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Isolation of Novel Bioactive Steroids from the Soft Coral *Alcyonium gracillimum*

Youngwan Seo, Jee H. Jung, Jung-Rae Rho, and Jongheon Shin*

Marine Natural Products Chemistry Laboratory, Korea Ocean Research & Development Institute
Ansan P.O. Box 29, Ansan 425-600, Korea

Jun-Im Song

Department of Biology, College of Natural Sciences,
Ewha Womans University, Seoul 120-750, Korea

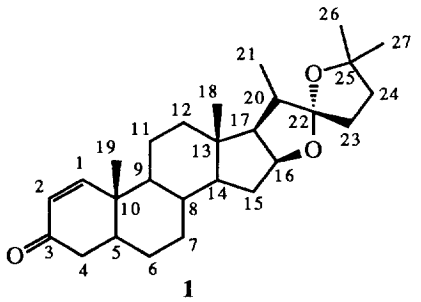
Abstract: Seven steroids including five new compounds (**1-5**) have been isolated from the soft coral *Alcyonium gracillimum*. Compound **1** is a spiroketal of the furospirostan class while compounds **2** and **3** are hemiketals of the corresponding furostan class. Compound **4** possesses an unusual cyclic enolether linkage between the C-18 and C-22 carbons of the cholestane skeleton. The structures of these compounds were determined by combined spectroscopic methods. The hemiketals **2** and **3** exhibited moderate cytotoxicity and antiviral activity.

Soft corals (order Alcyonacea) are recognized as very prolific sources of novel bioactive secondary metabolites.[1] In our search for bioactive substances from Korean Water marine organisms, we encountered the soft coral *Alcyonium gracillimum* whose organic crude extract exhibited moderate cytotoxicity and antiviral activity. In this paper we report the isolation and structure determination of seven steroids including five novel compounds.

Compound **1** is a steroid of the furospirostan class which possesses a spiroketal functionality between the ring D and side chain of the cholestane skeleton while **2** and **3** possess the corresponding hemiketal functionality. Compound **4** possesses an unusual dihydropyran ring formed by an oxidative linkage between the C-18 and C-22 carbons. In addition, compound **5** is a steroidal diketone. Finally, compounds **6** and **7** are known C_{21} steroids of the pregnane class.

A. gracillimum (91-18) specimens[2] were briefly air-dried, macerated and exhaustively extracted with dichloromethane and methanol. The combined extract was concentrated and fractionated by silica vacuum flash chromatography using sequential mixtures of n-hexane and ethyl acetate as elutants. Fractions eluted with non-polar and moderately polar solvents were subjected to silica and C_{18} reversed-phase HPLC to yield seven compounds.

Compound **1** was isolated as a white solid. The molecular formula $C_{27}H_{40}O_3$ was deduced by a combination of high-resolution mass and carbon NMR data. The presence of an α,β-unsaturated carbonyl group was readily recognized by carbon signals at δ 200.15 (s), 158.15 (d) and 127.39 (d) and a strong absorption band at 1690 cm^{-1} in IR spectrum (Table I). Corresponding ^1H NMR signals were found at δ 7.12 (1 H, d, J=10.3 Hz) and 5.84 (1 H, d, J=10.3 Hz). Downfield signals at δ 119.87 (s), 82.04 (s) and 80.36 (d) in the



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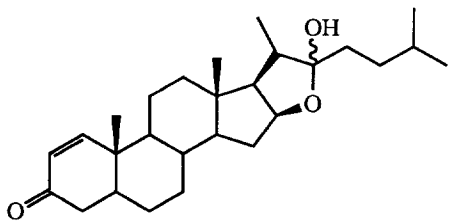
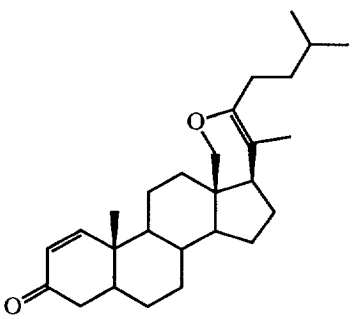


2

3 (4,5-didehydro)



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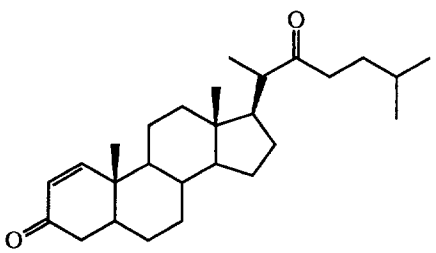

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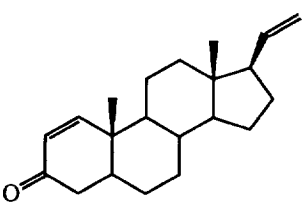
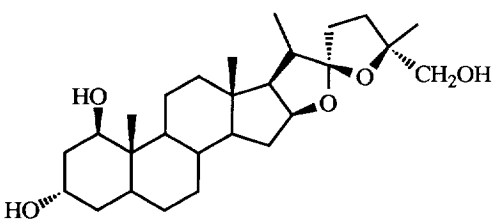


6

7 (1,2-dihydro)



wallogenin

^{13}C NMR spectrum indicated the presence of a ketal functionality. This interpretation was supported by the IR spectrum in which no absorption band was shown in the region of 3000-4000 cm^{-1}. Unusual downfield shift of the ketal center carbon (δ 119.87) suggested that the ketal must be composed of two tetrahydrofuran rings.

