Bioactive Polyoxygenated Steroids from the South China Sea Soft Coral, *Sarcophyton* sp.

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Abstract: Seven new polyoxygenated steroids (1–7) were isolated together with seven known analogues (8–14) from the South China Sea soft coral, *Sarcophyton* sp. The structures of the new compounds were identified on the basis of extensive spectroscopic analysis and comparison with reported data. All the steroids are characterized with 3β,5α,6β-hydroxy moiety, displaying carbon skeletons of cholestanne, ergostane, gorgostane and 23,24-dimethyl cholestanne. In the *in vitro* bioassay, metabolites exhibited different levels of antimicrobial activity against bacterial species *Escherichia coli* and *Bacillus megaterium*, and fungal species *Microbotryum violaceum* and *Septoria tritici*. No inhibition was detected towards microalga *Chlorella fusca*. Preliminary structure-activity analysis suggests that the 11α-acetoxy group may increase both antibacterial and antifungal activities. The terminal-double bond and the cyclopropane moiety at the side chain may also contribute to the bioactivity.

Keywords: *Sarcophyton* sp.; steroid; bioactivity; antibacterial; antifungal
1. Introduction

Soft corals are thought to produce various bioactive metabolites that chemically defend themselves from attack [1–3]. The soft coral *Sarcophyton* species (order Alcyonacea, family Alcyoniidae) are prolific in the South China Sea and are dominant in many coral reef areas [4]. Chemical research on the animals of this genus has established that it is a rich resource of steroids, diterpenes and tetraterpenes [5,6].

In the course of our ongoing screening for bioactive secondary metabolites from marine sources [7–11], we have made a collection of *Sarcophyton* sp. off Weizhou Island, Guangxi Province, China. Chemical investigation on the Et<sub>2</sub>O-soluble fraction of the acetone extract from *Sarcophyton* sp. resulted in the isolation of fourteen steroids (1–14, Figure 1) with 3β,5β,6β-hydroxy moiety. The sterols can be re-sorted into four clusters, due to their different carbon skeletons, namely cholesterol-, ergosterol-, gorgosterol- and 23,24-dimethyl cholesterol-types, displaying an excellent example of chemical diversity. The isolates were tested for *in vitro* antimicrobial activity against bacteria, fungi and a microalga. Here, we describe the isolation, structural elucidation and bioactivity of these new metabolites.

![Figure 1. The chemical structures of sterols 1–14.](image)

2. Results and Discussion

Freshly collected specimens of *Sarcophyton* sp. were kept at –20 °C before extraction. The acetone extract of the soft coral was partitioned between Et<sub>2</sub>O and H<sub>2</sub>O. Purification of the Et<sub>2</sub>O extract, by repeated column chromatography on silica and Sephadex LH-20 and followed by reversed-phase semi-preparative HPLC, yielded the pure compounds shown in 1–14 (Figure 1).

On the basis of detailed spectroscopic analysis and comparison with reported data, the structures of the known compounds were readily determined as (24S)-11α-acetoxy-ergostane-3β,5α,6β-triol (8, sarcoaldoster A) [12–14], (24S)-ergostane-3β,5α,6β-triol (9) [15], (24S)-ergostane-3β,5α,6β,11α-tetraol...
The large coupling constant between the olefinic protons indicated an assignment of three protons in the signals assigned to rings A and B (3H, d, J = 6.8 Hz, H27; 0.76, 3H, d, J = 6.8 Hz, H27) in 8 were replaced by two vinyl methyls (δ 1.59 and 1.67, each 3H, s) in 1 (Table 2). In correspondence, the doublet methyl signal of H3-28 (δ 0.76, 3H, d, J = 6.7 Hz) in 8 was replaced by a trisubstituted double bond linked to a methylene (δ 5.07, 1H, br t, J = 7.2 Hz) in 1, indicating a Δ24 cholestane side chain of the molecule. These data led 1 to the structure of 11α-acetoxy-cholesta-24-en-3β,5α,6β-triol. The 1H NMR and 13C NMR assignments of the side chain were identical to those of reported data [25–27] and were further confirmed by the proton sequence from H3-21 to H-24, as established by the 1H-1H correlation spectroscopy (COSY) experiment and the heteronuclear multiple bond (HMBC) correlation from H2-26 and H3-27 to both C-25 and C-24.

