Novel Polyhydroxysteroids from the Formosan Soft Coral *Sarcophyton glaucum*

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Two novel polyhydroxysteroids, (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 26-*n*-decanoate (**1**), (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 25,26-diacetate (**2**), and three known sterols (**3**-**5**), have been isolated from a Formosan soft coral *Sarcophyton glaucum*. The molecular structures of these compounds were determined by spectral methods. The cytotoxicity of these compounds toward various cancer cell lines has also been determined.

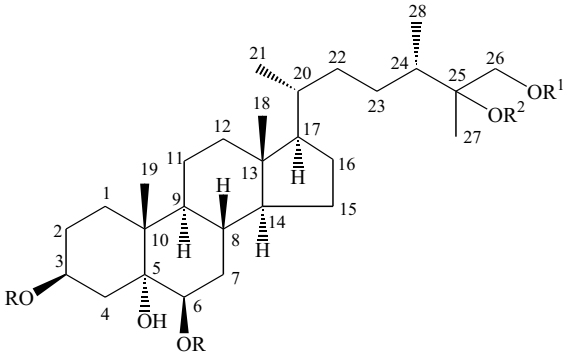
Keywords: *Sarcophyton glaucum*; Soft coral; Polyhydroxysteroids; Cytotoxicity.

INTRODUCTION

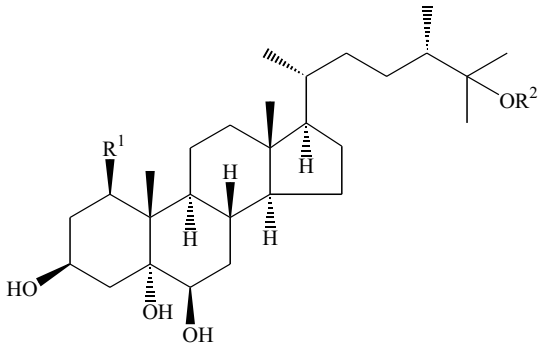
We have previously reported various types of bioactive metabolites from Formosan marine organisms including soft corals,[1] gorgonians,[2] and marine algae.[3] In our continuing search of bioactive natural products from marine organisms, a Formosan soft coral *Sarcophyton glaucum* Quoy & Gaimard was examined. Our investigation on the ethyl acetate extract of *S. glaucum* have led to the isolation of two novel steroids (**1**-**2**), which were characterized by extensive spectral analyses and by comparison of the spectral data with known compounds. Along with these two new metabolites, three known steroids (**3**-**5**) were also isolated. Cytotoxicity of these polyoxygenated steroids was described, too.

RESULTS AND DISCUSSION

Sterol **1** was obtained as a white gum. Its HREIMS established the molecular formula $C_{38}H_{68}O_6$ and thus requiring five degrees of unsaturation. The IR spectrum of **1** showed absorptions of ester carbonyls (ν_{max} 1731 cm^{-1}) and hydroxyl groups (ν_{max} 3400 cm^{-1}). The EIMS of **1** showed peaks at *m/z* 620 $[M]^+$, 566 $[M - 3\ H_2O]^+$, 493 $[M - C_9H_{19}]^+$, and 465 [M -