Careful examination of the ^{1}H and ^{13}C NMR data revealed that compound **1** was a steroid possessing the cholestane skeleton. Furthermore, comparison of spectral data with known metabolites revealed that **1** possessed the A-C rings related to minabeolides, a series of withanolides recently isolated from the soft coral *Minabea* sp.[3] The only difference was the replacement of α,β–α',β'-unsaturated carbonyl of minabeolides to α,β-unsaturated one in **1**. Comparison of the ^{13}C NMR data assigned the α,β-unsaturated carbonyl group to 1,2-didehydro-3-oxo of the cholestane skeleton. This interpretation was confirmed by combination of HMQC and HMBC experiments (Table I and II).

Table I. Carbon NMR Assignments for Compounds **1-5.**

C#	1	2	3	4	5
1	158.15 (CH)	158.21 (CH)	155.68 (CH)	158.33 (CH)	158.19 (CH)
2	127.39 (CH)	127.37 (CH)	127.53 (CH)	127.41 (CH)	127.40 (CH)
3	200.15 (C)	200.10 (C)	186.30 (C)	200.13 (C)	200.19 (C)
4	40.96 (CH_2)	40.89 (CH_2)	123.90 (CH)	40.96 (CH_2)	40.96 (CH_2)
5	44.22 (CH)	44.19 (CH)	168.90 (C)	44.31 (CH)	44.22 (CH)
6	27.51 (CH_2)	27.46 (CH_2)	32.77 (CH_2)	27.49 (CH_2)	27.58 (CH_2)[a]
7	31.70 (CH_2)	31.60 (CH_2)[b]	33.71 (CH_2)	31.54 (CH_2)	31.23 (CH_2)
8	35.26 (CH)	35.26 (CH)	35.16 (CH)	35.58 (CH)	35.63 (CH)
9	49.93 (CH)	49.91 (CH)	50.15 (CH)	50.15 (CH)	49.88 (CH)
10	39.03 (C)	38.99 (C)	43.55 (C)	39.11 (C)	38.94 (C)
11	21.02 (CH_2)	20.94 (CH_2)	22.66 (CH_2)	20.83 (CH_2)	21.28 (CH_2)
12	39.79 (CH_2)	39.78 (CH_2)	39.37 (CH_2)	31.66 (CH_2)	39.58 (CH_2)[c]
13	40.74 (C)	40.99 (C)	40.01 (C)	41.18 (C)	42.84 (C)
14	55.99 (CH)	56.01 (CH)	55.15 (CH)	54.34 (CH)	55.69 (CH)
15	31.42 (CH_2)	31.40 (CH_2)[b]	31.91 (CH_2)	24.20 (CH_2)	24.31 (CH_2)
16	80.36 (CH)	81.10 (CH)	80.99 (CH)	27.90 (CH_2)	27.51 (CH_2)[a]
17	61.79 (CH)	62.67 (CH)	62.59 (CH)	47.70 (CH)	52.05 (CH)
18	16.47 (CH_3)	16.51 (CH_3)	16.40 (CH_3)	63.80 (CH_2)	12.42 (CH_3)
19	13.06 (CH_3)	13.03 (CH_3)	18.75 (CH_3)	12.99 (CH_3)	12.99 (CH_3)
20	38.37 (CH)	39.60 (CH)	39.90 (CH)	106.04 (C)	49.40 (CH)
21	14.66 (CH_3)	15.48 (CH_3)	15.48 (CH_3)	16.79 (CH_3)	16.55 (CH_3)
22	119.87 (C)	110.49 (C)	110.54 (C)	146.15 (C)	214.83 (C)
23	33.66 (CH_2)	32.51 (CH_2)	32.53 (CH_2)	30.59 (CH_2)	32.39 (CH_2)
24	37.05 (CH_2)	36.87 (CH_2)	36.92 (CH_2)	36.81 (CH_2)	39.72 (CH_2)[c]
25	82.04 (C)	28.28 (CH)	28.33 (CH)	27.90 (CH)	27.70 (CH)
26	30.22 (CH_3)[d]	22.55 (CH_3)[d]	22.60 (CH_3)[d]	22.58 (CH_3)[d]	22.41 (CH_3)[d]
27	28.45 (CH_3)[d]	22.43 (CH_3)[d]	22.47 (CH_3)[d]	22.57 (CH_3)[d]	22.37 (CH_3)[d]

Carbon NMR spectra were obtained in $CDCl_3$ solution at 125 MHz. Numbers of attached protons were determined by DEPT experiments. Assignments for **1** and **4** were aided by HMQC and HMBC experiments. Assignments for others were aided by comparison with **1** and **4**. [a-d] Signals within a column may be reversed.