Compound 2 was obtained as an optically active, amorphous powder. Its formula was established as C30H48O5 by HR-ESI-MS. The NMR data of 2 also resembled those of 8, with a recognized difference in the signals assigned to the side chains. The methyls of the isopropyl group (δ1H 0.84, 3H, d, J = 6.8 Hz, H3-27; 0.76, 3H, d, J = 6.8 Hz, H3-27) in 8 were replaced by a vinyl methyl (δ 1.67, 3H, s) and a terminal double bond (δ 4.70, 2H, br s) in 2 (Table 2), leading to the assignment of the double bond as Δ25. In addition, a pair of signals for a disubstituted double bond were observed (δ 5.26, 1H, dd, J = 15.4, 6.2 Hz; δ 5.20, 1H, dd, J = 15.4, 7.3 Hz). The large coupling constant between the olefinic protons indicated an E geometry of the double bond, which was consequently assigned as Δ22. The marked-down field shift of H3-21 and H3-28 from δ 0.87 and 0.76 in 8 to δ 0.98 and 1.07 in 2
supported the above conclusion. Further structural evidence came from the proton sequence from H$_3$-21 to H$_2$-27, as established by the $^1$H-$^1$H COSY experiment and the HMBC correlation from H$_2$-28 to C-23, C-24 and C-25, H$_2$-26 and H$_2$-27 to both C-25 and C-24. The $^1$H NMR assignments of the side chain were identical to those of (22E,24S)-ergostane-5,22,25-trien-3β-ol, isolated from the plant, Clerodendrum fragrans (Verbenaceae family) [28], which further confirmed the established structure and suggested an S configuration at C-24. These lines of evidence led to the structure (22E,24S)-11α-acetoxy-ergostane-22,25-dien-3β,5α,6β-triol for compound 2.

**Table 1.** The $^{13}$C nuclear magnetic resonance (NMR) data of sterols 1–7 (100 $^a$ or 125 $^b$ MHz in CDCl$_3$).

| Carbon |
|--------|
|   1 | 33.5 t | 33.5 t | 76.8 d | 34.4 t | 32.3 t | 33.7 t | 33.9 t |
|   2 | 31.3 t | 31.3 t | 37.2 t | 31.2 t | 30.9 t | 31.3 t | 31.2 t |
|   3 | 67.2 d | 67.2 d | 64.2 d | 67.4 d | 67.6 d | 67.1 d | 67.5 d |
|   4 | 41.3 t | 41.3 t | 41.3 t | 41.0 t | 40.8 t | 41.3 t | 41.3 t |
|   5 | 76.6 s | 76.6 s | 76.8 s | 76.7 s | 76.7 s | 76.6 s | 76.7 s |
|   6 | 76.1 d | 76.1 d | 75.2 d | 76.1 d | 76.1 d | 76.0 d | 76.0 d |
|   7 | 34.8 t | 34.9 t | 35.2 t | 34.5 t | 34.8 t | 34.4 t | 34.0 t |
|   8 | 29.4 d | 29.4 d | 29.4 d | 29.1 d | 28.9 d | 29.4 d | 28.9 d |
|   9 | 48.6 d | 48.7 d | 46.6 d | 53.0 d | 45.9 d | 42.7 d | 46.3 d |
|  10 | 39.9 s | 39.9 s | 42.1 s | 40.0 s | 38.4 s | 39.6 s | 39.6 s |
|  11 | 71.1 d | 71.1 d | 68.0 d | 68.7 d | 21.5 t | 73.4 d | 70.7 d |
|  12 | 46.4 t | 46.3 t | 50.9 t | 51.8 t | 37.9 t | 75.0 d | 80.5 d |
|  13 | 42.9 s | 42.7 s | 43.2 s | 43.1 s | 44.7 s | 46.7 s | 46.0 s |
|  14 | 54.6 d | 54.7 d | 54.7 d | 55.1 d | 52.2 d | 45.3 d | 47.4 d |
|  15 | 24.2 t | 24.2 t | 24.2 t | 24.2 t | 72.2 d | 23.9 t | 23.8 t |
|  16 | 28.2 t | 28.5 t | 28.2 t | 28.0 t | 34.4 t | 27.5 t | 27.6 t |
|  17 | 56.0 d | 55.9 d | 55.8 d | 56.7 d | 149.0 s | 49.2 d | 50.1 d |
|  18 | 12.7 q | 12.9 q | 12.8 q | 13.4 q | 17.3 q | 11.8 q | 12.7 q |
|  19 | 16.8 q | 16.8 q | 16.3 q | 17.0 q | 16.8 q | 16.7 q | 16.7 q |
|  20 | 35.4 d | 39.8 d | 36.1 d | 34.5 d | 133.0 s | 35.1 d | 34.6 d |
|  21 | 18.6 q | 20.7 q | 18.9 q | 20.6 q | 17.1 q | 20.1 q | 20.6 q |
|  22 | 35.9 t | 135.5 d | 33.6 t | 131.3 d | 42.0 t | 31.9 d | 31.9 d |
|  23 | 24.7 t | 131.8 d | 30.6 t | 135.7 s | 32.7 d | 25.9 s | 25.9 s |
|  24 | 125.1 d | 43.6 d | 39.1 d | 50.2 d | 44.2 d | 50.8 d | 50.8 d |
|  25 | 131.1 s | 149.8 s | 31.5 d | 30.8 d | 30.1 d | 32.0 d | 32.0 d |
|  26 | 17.6 q | 108.8 t | 20.5 q | 21.7 q | 21.6 q | 21.5 q | 21.5 q |
|  27 | 25.7 q | 20.6 q | 17.6 q | 20.1 q | 19.1 q | 22.2 q | 21.3 q |
|  28 | 18.9 q | 15.5 q | 17.0 q | 11.5 q | 15.4 q | 15.5 q |
|  29 | 13.3 q | 13.8 q | 14.3 q | 14.3 q | 14.3 q | 14.3 q | 14.3 q |
|  30 | | | | | | | 21.3 t |
| COOCH$_3$ | 22.1 q | 22.1 q | | | | 22.1 q | 22.2 q |
| COOCH$_3$ | 170.4 s | 170.4 s | | | | 170.0 s | 170.4 s |