1 R = H, $R^1 = CO(CH_2)_8CH_3$, R^2 = H
2 R = H, $R^1 = R^2$ = Ac
6 R = $R^1 = R^2$ = H
7 R = $R^1 = R^2$ = Ac



3 R^1 = H, R^2 = Ac
4 $R^1 = R^2$ = H
5 R^1 = OH, R^2 = Ac

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$COC_9H_{19}]^+$, suggesting the presence of hydroxy and decanoyl groups in **1**. The ^{13}C NMR spectrum (Table 1) of **1** showed signals of five sp^3 oxygenated carbons, including one methylene at δ 69.8, two methines at δ 67.6 and 76.7, and two quaternary carbons at δ 74.2 and 76.7, and also exhibited the signal of an ester carbonyl carbon at δ 174.0. By the assistance of an HMQC experiment, it was found that the signals appearing at δ 3.54 (1H, brs) and 4.09 (1H, m) were due to the presence of two oxymethines. One triplet appearing at δ 2.08 (1H, J = 11.6 Hz) was assigned to H-4β, and two methyl protons at δ 0.67 (3H, s), 1.18 (3H, s) were due to H_3-18 and H_3-19 of a sterol, respectively. These peaks were considered to be the characteristic ^1H NMR signals of the 3β,5α,6β-trihydroxysteroids.[4-7] Two oxymethylene protons with *AB* spin coupling at δ 4.00 (1H, d, J = 11.3 Hz) and 4.10 (1H, d, J = 11.3 Hz) were due to an acylated methylene. The acyl group was assigned as an *n*-decanoyl group by inspection of the ^1H and ^{13}C NMR spectral data. A comparison of NMR data of **1** with those of **6**[4] suggested that **1** contains an acyl group at C-26 and the remaining tertiary hydroxyl has to be placed at C-25. The framework of **1** was further confirmed by an HMBC experiment (Fig. 1) which showed the following key correlations: H_3-18 to C-12, C-13, C-14, and C-17; H_3-19 to C-1, C-5, C-9, and C-10; H-6α to C-7; H_3-21 to C-17, C-20, and C-22; H_2-26 and H_2-30 to the ester carbonyl carbon, H_3-27 to C-24, C-25, and C-26; and H_3-28 to C-23. On the basis of the above observations, the structure of **1** was established unambiguously as (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 26-*n*-decanoate, and all three known metabolites **3**-**5**, isolated from the present investigation (later discussed), were found to possess 24*S* configuration in their structures.

Compound **2** was isolated as a white gum. Its HREIMS exhibited a molecular ion peak at *m/z* 550.3867, corresponding to a molecular formula of $C_{32}H_{54}O_7$, associated with six degrees of unsaturation. The EIMS of **1** showed peaks at *m/z* 550 $[M]^+$, 472 $[M - HOAc - H_2O]^+$, 454 $[M - HOAc - 2\ H_2O]^+$,

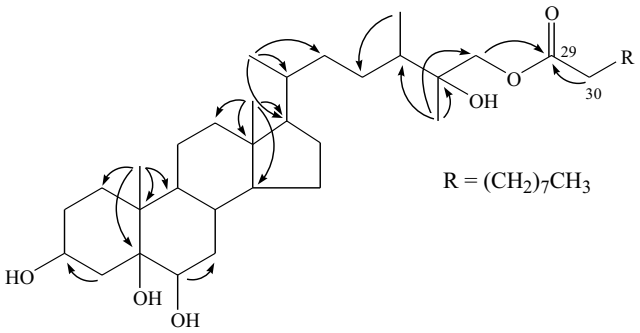

Fig. 1. Selective HMBC key correlations of steroid **1**.

Table 1. ^{13}C and Selective ^1H NMR Spectral Data for Steroids **1** and **2**

	1		**2**	
C/H	^1H[a]	^{13}C[b]	^1H[c]	^{13}C[d]
1		32.3 t[f]		32.4 t
2		30.8 t		30.8 t
3	4.09 (m)	67.6 d	4.14 (m)	67.6 d
4β	2.08 (t, 11.6)[e]	40.9 t	2.10 (t, 13.0)	40.7 t
5		76.7 s		76.7 s
6	3.54 (brs)	76.7 d	3.54 (brs)	76.7 d
7		34.6 t		34.5 t
8		30.1 d		30.2 d
9		45.8 d		45.8 d
10		38.3 s		38.3 s
11		21.1 t		21.1 t
12		39.9 t		39.9 t
13		42.7 s		42.7 s
14		55.8 d		55.9 d
15		24.1 t		24.1 t
16		28.1 t		28.1 t
17		55.8 d		55.8 d
18	0.67 (s)	12.1 q	0.68 (s)	12.1 q
19	1.18 (s)	16.9 q	1.19 (s)	16.9 q
20		36.2 d		36.1 d
21	0.91 (d, 6.8)	18.9 q	0.91 (d, 6.5)	18.9 q
22		34.5 t		34.5 t
23		27.9 t		27.5 t
24		41.1 d		38.4 d
25		74.2 s		85.4 s
26	4.00 (d, 11.3) 4.10 (d, 11.3)	69.8 t	4.42 (d, 12.0) 4.48 (d, 12.0)	65.7 t
27	1.11 (s)	20.7 q	1.19 (s)	18.1 q
28	0.94 (d, 6.8)	13.8 q	0.94 (d, 6.5)	14.0 q
29		174.0 s		
30	2.35 (t, 7.5)	34.3 t		
31		34.2 t		
32~36		29.2~29.7 t		
37		31.9 t		
38	0.88 (t, 6.9)	14.1 q		
25-acetate				
CH_3			2.08 (s)	18.1 q
CO				170.3 s
26-acetate				
CH_3			2.01 (s)	18.9 q
CO				170.8 s