Structure of the remaining components, D ring and the side chain, were determined by interpretation of combined spectral data. All of the proton-bearing carbons and their protons were precisely matched by HMQC experiment. Positions of the oxygenated carbons were assigned by ^1H decoupling and COSY experiments. Irradiation of a doublet methyl (δ 0.96, C-21) changed splitting of a methine proton signal (δ 2.08, C-20) from a broad quintet (J=7.0 Hz) to a sharp doublet (J=6.1 Hz). The downfield shift of this proton revealed that the adjacent C-22 carbon was oxidized. COSY data showed that both of the C-20 proton and a downfield proton at δ

4.45 were coupled to a common methine proton at δ 1.80 (C-17). This was interpreted as the C-16 carbon of D ring was oxidized. Finally, chemical shifts and splitting patterns of two methyl signals (δ 1.34 and 1.17, both singlets) revealed that the C-25 carbon was oxidized. Thus, the oxygen-bearing carbons were assigned to C-16, C-22 and C-25. From the molecular formula and carbon chemical shifts of oxygenated carbons, connectivity of these carbons by oxygen linkage was determined as a bicyclic ketal centered at C-22. This assignment was confirmed by HMBC experiment in which several long-range correlations were found among the key carbons and adjacent protons (Table II). Thus, the structure of compound **1** was unambiguously determined as a steroid of the furospirostan class.[4]

Table II. Results of HMBC Experiments with Compounds **1**, **4**, and **6**.[a]

H[b]	C		
	1	**4**	**6**
1	3, 5, 9, 10	5, 9, 10	3, 5, 9, 10
2	4, 10	---	4, 10
4	3, 5, 10	5, 6, 10	3, 5
15	---	---	8, 16
16	13	---	---
17	---	---	13, 18, 20, 21
18	13, 14, 17	12, 13, 17, 22	12, 13, 14
19	1, 5, 9, 10	5, 9, 10	1, 5, 9, 10
20	13, 17, 21	---	---
21	17, 22	17, 20, 22	17
26	24, 25, 27	24, 25, 27	
27	24, 25, 26	24, 25, 26	

[a]Experiments were performed at 125 MHz in $CDCl_3$ solutions. Parameters were optimized for couplings of 8 Hz. [b]Numbers are protons which exhibited H-C correlations. Other protons are omitted for clarity.

In addition to the common asymmetric centers of the cholestane type steroids, compound **1** possesses two new asymmetric carbon centers at C-16 and C-22. Configurations of these centers were determined by combination of NOEDS experiments and comparison of proton NMR data with known compounds. Irradiation of the C-16 methine proton and C-21 methyl protons significantly enhanced the C-17 proton resonance. In addition, irradiation of the C-20 proton strongly enhanced the C-18 methyl protons. However, there was no nOe between the C-16 and C-20 protons. Therefore, orientation of the oxygen at C-16 was determined as β that is identical with other furostans. Irradiation of the C-21 methyl protons did not significantly enhance the C-23 methylene protons that was indicative of the 22*S* configuration. Due to the severe overlapping of upfield signals, however, further nOe results supporting this interpretation were not obtained.

The configuration of the C-22 carbon was determined by comparison of NMR data with structurally similar compounds. Literature survey revealed that furospirostans possess either *R* or *S* configurations at this

asymmetric center.[5] Due to the diamagnetic effect of the ketal oxygen, however, chemical shifts of the adjacent protons and carbons were significantly changed depending on stereochemistry of the ketal. The C-16 proton of wallogenin, a furospirostan possessing the 22*R* configuration was found at δ 4.15 while the same proton of the corresponding C-22 epimer was found at δ 4.45.[5] Signal of the same proton of **1** was found at δ 4.45 which was identical with that of the C-22 epimer. In ^{13}C NMR spectrum, resonances of the C-20, -21, -22, and -23 carbons of wallogenin were found at δ 37.5, 16.7, 120.4, and 30.0, respectively, while the same carbons of the C-22 epimer were found at δ 38.3, 14.7, 120.2, and 32.2, respectively. In compound **1**, signals of the same carbons were found at δ 38.4, 14.7, 119.9, and 33.7, respectively, which were very similar to those of the C-22 epimer. Therefore, the configuration of the C-22 of **1** must be *S*.