$s$ = singlet; $d$ = doublet, $t$ = triplet; $q$ = quartet.
Table 2. The $^1$H NMR data of sterols 1–4 (400$^a$ or 500$^b$ MHz in CDCl$_3$; $J$ in Hz).

| Position | 1$^a$ | 2$^a$ | 3$^b$ | 4$^a$ |
|----------|------|------|------|------|
| 1        | 1.26 (m), 1.81 (m) | 1.22 (m), 1.76 (m) | 4.11 (br s) | 1.50 (m), 1.71 (m) |
| 2        | 1.47 (m), 1.82 (m) | 1.53 (m), 1.77 (m) | 1.86 (m), 2.18 (m) | 1.56 (m), 1.86 (m) |
| 3        | 4.05 (m) | 4.05 (m) | 4.40 (m) | 4.07 (m) |
| 4        | 1.62 (ov), 2.07 (t, 12.2) | 1.58 (ov), 2.08 (d, 11.8) | 1.77 (ov), 2.08 (ov) | 1.67 (ov), 2.11 (ov) |
| 5        | 6.25 (m) | 6.25 (m) | 6.25 (m) | 6.25 (m) |
| 6        | 1.61 (m), 1.76 (m) | 1.58 (m), 1.74 (m) | 1.52 (m), 1.88 (m) | 1.73 (m), 2.08 (m) |
| 7        | 1.87 (m) | 1.76 (m) | 1.88 (m) | 1.76 (m) |
| 8        | 1.78 (m) | 1.82 (m) | 1.99 (m) | 1.34 (m) |
| 9        | 1.74 (m) | 1.76 (m) | 1.80 (m) | 1.76 (m) |
| 10       | 5.14 (dd, 10.3, 5.4) | 5.14 (dd, 10.5, 5.4) | 4.01 (11.0, 5.5) | 3.94 (9.7, 4.9) |
| 11       | 1.16 (ov) | 1.18 (ov) | 1.26 (ov) | 1.19 (ov) |
| 12       | 2.31 (dd, 12.2, 5.4) | 2.30 (dd, 12.1, 5.4) | 2.33 (dd, 11.5, 5.5) | 2.34 (11.5, 4.9) |
| 13       | 1.28 (m) | 1.18 (m) | 1.19 (m) | 1.16 (m) |
| 14       | 1.06 (m), 1.56 (m) | 1.07 (m), 1.79 (m) | 1.09 (m), 1.60 (m) | 1.03 (m), 1.59 (m) |
| 15       | 1.20 (m), 1.88 (m) | 1.26 (m), 1.50 (m) | 1.26 (m), 1.90 (m) | 1.19 (m), 1.78 (m) |
| 16       | 1.15 (m) | 1.18 (m) | 1.19 (m) | 1.19 (m) |
| 17       | 0.73 (s) | 0.75 (s) | 0.66 (s) | 0.73 (s) |
| 18       | 1.28 (s) | 1.28 (s) | 1.21 (s) | 1.32 (s) |
| 19       | 1.38 (m) | 2.00 (m) | 1.37 (m) | 2.30 (m) |
| 20       | 0.90 (d, 6.5) | 0.98 (d, 6.6) | 0.94 (d, 6.5) | 0.92 (d, 6.9) |
| 21       | 1.02 (m), 1.39 (m) | 5.26 (dd, 15.4, 6.2) | 0.89 (m), 1.39 (m) | 4.87 (d, 9.5) |
| 22       | 1.87 (m), 1.99 (m) | 5.20 (dd, 15.4, 7.3) | 0.89 (m), 1.38 (m) | 4.87 (d, 9.5) |
| 23       | 5.07 (t, 7.2) | 2.69 (m) | 1.19 (m) | 1.67 (m) |
| 24       | 5.16 (s) | 1.60 (m) | 1.53 (m) | 1.53 (m) |
| 25       | 0.86 (d, 7.0) | 0.86 (d, 7.0) | 0.84 (d, 6.5) | 0.84 (d, 6.5) |
| 26       | 1.07 (d, 6.9) | 0.78 (d, 7.0) | 0.78 (d, 7.0) | 0.93 (d, 7.1) |
| 27       | 1.50 (s) | 1.50 (s) | 1.50 (s) | 1.50 (s) |

$^a$ = singlet; $d$ = doublet; $t$ = triplet; $m$ = multiplet; $br$ s = broad singlet; $dd$ = doublet of doublets; $ov$ = overlapped signals.