[a] Spectra recorded at 400 MHz in $CDCl_3$. [b] Spectra recorded at 100 MHz in $CDCl_3$. [c] Spectra recorded at 500 MHz in $CDCl_3$. [d] Spectra recorded at 125 MHz in $CDCl_3$. [e] Multiplicities and J values (in Hz) in parenthese. [f] Multiplicities deduced by DEPT and indicated by usual symbols. Chemical shifts are downfield in ppm from TMS.

430 $[M - 2\ HOAc]^+$, and 412 $[M - 2\ HOAc - H_2O]^+$, suggesting the presence of at least two acetoxyl groups and two hydroxyl groups in compound **2**. Its ^1H NMR (Table 1) spec-

tral data revealed the presence of seven methyl groups, including two singlets at δ 0.68 (H_3-18) and 1.19 (H_3-19), two doublets at δ 0.91 (3H, J = 6.5 Hz), and 0.92 (3H, J = 6.0 Hz), which were assigned to H_3-21 and H_3-28, and also two acetyl methyls at δ 2.01 (3H, s) and 2.08 (3H, s). Two signals at δ 3.54 (1H, brs) and 4.14 (1H, m) were due to protons of the hydroxyl-bearing methines, H-6 and H-3, respectively. A triplet appearing at δ 2.10 (1H, J = 13.0 Hz) was attributed to H-4β of **2**. Two oxymethylene signals appearing at β 4.42 (1H, d, J = 12.0 Hz) and 4.48 (1H, d, J = 12.0 Hz) were due to H_2-26. The ^{13}C NMR spectral data of **2** (Table 1) showed signals of 32 carbons, including seven methyls, eleven methylenes, eight methines, and six quarternary carbons. Among them, five were oxygenated carbons, which include two methines (δ 67.6, and 76.7), two quarternary carbons (δ 76.7, and 85.4), and one oxymethylene (δ 65.7). These data were found to be very similar to those of 3β,5α,6β,25ξ-tetrahydroxysteroid (**1**), suggesting that **2** could be the acetyl derivative of **1**. The side chain of sterol **2** was further clarified by comparison of its ^{1}H and ^{13}C NMR data with those of a known sterol **7**.[5] Thus, the structure of **2** was established as (24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 25,26-diacetate.

Along with the above two new polyhydroxysteroids, three known sterols, (24*S*)-24-methylcholestane-3β,5α,6β,25ξ-tetrol 25-monoacetate (**3**),[5-8] (24*S*)-24-methylcholestane-3β,5α,6β,25ξ-tetrol (**4**),[5-8] and (24*S*)-24- methylcholestane-1β,3β,5α,6β,25ξ-pentol 25-monoacetate (**5**),[6-8] also have been isolated in this investigation. The structures of **3**-**5** were identified by comparison of their spectral (IR, MS, and NMR) data with those published previously.