Steroids of the furospirostan class have been isolated from several terrestrial plants. However, all of these compounds have been isolated not as free steroids but as aglycons of steroidal saponins. Furospirostan steroids were obtained only by hydrolysis of the corresponding saponins. To the best of our knowledge, the only other furospirostans isolated as free steroids are hippurins which are metabolites of the soft coral *Isis hippuris*.[6-9]

Compound **2** was isolated as a white solid which was analyzed for $C_{27}H_{42}O_3$ by high-resolution mass and ^{13}C NMR spectroscopic methods. Spectral data of **2** were highly compatible with those derived from **1**. Careful examination of NMR data revealed that **2** possessed the same A-D rings as **1**. However, there were significant differences in the ^{13}C NMR spectrum. Signal of the quaternary carbon at δ 82.04 (C-25) of **1** was replaced by an upfield methine signal at δ 28.28 in **2**. In addition, the ketal carbon (C-22) at δ 119.87 of **1** was upfield shifted to δ 110.49. Corresponding changes were found in the ^1H NMR spectrum. Singlet methyl signals at δ 1.34 and 1.17 were changed to doublets at δ 0.88 (*J*=6.4 Hz) and 0.87 (*J*=6.4 Hz). Also, a strong absorption band appeared at 3450 cm^{-1} in the IR spectrum. All of the differences were accommodated by replacement of the ketal of **1** with a hemiketal group formed by ring-opening of the terminal tetrahydrofuran ring in **2**. Thus, the structure of **2** was confidently assigned as a hemiketal of the furostan class. Compound **2** possessed the same asymmetric centers as **1**. Chemical shifts and coupling constants of key protons of **2** were very similar to **1**. Therefore, configuration of the asymmetric center at C-16 of **2** must be the same as **1**. However, stereochemistry of the C-22 carbon was not assigned. Although all of the known furostanols have been reported as possessing the 22*R* configuration, we found that evidences have not been enough to support this conclusion. Due to ring-opening of the terminal tetrahydrofuran, irradiation of either the C-20 or C-21 protons failed to enhance the C-23 protons significantly.

A closely related compound, **3** was isolated as a white solid which was analyzed for $C_{27}H_{40}O_3$ by high-resolution mass and ^{13}C NMR spectroscopic methods. Spectral data of **3** were analogous to those of **2**. The only significant differences in the ^{13}C NMR spectrum were replacements of two upfield signals at δ 40.89 (CH_2) and 44.19 (CH) of **2** with the olefinic signals at δ 168.90 (C) and 123.90 (CH). Corresponding difference was found in the ^1H NMR spectrum in which a new olefinic proton signal appeared at δ 6.07 (broad singlet). In addition, the carbonyl absorption band at 1680 cm^{-1} in the IR spectrum of **2** was shifted to 1665 cm^{-1} in **3**. All of the differences were accommodated by replacement of the α,β-unsaturated carbonyl group of **2** to an α,β-α',β'-unsaturated carbonyl group in **3**. Comparison of NMR data with known compounds possessing the same functional group fully supported this interpretation.[3] Thus, compound **3** was unambiguously determined as a hemiketal of the furostan class.

Compound **4** was isolated as a white solid by HPLC. The molecular formula $C_{27}H_{40}O_2$ was deduced by

high- resolution mass and analysis of the ^{13}C NMR spectrum. Careful examination of the ^{1}H and ^{13}C NMR spectra revealed that **4** possessed the same A-C rings and terminal isopropyl group as **2**. However, there were several significant differences in the ^{13}C NMR spectrum. One of the methyl signals at δ 16.51 (C-18) and 15.48 (C-21) of **2** was replaced by an oxygen-bearing methylene at δ 63.80. In addition, signals of an asymmetric double bond were found at δ 146.15 (s) and 106.04 (s). Finally, the C-17 methine carbon signal at δ 62.67 of **2** was shifted to δ 47.70 in **4**. Also, in the ^{1}H NMR spectrum, methyl signals at δ 1.02 (d, *J*=7.3 Hz, H-21) and 0.81 (s, H-18) of **2** were replaced by a vinyl methyl at δ 1.59 (s) and methylene at δ 3.91 (dd, *J*=10.6, 1.7 Hz) and 3.31 (d, *J*=10.6 Hz). These differences were accommodated by formation of a double bond at C-20 and connection of the C-18 and C-22 carbons by an oxygen atom.

This interpretation was confirmed by 2-D NMR experiments. All of the protons and proton-bearing carbons were confidently assigned by ^{1}H COSY and HMQC experiments. The presence of a dihydropyran moiety between the ring D and side chain was confirmed by HMBC experiments (Table II). Long-range correlations between the vinyl methyl protons and olefinic carbons showed the presence of a double bond at C-20. Correlations among the protons at δ 3.91 and 3.31 and the C-12, C-13 and C-17 carbons confirmed oxidation of the C-18 carbon. In addition, correlations between the proton at δ 3.91 and the C-22 carbon confirmed the presence of an oxygen bridge between these carbons. Thus, the structure of **4** was unambiguously assigned as a steroid possessing an enolether functionality.