Compound 3 was also obtained as an optically active, amorphous powder. The HR-ESI-MS of compound 3 exhibited a pseudo-molecular ion peak at $m/z$ 489.4582 [M + Na]$^+$, leading to the determination of the molecular formula as $C_{29}H_{50}O_5$. Analysis of the NMR spectra of 3 (Tables 1 and 2) revealed a remarkable similarity to those of the co-occurring sterol 10, except for the replacement of a methylene group ($\delta_H$ 1.49, 1H, m, H-1a 1.73, 1H, m, H-1β; $\delta_C$ 34.4, t) in 10 by a secondary hydroxyl group ($\delta_H$ 4.11, 1H, br s; $\delta_C$ 76.8, d) in 3. This hydroxyl group was assigned at C-1, due to the proton connectivity from H-1 to H$_2$-4, as established by the $^1$H-$^1$H COSY spectrum and the obvious HMBC correlations from H$_3$-19 to C-1, C-5, C-9 and C-10. A β configuration of H-1 was deduced from its coupling constant pattern (br s) and supported by the observation of NOE interactions between H$_3$-19 and H-1. A comparison of the NMR data for the core structure of 3 with those of co-isolated gorgostane-1α,3β,5α,6β,11α-pentaol (14) [22–24] further confirmed the established structure. The absolute configuration at C-24 was determined as $S$, due to the $^1$H NMR shift values of H$_3$-21
(δ 0.936, s), H3-27 (δ 0.790, s) and H3-26 (δ 0.856, s), similar to those reported for 8–11 [13,16,18–21], instead of those for the C-24 epimer (δ 0.925, s, H3-21; 0.805, s, H3-27; 0.853, s, H3-26) [23]. The structure of 3 was evidently established as (24S)-ergostane-1α,3β,5α,6β,11α-pentaol.

Furthermore, compound 4 was also obtained as an optically active, amorphous powder. Its formula C29H50O4 was deduced from the molecular ion at m/z 485.3532 [M + Na]+ in the HR-ESI-MS spectrum. A comparison of the 1H and 13C NMR spectra of 4 (Tables 1 and 2) and the co-isolated 10 revealed a great similarity with the exception of an appearance of a trisubstituted double bond (δH 4.87, d, J = 9.5 Hz; δC 131.3, d, 135.7, s) and an additional vinylic methyl group (δH 1.50, s) in the side chain. The double bond was placed at C-22, due to the proton spin system of H3-21/H-20/H-22 and the long-range correlations of H3-29/C-22, C-23 and C-24, as deduced from the 1H-1H COSY and the HMBC spectra, respectively. The downfield shift of H3-28 from δ 0.77 in 10 to δ 0.93 in 4 supported the above conclusion. The 1H NMR and 13C NMR assignments of the side chain were identical to those of model compounds [29–34]. An S absolute stereochemistry at C-24 in 4 was proposed by careful comparison of 1H NMR data with those of in dinosterol and its C-24 epimer [31], where the 1H signals for H3-21 (δ 0.921, d, J = 6.9 Hz), H3-27 (δ 0.779, d, J = 6.8 Hz) and H3-26 (δ 0.836, d, J = 6.5 Hz) were close to those of dinosterol (δ 0.919, d, J= 6.5 Hz, H3-21; 0.778, d, J = 6.5 Hz, H3-26; 0.837, d, J = 6.5 Hz, H3-27) rather than its C-24 epimer (δ 0.910, d, J = 6.8 Hz, H3-21; 0.772, d, J = 6.8 Hz, H3-26; 0.848, d, J = 6.5 Hz, H3-27). Therefore, the structure of 4 is (24S)-23,24-dimethylcholesta-22-en-3β,5α,6β,11α-tetraol.