The cancer cells growth inhibitory properties of sterols **1**-**5** were evaluated using P-388 (mouse lymphocytic leukemia), A549 (human lung carcinoma), and HT-29 (human colon carcinioma) cell lines. Sterol **3** was found to exhibit significant inhibitory activity against HT-29 (ED_{50} 0.6 μg/mL), but showed moderate cytotoxicity against the growth of P-388 (ED_{50} 12.6 μg/mL), and A549 (ED_{50} 6.6 μg/mL) cells. Sterol **6** exhibited moderate cytotoxicity against A549 (ED_{50} 8.0 μg/mL), and HT-29 (ED_{50} 10.3 μg/mL) cells, whereas sterols **1**, **2**, and **4** were found to be inactive ($ED_{50's}$ > 20 μg/mL) toward the above cancer cell lines.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined using a Fisher-Johns melting points apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. The NMR spectra were recorded on a Bruker AMX-400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. EIMS and FABMS were obtained with a VG Quattro GC/MS spectrometer. Si gel 60 (Merck, 230-400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC.

Organism

Sarcophyton glaucum was collected by hand *via* SCUBA on the coast of Green Island, Taiwan in July, 1998, at a depth of 8 to 15 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Resources, National Sun Yat-Sen University.

Extraction and Separation

The marine soft coral, *Sarcophyton glaucum* (860 g, dry wt.) was exhaustively extracted with EtOAc. The combined EtOAc extract was then filtered and concentrated under vacuum to provide a brownish semisolid residue (20.6 g). The above residue was triturated with hexanes to give the hexanes-soluble and insoluble layers. Cytotoxicity testing revealed that only the hexanes-insoluble layer (14.2 g) exhibited cytotoxicity against the growth of P-388 cells with an ED_{50} of 14.3 μg/mL. This layer was then separated by column chromatography on Si gel. Elution was performed with EtOAc-hexanes (stepwise, 0-100% EtOAc) to yield 20 fractions. Fraction 6 eluted with 50% EtOAc was further chromatographed on Si gel using EtOAc-hexanes gradient to yield **1** (2.1 mg) and impure **2**. The impure sterol **2** was further chromatographed on Si gel using EtOAc-CH_2Cl_2 (1:1) to yield pure **2** (2.0 mg). Fraction 7 eluted with 50% EtOAc was further chromatographed on Si gel using EtOAc-CH_2Cl_2 (1:20) to yield **4** (2.1 mg) and **5** (2.0 mg). Fraction 8 eluted with 70% EtOAc was further chromatographed on Si gel using MeOH-CH_2Cl_2 (1:15) to yield **3** (8.5 mg).

(24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 26-*n*-decanoate (1)

White gum; $[\alpha]_D^{30}$ -11.2° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3400, 1731 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 1; EIMS *m/z* (rel. int %) 620 (0.1, $[M]^+$), 566 (0.5), 493 (0.6), 465 (0.4); HREIMS *m/z* 620.5020 (calcd for $C_{38}H_{68}O_6$, 620.5018).

(24*S*)-24-methylcholestane-3β,5α,6β,25ξ,26-pentol 25,26-diacetate (2)

White gum; $[\alpha]_D^{30}$ -3.0° (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3400, 1733 cm^{-1}; ^1H and ^{13}C NMR data in Table 1; EIMS *m/z* (rel. int %) 550 (0.2, $[M]^+$), 472 (0.2), 454 (0.3), 430 (1.2), 412 (0.5), 285 (2.4); HREIMS *m/z* 550.3867 (calcd for $C_{32}H_{54}O_7$, 550.3871).

(24*S*)-24-methylcholestane-3β,5α,6β,25ξ-tetrol 25-monoacetate (3)

White powder; $[\alpha]_D^{30}$ -19.0° (*c* 0.1, $CHCl_3$); mp 247 °C; IR (neat) ν_{max} 3400, 1730 cm^{-1}; EIMS *m/z* (rel. int %) 474 (0.04, $[M - H_2O]^+$), 456, 438 (0.1), 432 (2.7), 414 (5.1), 396 (2.6), 287 (2.6). ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported.[5,6]

(24*S*)-24-methylcholestane-3β,5α,6β,25ξ-tetrol (4)