Compound **5** was isolated as a white solid which was analyzed for $C_{27}H_{42}O_2$ by high resolution mass and ^{13}C NMR spectroscopic methods. Spectral data of **5** were highly comparable with those of **2**. The only significant differences were the appearance of a new carbonyl signal at δ 214.83 in the ^{13}C NMR spectrum and absorption band at 1710 cm^{-1} in the IR spectrum. Therefore, **5** must be a derivative possessing an additional carbonyl group. Position of the carbonyl group was assigned by ^{1}H COSY and decoupling experiments. ^{1}H COSY correlation readily assigned the C-20 proton (δ 2.52). Irradiation of C-21 methyl protons at δ 1.10 (d, *J*=6.8 Hz) changed splitting of the C-20 proton from double-quartet (*J*=10.3, 6.8 Hz) to sharp doublet (*J*=10.3 Hz). Downfield chemical shift and coupling pattern of the C-20 proton revealed that the adjacent C-22 carbon was oxidized to carbonyl. Thus, **5** was assigned as a C_{27} steroid possessing carbonyl groups at C-3 and C-22.

Compound **6** was isolated as a white solid. In contrast to compounds **1-5**, ^{13}C NMR spectrum of **6** showed only twenty one carbon signals. Combination of the ^{13}C NMR and mass data deduced the molecular formula $C_{21}H_{30}O$ for **6**. Careful examination of the NMR spectra revealed that **6** possessed the same A-D rings as compounds **1**, **2**, and **5**. Combination of the ^{1}H NMR COSY, HMQC and HMBC experiments determined the structure of **6** to be pregna-1,20-dien-3-one which was previously isolated from an unidentified Pacific soft coral.[10] Comparison of spectral data showed very good correlation with published data for this compound.

An apparently related pregnane, compound **7** was isolated as a white solid which was analyzed for $C_{21}H_{32}O$ by high resolution mass and ^{13}C NMR spectroscopic data. Spectral data of **7** were very similar to those from **6**. However, ^{13}C NMR spectrum of **2** showed that the olefinic carbon signals at δ 158.45 (d) and 127.26 (d) were replaced by upfield signals at δ 38.92 (t) and 38.19 (t) while the carbonyl carbon at δ 200.09 (s) was shifted downfield to δ 212.15 (s). Corresponding differences were found in the ^{1}H NMR spectrum in which the olefinic proton signals at δ 7.15 and 5.85 of **6** were replaced by upfield signals. In addition, the carbonyl absorption band at 1685 cm^{-1} in the IR spectrum of **6** was shifted to 1715 cm^{-1} in **7**. Therefore, **7** must be pregna-20-en-30-one. Literature survey revealed that compound **2** has not been isolated as a natural

product. However, this compound has been synthesized as a key intermediate for the synthesis of **6**.[11]

Crude extract of *A. gracillimum* exhibited moderate cytotoxicity (IC_{50} 22.4 μg/ml) and antiviral activity (IC_{50} 7.8 μg/ml) against P388 and HSV-I, respectively. Hemiketal **2** displayed moderate cytotoxicity against P388 strain (IC_{50} 7.8 μg/ml). In addition, **2** and **3** exhibited moderate inhibition against human cytomegalovirus (IC_{50} 3.7 and 7.2 μg/ml, respectively).

EXPERIMENTAL

General. NMR spectra were recorded in $CDCl_3$ solutions on a 500-MHz Varian Unity spectrometer. All chemical shifts were recorded with respect to internal Me_4Si. Infrared spectra were recorded on a Matteson GALAXY spectrophotometer. Ultraviolet spectra were obtained in CH_3CN using a Milton-Roy spectrophotometer. Mass measurements were supplied by Department of Chemistry, Seoul National University and Mass Spectrometry Facility, University of California, Riverside. Optical rotations were measured on a JASCO digital polarimeter with a 5-cm microcell. Melting points were measured on a Fisher-Johns Apparatus and reported without calibration. All solvents used were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Isolation. *Alcyonium gracillimum* was collected by hand using SCUBA at 15-25 m depth in November, 1991, along the offshore of Geomun Islands, Korea. The collection was frozen immediately and kept in freezer until chemically investigated. The soft coral (3 kg) was defrosted and repeatedly extracted with MeOH and CH_2Cl_2. The combined extracts were partitioned between water and n-butanol. The n-butanol layer was dried under vacuum to yield 9.2 g of crude organic material. Compounds **1-7** were separated by using silica vacuum flash chromatography eluting with 10-20% EtOAc in hexane. Final purification was made by silica and reversed-phase HPLC.