Compound 5, an optically active, amorphous powder, had the same molecular formula (C29H50O4) as that of 4, as deduced by HR-ESI-MS. Its NMR spectra also showed the characteristic signals for the 3β,5α,6β-trihydroxyl moiety. However, the trisubstituted double bond (δH 4.87, d, J = 9.5 Hz; δC 131.3, d, 135.7, s) in 4 was replaced by a tetrasubstituted double bond (δ 149.0, s; 133.0, s) in 5 (Tables 1 and 3). The double bond was readily assigned as Δ17(20), due to the observation of the vinyl methyl singulate for H3-21 (δ 1.71, s) and its HMBC correlation with C-17, C-20 and C-22, and the HMBC correlation from H3-18 to C-17 provided further evidence. Meanwhile, the secondary alcohol at C-11 had to be assigned to C-15, due to the proton sequence from H-9 to H2-12 and from H-14 to H2-16, as established by the 1H-1H COSY experiment. The location of 15-OH was confirmed by the HMBC correlation from H-15 to both C-13 and C-17. The diagnostic interactions between H3-18 and H-15 in the NOESY spectrum indicated a β configuration of the proton. The R configuration at both C-23 and C-24 were suggested to be the same, biogenetically, as those in sarcosterol, 25-hydroxy sarcosterol and peridinosterol, produced by the soft corals, S. glaucum [35] and Sinularia mayi [36] and the symbiotic dinoflagellate [37], respectively. Biosynthetic studies suggested the same C-23 stereochemistry in sarcosterol and peridinosterol, due to the symbiotic relationship of soft coral and dinoflagellate [36]. The structure of compound 5 was tentatively determined to be (23R,24R)-23,24-dimethylcholesta-17(20)-en-3β,5α,6β-triol.

Compounds 6, 7 and 12–14 belong to the cluster of gorgosteroids, which are characterized as having a cyclopropane ring in the side chain. Compound 6 was isolated as an optically active, white amorphous solid. The molecular formula was established as C33H54O6 by HR-ESI-MS. The NMR data of 6 were almost identical to those of 12, displaying characteristic proton signals for the cyclopropane ring at the very high field (δH −0.13–0.46) (Table 3) in the 1H NMR spectrum. One methylene group (δH 2.33, dd, J = 12.2, 5.3 Hz, H-12β and 1.17, m, H-12α; δC 46.5) in 12 was replaced by a oxygenated
methine group ($\delta_c$ 75.0, CH; $\delta_H$ 3.95, d, $J = 3.0$ Hz, 1H) in 6. This hydroxyl group was assigned at C-12, due to HMBC correlation from H-18 to H-12 and the proton connectivity of H-9/H-11/H-12. The $\beta$ configuration of H-12 was deduced from its small coupling constant with H-11 and its nuclear Overhauser effect (NOE) effect with H-18. The structure of 6 was thus elucidated as 11$\alpha$-acetoxy-gorgostane-3$\beta$,5$\alpha$,6$\beta$,12$\alpha$-tetraol.

Table 3. The $^1$H NMR data of sterols 5–8 (400$^a$ or 500$^b$ Hz in CDCl$_3$; $J$ values are in Hz).

| Position | 5$^a$ | 6$^a$ | 7$^a$ |
|----------|------|------|------|
| 1        | 1.43 (m), 1.58 (m) | 1.27 (m), 1.80 (m) | 1.63 (m), 2.16 (m) |
| 2        | 1.55 (m), 1.82 (m) | 1.52 (m), 1.81 (m) | 1.47 (m), 1.85 (m) |
| 3        | 4.11 (m) | 4.06 (m) | 4.05 (m) |
| 4        | 1.63 (ov), 2.09 (ov) | 1.61 (ov), 2.10 (ov) | 1.63 (ov), 2.15 (ov) |
| 6        | 3.59 (br s) | 3.52 (br s) | 3.54 (br s) |
| 7        | 1.50 (m), 1.69 (m) | 1.53 (m), 1.76 (m) | 1.60 (m), 1.74 (m) |
| 8        | 1.72 (m) | 1.84 (m) | 1.85 (m) |
| 9        | 1.40 (m) | 2.08 (m) | 1.62 (m) |
| 11       | 1.40 (m), 1.49 (m) | 5.27 (dd, 11.4, 3.0) | 4.12 (dd, 10.2, 3.0) |
| 12       | 1.51 (m), 2.35 (m) | 3.95 (d, 3.0) | 5.27 (d, 3.0) |
| 14       | 1.74 (m) | 1.88 (m) | 1.72 (m) |
| 15       | 4.64 (d, 5.0) | 1.08 (m), 1.61 (m) | 1.12 (m), 1.63 (m) |
| 16       | 1.65 (m), 1.78 (m) | 1.31 (m), 2.05 (m) | 1.39 (m), 2.00 (m) |
| 17       | 1.96 (m) | 1.68 (m) | |
| 18       | 0.90 (s) | 0.76 (s) | 0.79 (s) |
| 19       | 1.20 (s) | 1.27 (s) | 1.29 (s) |
| 20       | 1.02 (m) | 1.01 (m) | |
| 21       | 1.71 (s) | 1.01 (br s) | 0.93 (d, 6.5) |
| 22       | 1.95 (dd, 13.3, 4.3), 0.23 (ov) | 0.13 (ddd, 9.3, 6.1, 5.7) | 2.25 (dd, 12.7, 9.5) |
| 23       | 1.80 (m) | | |
| 24       | 1.05 (m) | 0.23 (ov) | 0.24 (m) |
| 25       | 1.62 (m) | 1.50 (m) | 1.63 (m) |
| 26       | 0.83 (d, 6.7) | 0.85 (d, 6.5) | 0.85 (d, 6.9) |
| 27       | 0.89 (d, 8.1) | 0.94 (d, 6.5) | 0.95 (d, 6.5) |
| 28       | 0.79 (d, 6.9) | 0.93 (d, 6.7) | 0.94 (d, 6.4) |
| 29       | 0.70 (d, 6.8) | 0.89 (s) | 0.89 (s) |
| 30       | 0.46 (dd, 8.8, 4.2), 0.46 (dd, 8.7, 4.4), | −0.14 (d, 5.0) | −0.13 (d, 5.0) |
| COOCH$_3$ | | 2.08 (s) | 2.17 (s) |

