 169 (18), 125 (30), 115 (100). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.56 (1H, d, J = 4.9 Hz, H-6), 5.08 (1H, t, J = 9.5 Hz, H-3′), 4.92 (1H, t, J = 9.5 Hz, H-4′), 4.83 (1H, dd, J = 9.5, 7.9, H-2′), 4.78 (1H, dd, J = 11.4, 4.9, H-22), 4.49 (1H, d, J = 7.9 Hz, H-1′), 4.14 (1H, dd, J = 12.2, 5.1 Hz, H-6′), 3.99 (1H, dd, J = 12.2, 2.5 Hz, H′-6′), 3.70 (1H, m, 3-H), 3.61 (1H, ddd, J = 9.5, 5.1, 2.5 Hz, H-5′), 1.99, 1.95, 1.92, 1.90 (4 × 3H, s, $COCH_3$), 1.86 (3H, s, H-28), 1.76 (3H, s, H-27), 1.30 (3H, s, H-21), 1.16 (3H, s, H-19), 1.00 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 210.3 (C-1), 170.7, 170.2, 169.4, 169.2 (4 × $COCH_3$), 166.3 (C-26), 150.8 (C-24), 133.9 (C-5), 126.8 (C-6), 121.3 (C-25), 100.0 (C-1′), 87.9 (C-17), 81.8 (C-14), 80.3 (C-22), 78.9 (C-20), 77.0 (C-3), 72.8 (C-3′), 71.8 (C-5′), 71.4 (C-2′), 68.5 (C-4′), 62.0 (C-6′), 54.6 (C-10), 52.7 (C-13), 46.0 (C-2), 38.2 (C-4), 37.5 (C-16), 36.1 (C-8), 35.5 (C-9), 34.2 (C-23), 32.3 (C-12), 30.0 (C-15), 25.8 (C-7), 21.9 (C-11), 20.7, 20.6, 20.5, 20.4 (4 × $COCH_3$), 20.3 (C-28), 20.2 (C-18), 19.5 (C-21), 18.7 (C-19), 12.2 (C-27).

Acid hydrolysis of coagulin L (5): Compound **5** (5 mg) was refluxed for 4 h with 1 M methanolic HCl (3 mL). Analogous work-up (as described for **4**) yielded methyl glucoside.

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