White powder; $[\alpha]_D^{30}$ -18.7° (*c* 0.1, $CHCl_3$); mp 252-253 °C; IR (neat) ν_{max} 3400 cm^{-1}; EIMS *m/z* (rel. int %) 432 (3.4, $[M - H_2O]^+$), 414 (6.4), 399 (2.6), 396 (3.0), 287 (3.8). ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported.[5]

(24*S*)-24-methylcholestane-1β,3β,5α,6β,25ξ-pentol 25-monoacetate (5)

White powder; $[\alpha]_D^{30}$ -85.7° (*c* 0.1, $CHCl_3$); mp 263-265 °C; IR (neat) ν_{max} 3440, 1730 cm^{-1}; EIMS *m/z* (rel. int %) 508 (0.1, $[M - H_2O]^+$), 430 (0.3), 412 (1.0), 285 (3.4). ^1H and ^{13}C NMR spectral data were found to be in full agreement with those reported.[7]

Cytotoxicity Testing

P-388 cells were kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 cells were purchased from the American Type Culture Collection. The cytotoxic activities of tested compounds against the above three cancer cells were assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to a procedure described previously.[9]

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REFERENCES

1. (a) Sheu, J.-H.; Wang, G.-H.; Sung, P.-J.; Duh, C.-Y.; Chiang, M. Y. *Tetrahedron*, **2001**, *57*, 7639. (b) Wang, G.-H.; Sheu, J.-H.; Chiang, M. Y.; Lee, T.-J. *Tetrahedron Lett*. **2001**, *42*, 2333. (c) Sheu, J.-H.; Chang, K.-C.; Duh, C.-Y. *J. Nat., Prod*. **2000**, *63*, 149. (d) Sheu, J.-H.; Chang, K.-C.; Sung, P.-J.; Duh, C.-Y.; Shen, Y.-C. *J. Chin. Chem. Soc*. **1999**, *46*, 253. (e) Sheu, J.-H.; Yeh, T.-H. *J. Chin. Chem. Soc.* **1991**, *38*, 397.
2. Sung, P.-J.; Su, J.-H.; Duh, C.-Y.; Chiang, M. Y.; Sheu, J.-H. *J. Nat. Prod.* **2001**, *64*, 318, and previous reports in this series.
3. (a) Sheu. J.-H.; Wang, G.-H.; Sung, P.-J.; Duh, C.-Y.; Shen, Y.-C. *J. Nat. Prod*. **1999**, *63*, 224. (b) Sheu, J.-H.; Wang, G.-H.; Sung, P.-J.; Chiu, Y.-H.; Duh, C.-Y. *Planta Med*. **1997**, *63*, 571. (c) Sheu, J.-H.; Huang, S.-Y.; Wang, G.-H.; Duh. C.-Y. *J. Nat. Prod*. **1997**, *60*, 900.
4. Kobayashi, M.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1983**, *31*, 4127.
5. Raju, B. L.; Subbaraju, G. V.; Reddy, M. C.; Rao, D. V.; Rao, C. B.; Raju, V. S. *J. Nat. Prod*. **1992**, *55*, 904.
6. Dong, H.; Gou, Y.-L.; Kini, R. M.; Xu, H.-X.; Chen, S.-X.; Teo, S. M.; Paul, P.-H. *Chem. Pharm. Bull*. **2000**, *48*, 1087.
7. Kobayashi, M.; Hayashi, T.; Hayashi, K.; Tanabe, M.; Nakagawa, T.; Mitsuhashi, H. *Chem. Pharm. Bull.* **1983**, *31*, 1848.
8. Anjaneyulu, A. S. R.; Gowri, P. M.; Venugopal, M. J. R.; Sarada, P.; Murthy, M. V. R. K.; Rao, G. V.; Murthy, P. S. N.; Rao, C. V.; Kumar, G. *J. Indian Chem. Soc*. **1999**, *76*, 651.
9. Sheu, J.-H.; Liaw, C.-C.; Duh, C.-Y. *J. Nat. Prod.* **1995**, *58*, 1521.