$s$ = singlet; $d$ = doublet; $t$ = triplet; $m$ = multiplet; br $s$ = broad singlet; dd = doublet of doublets; ov = overlapped signals.

Compound 7 was isolated as an optically active, white amorphous solid. The compound had the same formula ($C_{32}H_{54}O_6$) as 6 on the basis of HR-ESI-MS. Inspection of NMR spectra for 7 revealed great similarity with those of 6 concerning the signals in rings A, C, D and the side chain. Analysis of the $^1$H-$^1$H COSY spectrum for the proton sequence H-9/H-11/H-12 and the HMBC correlation from
H$_{3}$-18 to C-12 led the assignment of 12-OAc and 11-OH in 7 instead of 11-OAc and 12-OH in 6. The configuration at chiral centers remains intact due to the analysis of related proton coupling constants and the NOE effects, establishing the structure of 7 as 12α-acetoxy-gorgostane-3β,5α,6β,11α-tetraol.

The isolation of an array of sterols demonstrates an excellent example of chemical diversity, displaying carbon skeletons of cholestanne, ergostane, gorgostane and 23,24-dimethyl cholestanne. The co-occurrence of these metabolites supported the biosynthesis proposal of side chains in dinosterol, peridinosterol and gorgosterol [32]. The methylation of the side chain of 1 may yield the side chain of 2, which further would be methylated to the side chain of the dinosterol derivative 4 and the peridinosterol analogues, 5 and 6. The side chain of 4 was then methylated to the side chain of sterols belonging to the gorgosterol family (6, 7, 12–14).

All isolated compounds, except 5, were tested in an agar diffusion assay for their antibacterial, antifungal and algicidal properties (Table 4). All the metabolites exhibited antibacterial activity against the Gram-negative bacterium, Escherichia coli, the Gram-positive bacterium, Bacillus megaterium, and antifungal activity against the fungi, Microbotryum violaceum and Septoria tritici. However, 1, 2, 6, 7 and 8 exhibited considerable growth inhibitory activity with regard to bacteria and/or fungi species. This suggests that the 11α-acetoxy group may increase both antibacterial and antifungal activities. The terminal-double bond and the cyclopropane side chain seem to also contribute to this bioactivity. None of the sterols showed inhibition of green alga Chlorella fusca.

Table 4. Agar diffusion assays for antibacterial, antifungal and antialgal activities$^{a,b}$.

| Compound | Escherichia coli | Bacillus megaterium | Microbotryum violaceum | Septoria tritici | Botrytis cinerea | Chlorella fusca |
|----------|-----------------|---------------------|------------------------|------------------|----------------|----------------|
| 1        | 12.0            | 10.0                | 6.5                    | 10.5             | 10.5           | 0              |
| 2        | 14.5            | 12.0                | 10.0                   | 7.5              | 5.5            | 0              |
| 3        | 7.5             | 4.5                 | 7.0                    | 10.5             | 5.5            | 0              |
| 4        | 6.0             | 7.0                 | 7.5                    | 9.0              | 0              | 0              |
| 6        | 9.5             | 10.0                | 8.5                    | 10.0             | 8.0            | 0              |
| 7        | 8.0             | 10.0                | 7.0                    | 4.5              | 12.0           | 0              |
| 8        | 7.0             | 7.5                 | 6.0                    | 6.5              | 0              | 0              |
| 9        | 4.5             | 4.5                 | 7.0                    | 6.5              | 0              | 0              |
| 10       | 6.0             | 8.5                 | 8.5                    | 4.5              | 0              | 0              |
| 11       | 6.0             | 7.0                 | 6.0                    | 6.0              | 0              | 0              |
| 12       | 7.0             | 8.0                 | 9.5                    | 10.0             | 5.0            | 0              |
| 13       | 10.0            | 6.5                 | 11.5                   | 7.5              | 0              | 0              |
| 14       | 5.0             | 4.0                 | 8.5                    | 7.0              | 0              | 0              |
| Penicillin | 17.5            | 22.5                | 11.5                   | 9.0              | 0              | 0              |
| Strepolin  | 8.0             | 7.5                 | 6.5                    | 9.0              | 0              | 0              |
| Ketoconazole | 10.0            | 10.0                | 20.5                   | 20.0             | 26.5           | 0              |
| Acetone  | 0               | 0                   | 0                      | 0                | 0              | 0              |