Compound 1. The spiroketal **1** (39 mg, 0.4% of the crude extract) was isolated as a white solid by silica HPLC (12 % EtOAc in hexane) followed by reversed-phase HPLC (100% CH_3CN), mp. 181-183°. Compound **1** exhibited the following spectral features: $[\alpha]_D$ -20.7° (c 0.5, $CHCl_3$); UV (CH_3CN) λmax 228 nm (ε 11500); HRCIMS: $(M + H)^+$ obsd 413.3048, $C_{27}H_{41}O_3$ requires 413.3055; LREIMS: m/z (relative intensity) 412 (4), 397 (9), 354 (6), 343 (8), 298 (45), 283 (15), 269 (27), 177 (8), 139 (100), 122 (9); IR (KBr) 2930, 2870, 1690, 1450, 1380, 1260, 1170, 1135, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12 (1 H, d, 10.3, H-1), 5.84 (1 H, d, 10.3, H-2), 4.45 (1 H, ddd, 7.5, 7.4, 7.2, H-16), 2.37 (1 H, dd, 17.6, 14.2, H-4), 2.22 (1 H, dd, 17.6, 3.9, H-4), 2.08 (1 H, m, H-20), 1.98 (4 H, m, H-15, -23, -23, -24), 1.89 (1 H, m, H-5), 1.80 (1 H, m, H-17), 1.77 (1 H, m, H-12), 1.72 - 1.70 (3 H, m, H-7, -11, -24), 1.63 (1 H, m, H-8), 1.45 - 1.44 (3 H, m, H-11, -6, -6), 1.34 (3 H, s, H-26/-27), 1.23 (1 H, m, H-15), 1.20 (1 H, m, H-12), 1.18 (1 H, m, H-14), 1.17 (3 H, s, H-26/-27), 1.01 (3 H, s, H-19), 1.01 (1 H, m, H-9), 0.96 (3 H, d, 7.3, H-21), 0.96 (1 H, m, H-7), 0.80 (3 H, s, H-18).

Compound 2. The hemiketal **2** (30 mg, 0.3% of the crude extract) was isolated as a white solid by silica HPLC (35 % EtOAc in hexane) followed by reversed-phase HPLC (100% CH_3CN), mp. 43-45°. Compound **2** showed the following spectral features: $[\alpha]_D$ -3.8° (c 0.5, $CHCl_3$); UV (CH_3CN) λmax 225 nm (ε 11100); HRCIMS: $(M + NH_4^+ - H_2O)^+$ obsd 414.3357, $C_{27}H_{44}NO_2$ requires 414.3372; LREIMS: m/z (relative intensity) 414 (1), 396 (18), 353 (100), 298 (6), 283 (6), 269 (83), 149 (5), 121 (6); IR (KBr) 3450, 2950,

2930, 2870, 1680, 1450, 1380, 1260, 1170, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.11 (1 H, d, 10.3, H-1), 5.84 (1 H, brd, 10.3, H-2), 4.58 (1 H, ddd, 8.5, 7.3, 6.3, H-16), 2.34 (1 H, dd, 17.6, 14.3, H-4), 2.20 (1 H, dd, 17.6, 3.9, H-4), 2.04 (1 H, m, H-20), 1.97 (1 H, ddd, 12.2, 7.3, 5.1, H-15), 1.89 (1 H, m, H-5), 1.82 - 1.75 (2 H, m), 1.74 - 1.60 (5 H, m), 1.52 (1 H, m, H-25), 1.48 - 1.38 (3 H, m), 1.36 - 1.29 (2 H, m), 1.26 (1 H, m, H-15), 1.21 - 1.11 (2 H, m), 1.02 (3 H, d, 7.3, H-21), 1.00 (3 H, s, H-19), 1.02 - 0.93 (2 H, m), 0.88 (3 H, d, 6.4, H-26/-27), 0.87 (3 H, d, 6.4, H-26/-27), 0.81 (3 H, s, H-18).

Compound 3. The hemiketal **3** (34 mg, 0.4% of the crude extract) was isolated as a white solid by silica HPLC (45 % EtOAc in hexane) followed by reversed-phase HPLC (100% CH_3CN), mp. 123-124°. Compound **3** displayed the following spectral features: $[\alpha]_D$ -10.2° (c 0.5, $CHCl_3$); UV (CH_3CN) λmax 236 nm (ε 13000); HRCIMS: $(M + H)^+$ obsd 413.3054, $C_{27}H_{41}O_2$ requires 413.3055; LREIMS: m/z (relative intensity) 412 (0.2), 410 (2), 394 (48), 351 (42), 267 (26), 248 (100), 171 (10), 165 (48), 121 (11), 115 (8); IR (KBr) 3450, 2950, 2870, 1665, 1625, 1450, 1380, 1250, 1070, 890 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.04 (1 H, d, 10.0, H-1), 6.07 (1 H, brs, H-4), 6.02 (1 H, dd, 10.0, 1.7, H-2), 4.59 (1 H, ddd, 7.3, 7.3, 7.3,-16), 2.48 (1 H, ddd, 12.9, 12.9, 4.9, H-6), 2.36 (1 H, ddd, 12.9, 3.3, 3.3, H-6), 2.06 (1 H, m, H-20), 2.03 - 1.94 (2 H, m), 1.85 - 1.76 (3 H, m), 1.72 - 1.62 (4 H, m), 1.54 (1 H, septet, 6.7, H-25), 1.38 - 1.28 (3 H, m), 1.24 (3 H, s, H-19), 1.21 - 0.98 (4 H, m), 1.03 (3 H, d, 7.5, H-21), 0.90 (6 H, d, 6.5, H-26, -27), 0.87 (3 H, s, H-18).