$^{a}$ 0.05 mg of the test or control substances dissolved in acetone were applied to a filter disc and sprayed with the respective test organism at a concentration of 10$^{5}$ cells/mL. $^{b}$ Radii of the zones of inhibition are given in mm (gi = growth inhibition); that is, some growth within the zone of inhibition. Otherwise, the inhibition zone was clear.
3. Experimental Section

3.1. General Experimental Procedures

Commercial silica gel (200–300 mesh, 10–40 mm; Yantai, China) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Precoated silica gel plates (Yantai, GF$_{254}$ plate, 10–40 mm) were used for analytical thin-layer chromatography (TLC). Spots were visualized by heating Si gel plates sprayed with 10% H$_2$SO$_4$ in EtOH. Semipreparative HPLC was carried out on an Agilent 1100 liquid chromatography equipped with a refractive index detector using a Zorbax 300 SB-C18 column (25 cm × 9.4 mm i.d.). Melting points were determined on an XT5-XMT apparatus. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. NMR spectra were recorded in CHCl$_3$ on a Bruker DRX 400 or 500 spectrometer, and the 2D NMR spectra were obtained using standard pulse sequences. Chemical shifts are reported in parts per million (δ), with use of the residual CHCl$_3$ signal (δ$_H$ 7.26 ppm) as an internal standard for $^1$H NMR and CDCl$_3$ (δ$_C$ 77.0 ppm) for $^{13}$C NMR; Coupling constants (J) are reported in Hz. $^1$H NMR and $^{13}$C NMR assignments were complemented by heteronuclear single quantum correlation (HSQC), HMBC, $^1$H-$^1$H COSY and nuclear Overhauser effect spectroscopy (NOESY) experiments. The following abbreviations were used to describe spin multiplicity: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br s = broad singlet; dd = doublet of doublets; ov = overlapped signals. HR-ESI-MS were recorded on a Micromass Quattro mass spectrometer. Optical rotations were measured in CHCl$_3$ with an Autopol IV polarimeter at the sodium D line (590 nm). Infrared spectra were recorded in thin polymer films on a Nexus 470 FTIR spectrophotometer (Nicolet, USA); peaks are reported in cm$^{-1}$.

3.2. Material and Methods

The soft coral Sarcophyton sp. was collected by hand using SCUBA off the coast of Weizhou Island, Guangxi Province of China, in October 2008, at a depth of 20 m and identified by Dr. Xiu-Bao Li, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (No. S-7-1) was deposited at the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai, China.

3.3. Extraction and Isolation

The frozen bodies of Sarcophyton sp. (1642 g, wet weight) were cut into small pieces, exhaustively extracted with acetone (5 L × 5) and then evaporated under reduced pressure. The acetone extract was resuspended in H$_2$O and partitioned with Et$_2$O 5 times. The concentrated Et$_2$O extract (21.6 g) was subjected to column chromatography (CC) on a Si gel using petroleum ether/acetone (99:1–1:10) as eluent to yield seven fractions (Fr. A–G) on the basis of thin layer chromatography (TLC) analysis. Fractions D–F were gel-filtered on Sephadex LH-20 (n-hexane/CH$_2$Cl$_2$/MeOH, 2:1:1), followed by CC on Si gel eluting with gradient CHCl$_3$/MeOH (50:1, 30:1, 20:1, 10:1) and further purification by semipreparative HPLC to yield 1 (2.3 mg, 28.4 min, 95% MeOH, 1.0 mL/min), 2 (3.3 mg, 29.6 min, 95% MeOH, 1.0 mL/min), Fr. 8 (33.7 mg, 33.9 min, 95% MeOH, 1.0 mL/min), 9 (25.5 mg, 31.2 min, 96% MeOH, 1.5 mL/min), 11 (1.2 mg, 25.1 min, 96% MeOH, 1.5 mL/min) and 12 (5.4 mg, 38.8 min,
95% MeOH, 1.0 mL/min) from fraction D, 3 (3.4 mg, 16.2 min, 95% MeOH, 1.0 mL/min), 6 (3.4 mg, 15.3 min, 95% MeOH, 1.0 mL/min) and 14 (0.5 mg, 16.7 min, 95% MeOH, 1.0 mL/min) from fraction E and 4 (1.4 mg, 17.4 min), 5 (13.2 mg, 20.1 min), 7 (9.5 mg, 18.7 min), 10 (11.4 mg, 18.0 min) and 13 (4.8 mg, 20.8 min) from fraction F (94% MeOH, 1.5 mL/min).