Compound 4. The cyclic enolether **4** (16 mg, 0.2% of the crude extract) was isolated as a white solid by silica HPLC (8 % EtOAc in hexane) followed by reversed-phase HPLC (100% CH_3CN), mp. 52-53°. Compound **4** exhibited the following spectral features: $[\alpha]_D$ +34.0° (c 0.5, $CDCl_3$); UV (CH_3CN) λmax 224 nm (ε 10600); HREIMS: M^+ obsd 396.3034, $C_{27}H_{40}O_2$ requires 396.3028; LREIMS: m/z (relative intensity) 396 (100), 340 (10), 292 (7), 268 (8), 191 (7), 149 (7), 110 (9); IR (KBr) 2930, 2870, 1680, 1630, 1445, 1260, 1170, 785 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.16 (1 H, d, 10.0, H-1), 5.86 (1 H, brd, 10.0, H-2), 3.91 (1 H, dd, 10.6, 1.7, H-18), 3.31 (1 H, d, 10.6, H-18), 2.37 (1 H, dd, 17.6, 14.2, H-4), 2.22 (1 H, dd, 17.6, 3.9, H-4), 2.12 (1 H, m, H-12), 2.10 (1 H, m, H-23), 2.08 (2 H, m, H-6, -16), 1.93 (1 H, m, H-5), 1.83 (1 H, m, H-11), 1.73 (1 H, m, H-7), 1.69 (1 H, m, H-15), 1.59 (3 H, s, H-21), 1.58 (1 H, m, H-17), 1.52 (1 H, m, H-25), 1.48 (1 H, m, H-23), 1.42 (2 H, m, H-6, -16), 1.38 (1 H, m, H-8), 1.35 (2 H, m, H-14, -15), 1.33 (2 H, m, H-24), 1.07 (1 H, m, H-9), 1.00 (3 H, s, H-19), 0.98 (1 H, m, H-7), 0.92 (1 H, m, H-12), 0.90 (1 H, m, H-11), 0.89 (6 H, d, 6.4, H-26, -27).

Compound 5. The diketone **5** (10 mg, 0.1% of the crude extract) was isolated as a white solid by silica HPLC (9 % EtOAc in hexane) followed by reversed-phase HPLC (100 % CH_3CN), mp. 55-57°. Compound **5** exhibited the following spectral features: $[\alpha]_D$ +22.4° (c 0.5, $CHCl_3$); UV (CH_3CN) λmax 229 nm (ε 10600); HREIMS M^+ obsd 398.3161, $C_{27}H_{42}O_2$ requires 398.3184; LREIMS: m/z (relative intensity) 398 (100), 383 (7), 342 (11), 327 (23), 299 (31), 271 (61), 245 (8), 229 (8), 161 (9), 122 (25), 99 (23), 81 (17); IR (KBr) 2930, 2870, 1710, 1690, 1450, 1370, 1265, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.13 (1 H, d, 10.3, H-1), 5.85 (1 H, d, 10.3, H-2), 2.52 (1 H, dq, 10.3, 6.8, H-20), 2.45 (1 H, ddd, 17.1, 9.3, 6.4, H-23), 2.36 (1 H, dd, 17.6, 14.2, H-4), 2.35 (1 H, ddd, 17.1, 9.3, 6.3, H-23), 2.21 (1 H, dd, 17.6, 3.9, H-4), 1.98 (1 H, ddd, 12.6, 3.4, 3.4, H-12), 1.92 (1 H, m, H-5), 1.75 (1 H, m, H-11), 1.70 (1 H, m, H-7), 1.67 (2 H, m, H-15, -16), 1.64 (1 H, m, H-17), 1.62 (1 H, m, H-16), 1.53 (1 H, m, H-25), 1.47 - 1.38 (6 H, m, H-6,-6, -8, -11, -24, -24), 1.30 (1 H, ddd, 12.6, 12.6, 3.4, H-12), 1.12 (1 H, m, H-15), 1.10 (3 H, d, 6.8, H-21), 1.10 (1 H, m, H-14), 1.01 (3 H, s, H-19), 0.99 (2 H, m, H-7, -9), 0.89 (6 H, d, 6.8, H-26, -27), 0.72 (3 H, s, H-18).

Compound 6. The pregnadienone **6** (83 mg, 0.9% of the crude extract) was isolated as a white solid by silica HPLC (7 % EtOAC in hexane), mp. 126-127°. Compound **6** exhibited the following spectral features: $[\alpha]_D$ +35.4° (c 0.5, $CHCl_3$); UV (CH_3CN) λmax 228 nm (ε 12000); LREIMS: m/z (relative intensity) 298 (74), 283 (100), 270 (12), 229 (31), 177 (20), 163 (19), 134 (24), 122 (60), 107 (22); IR (KBr) 2920, 2850, 1685, 1630, 1440, 1380, 1270, 1010, 930 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.15 (1 H, d, 10.3, H-1), 5.85 (1 H, d, 10.3, H-2), 5.76 (1 H, ddd, 17.1, 10.3, 7.3, H-20), 4.99 (1 H, dd, 10.3, 1.0, H-21), 4.96 (1 H, ddd, 17.1, 1.0, 1.0, H-21), 2.37 (1 H, dd, 17.6, 14.2, H-4), 2.22 (1 H, dd, 17.6, 3.9, H-4), 1.98 (1 H, m, H-17), 1.92 (1 H, m, H-5), 1.79 (2 H, m, H-11, -16), 1.77 (1 H, m, H-12), 1.75 (1 H, m, H-7), 1.69 (1 H, m, H-15), 1.58 (1 H, m, H-16), 1.46 (1 H, m, H-8), 1.42 (2 H, m, H-6), 1.40 (1 H, m, H-11), 1.20 (1 H, m, H-15), 1.10 (2 H, m, H-12, -14), 1.02 (3 H, s, H-19), 0.99 (2 H, m, H-7, -9), 0.63 (3 H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 200.09 (C, C-3), 158.45 (CH, C-1), 139.46 (CH, C-20), 127.26 (CH, C-2), 114.64 (CH_2, C-21), 55.45 (CH, C-14), 55.19 (CH, C-14), 50.17 (CH, C-9), 44.28 (CH, C-5), 43.61 (C, C-13), 40.91 (CH_2, C-4), 39.00 (C, C-10), 37.26 (CH_2, C-12), 35.70 (CH, C-8), 31.33 (CH_2, C-7), 27.55 (CH_2, C-6), 27.11 (CH_2, C-16), 24.59 (CH_2, C-15), 20.75 (CH_2, C-11), 12.99 (CH_3, C-19), 12.97 (CH_3, C-18).

Compound 7. The pregnenone **7** (5 mg, 0.05% of the crude extract) was isolated as a white solid by silica HPLC (7 % EtOAc in hexane) followed by reversed-phase HPLC (100 % CH_3CN), mp. 113-115°. Compound **7** displayed the following spectral features: $[\alpha]_D$ +12.5° (c 0.2, $CHCl_3$); UV (CH_3CN) no λmax; LREIMS: m/z (relative intensity) 300 (100), 285 (36), 272 (16), 231 (51), 217 (10), 163 (10), 121 (12); IR (KBr) 2940, 2850, 1715, 1640, 1440, 1250, 910, 685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.75 (1 H, ddd, 16.9, 10.7, 7.6, H-20), 4.97 (1 H, dd, 10.7, 2.2, H-21), 4.96 (1 H, ddd, 16.9, 1.0, 1.0, H-21), 2.38 (1 H, ddd, 15.6, 13.7, 6.4, H-2), 2.29 (1 H, brddd, 15.6, 5.4, 2.9, H-2), 2.27 (1 H, dd, 14.9, 14.7, H-4), 2.08 (1 H, ddd, 14.9, 3.9, 2.2, H-4), 2.02 (1 H, ddd, 15.1, 6.4, 2.0, H-1), 1.96 (1 H, m, H-17), 1.79 (1 H, m, H-16), 1.73 (1 H, m, H-7), 1.69 (1 H, m, H-12), 1.67 (1 H, m, H-15), 1.56 (1 H, m, H-16), 1.54 (1 H, m, H-11), 1.53 (1 H, m, H-5), 1.41 (1 H, m, H-8), 1.36 (1 H, m, H-1), 1.33 (2 H, m, H-6), 1.31 (1 H, m, H-11), 1.19 (1 H, m, H-15), 1.04 (2 H, m, H-12, -14), 1.01 (3 H, s, H-19), 0.94 (1 H, m, H-7), 0.76 (1 H, ddd, 12.7, 10.3, 4.4, H-9), 0.61 (3 H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 212.15 (C, C-3), 139.74 (CH, C-20), 114.55 (CH_2, C-21), 55.44 (CH, C-14), 55.38 (CH, C-17), 54.09 (CH, C-9), 46.78 (CH, C-5), 44.73 (CH_2, C-4), 43.61 (C, C-13), 38.62 (CH_2, C-1), 38.19 (CH_2, C-2), 37.44 (CH_2, C-12), 35.77 (C, C-10), 35.52 (CH, C-8), 31.82 (CH_2, C-7), 28.95 (CH_2, C-6), 27.20 (CH_2, C-16), 24.79 (CH_2, C-15), 21.05 (CH_2, C-11), 12.94 (CH_3, C-18), 11.50 (CH_3, C-19).

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