Compound 1: white amorphous solid; melting point (m.p.) 108–110 °C; [α]D 20 –34.5 (c 0.165, CHCl3); IR (film) νmax 3408 (board), 2927, 2869, 1712, 1376, 1263, 1027, 757; 1H and 13C-NMR data, see Tables 1 and 2; HRESI-MS m/z: 499.3397 [M + Na]+ (calcd. C29H48O5Na, 499.3399).

Compound 2: white amorphous solid; m.p. 173–175 °C; [α]D 20 –44.3 (c 0.115, CHCl3); IR (film) νmax 3401 (board), 2961, 2927, 2869, 1715, 1375, 1216, 1026, 801; 1H and 13C-NMR data, see Tables 1 and 2; HRESI-MS m/z: 511.3403 [M + Na]+ (calcd. C30H48O5Na, 511.3399).

Compound 3: white amorphous solid; m.p. 264–265 °C; [α]D 20 –35.3 (c 0.085, CHCl3); IR (film) νmax 3362 (board), 2956, 2927, 2870, 1464, 1378, 1067, 1042; 1H and 13C-NMR data, see Tables 1 and 2; HRESI-MS m/z: 467.3731 [M + H]+ (calcd. C29H50O5Na, 467.3737).

Compound 4: white amorphous solid; m.p. 245–247 °C; [α]D 20 –38.9 (c 0.095, CHCl3); IR (film) νmax 3412 (board), 2960, 2926, 2869, 1462, 1261, 1096, 801; 1H and 13C-NMR data, see Tables 1 and 2; HRESI-MS m/z: 485.3606 [M + Na]+ (calcd. C29H50O4Na, 485.3607).

Compound 5: white amorphous solid; m.p. 150–152 °C; [α]D 20 –12.1 (c 0.165, CHCl3); 1H and 13C-NMR data, see Tables 1 and 3; HRESI-MS m/z: 485.3609 [M + Na]+ (calcd. C29H50O4Na, 485.3607).

Compound 6: white amorphous solid; m.p. 170–172 °C; [α]D 20 –20.0 (c 0.17, CHCl3); IR (film) νmax 3434 (board), 2957, 2931, 2874, 1716, 1460, 1372, 1255; 1H and 13C-NMR data, see Tables 1 and 3; HRESI-MS m/z: 557.3819 [M + Na]+ (calcd. C32H54O6Na, 557.3818).

Compound 7: white amorphous solid; m.p. 150–152 °C; [α]D 20 –9.8 (c 0.395, CHCl3); IR (film) νmax 3422, 2956, 2926, 2872, 1719, 1459, 1375, 1254; 1H and 13C-NMR data, see Tables 1 and 3; HRESI-MS m/z: 557.3829 [M + Na]+ (calcd. C32H54O6Na, 557.3818).

3.4. Agar Diffusion Test for Biological Activity

An agar diffusion test was applied to determine the biological activities, as previously reported [38]. Briefly, compounds were dissolved in acetone at 1.0 mg/mL (2.1 mM for 1, 2.0 mM for 2, 2.3 mM for 3, 2.2 mM for 4 and 5, 1.9 mM for 6 and 7, 2.0 mM for 8, 2.3 mM for 9, 2.2 mM for 10, 2.3 mM for 11, 1.9 mM for 12, 2.1 mM for 13 and 2.0 mM for 14); 50 μL of the solution (0.05 mg) were pipetted onto a sterile filter disc (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism at a concentration of 10^5 cells/mL. The test organisms were bacteria, the Gram-negative bacterium, Escherichia coli, and the Gram-positive bacterium, Bacillus megaterium (both grown on NB medium), and fungi, Microbotryum violaceum, Septoria tritici and Botrytis cinerea (all grown on MPY medium), and the microalga, Chlorella fusca (grown on CP medium). Penicillin (1.0 mg/mL, 0.05 mg), strepolin (1.0 mg/mL, 0.05 mg) and ketoconazole (1.0 mg/mL, 0.05 mg) were used as positive controls. These microorganisms were chosen because (a) they are non-pathogenic and (b) had
in the past proved to be accurate as initial test organisms for antibacterial and antifungal activities [38,39]. Commencing at the outer edge of the filter disc, the radius of the zone of inhibition was measured in mm.

4. Conclusions

Chemical investigation of the soft coral Sarcophyton sp. from the South China Sea led to the isolation and structural elucidation of fourteen polyoxygenated steroids (1–14) with 3β,5α,6β-hydroxy moiety, demonstrating an excellent example of chemical diversity. These compounds displayed different levels of antibacterial and antifungal bioactivities in the in vitro bioassay. Preliminary structure-activity analysis suggests that the 11α-acetoxy group may increase both antibacterial and antifungal activities. The terminal-double bond and the cyclopropane moiety at the side chain may also contribute to the bioactivity. The interesting discovery may encourage further investigations on the sterols, the antibacterial and antifungal activity, and the structure-activity relationship.

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