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Steroidal and α-Tocopherylhydroquinone Glycosides from two Soft Corals *Cladiella hirsuta and Sinularia nanolobata*†

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Two new pregnane glycosides, hirsutosterosides A and B (**1** and **2**), as well as two new α-tocopherylhydroquinone glycosides, cladophenol glycosides A and B (**3** and **4**) were isolated from a soft coral *Cladiella hirsuta*. In addition, a new steroidal glycoside, lobatasteroside A (**5**) along with six known steroids (**6–11**), were discovered from the other soft coral *Sinularia nanolobata*. Their structures were elucidated by extensive spectroscopic analysis. Cytotoxicities of the isolates against a limited panel of cancer cell lines were reported. The inhibitory activities for superoxide anion generation and elastase release for these compounds were also examined to evaluate the anti-inflammatory potential.

Keywords: *Cladiella hirsuta*; *Sinularia nanolobata*; glycoside

Introduction

Soft corals have been proven to be the important sources for bioactive natural products.[1] *Sinularia* species is one of the most widely distributed soft corals.[2] It constitutes sesquiterpenes,[3,4] diterpenes,[5,6] and steroids[7,8] endowed with structural diversity and a wide spectrum of biological activities. Eunicellin-based diterpenes are known to be enriched in *Cladiella* species and some of the eunicellins exhibited potent anti-inflammatory and cytotoxic activities.[9–12] Polyoxygenated steroids occur mainly in marine invertebrates; however, glycosides are elaborated as a minor group by marine soft corals.[13] Some of the metabolites from our prior investigations on the soft corals *Cladiella hirsuta and Sinularia nanolobata* showed potent cytotoxic and anti-inflammatory activities. Thus, we continued our studies on these two marine organisms in an attempt to discovering other biologically active natural products, leading to the isolation of two new pragnane glycosides (**1** and **2**) and two new α-tocopherylhydroquinone glycosides (**3** and **4**) from *C. hirsuta* as well as one new steroidal glycoside (**5**) and six known steroids (**6–11**) from *S. nanolobata* (Chart 1). Their structures have been established by extensive spectroscopic analysis, including 2D NMR (^{1}H–^{1}H COSY, HMBC, HSQC, and NOESY) correlations. The absolute configurations of sugar residues were determined by HPLC analysis with a pre-column derivatization reaction to produce a chromophore.[14] Moreover, the cytotoxic activities of the isolates were evaluated by the inhibition of cell proliferation, including, K562 (human erythromyeloblastoid leukemia), P388 (murine leukemia), HT-29 (human colon adenocarcinoma), and A549 (human lung adenocarcinoma). The anti-inflammatory activity was evaluated by suppressing superoxide aniongeneration and elastase release by human neutrophils in response to fMLP/CB. Compounds **1**–**4** and **8** were shown to exhibit *in vitro* cytotoxicity against some of the above cancer cell lines and **1**, **7**, and **8** displayed interesting anti-inflammatory activities.

Results and discussion

The EtOAc-soluble portions of the EtOH extracts were repeatedly chromatographed over silica gel,

Sephadex LH-20, and RP-HPLC to afford four new glycosides, hirsutosterosides A and B (**1** and **2**) and cladophenol glycosides A and B (**3** and **4**) from *C. hirsuta*. In addition, one new steroidal glycoside, lobatasteroside A (**5**), along with six known steroids (**6**–**11**) were isolated from the EtOAc extract of *S. nanolobata* by the same manner. The known steroids were identified as 24-methylenecholest-5-en-3β-ol-3-*O*-α-L-fucoside (**6**),[15] 24-methylenecholest-5-ene-3β,16β-diol-3-*O*-α-L-fucoside (**7**),[13,16] 5β,6β-epoxy-3β,11-dihydroxy-24-methylene-9,11-secocholestan-9-one (**8**),[17] crassarosterol A (**9**),[16] 3β,7α,16β- trihydroxyergosta-5,24(28)-diene (**10**),[18] and 3β,7β,16β-trihydroxyergosta-5,24(28)-diene (**11**)[18] by comparison of their NMR data and $[\alpha]_D$ values with literature data.

The HRESIMS of hirsutosteroside A (**1**) showed a pseudomolecular ion peak at *m/z* 569.3087 $[M+Na]^+$, consistent with a molecular formula of $C_{31}H_{46}O_8$ and requiring nine degrees of unsaturation. The IR spectrum revealed the presence of ester carbonyl (1744 cm^{-1}) and hydroxy groups (3393 cm^{-1}) in **1**. The former was identified as two acetoxy groups according to the carbon resonances at δ_C 170.8 (C), 170.6 (C), 20.9 (CH_3), and 20.8 (CH_3), and proton signals at δ_H 2.12 and 2.07 (each 3H, s) (Table 1). The ^{13}C NMR and DEPT spectroscopic data (Table 1) showed the presence of 31 carbon signals, including four methyls, 10 methylenes, 12 methines, and five quaternary carbons. The ^{13}C NMR spectra showed characteristic signals for two double bonds [δ_C 140.3 (C), 122.1(CH); 139.7 (CH), 114.6 (CH_2)] and a hexose [δ_C 100.9 (CH), 74.2 (CH), 74.6 (CH), 70.6 (CH), 71.9 (CH), 62.5 (CH_2)]. The aforementioned spectroscopic data implied that **1** might be a pregnane with an acetyl-substituted hexose residue. Acid-catalyzed hydrolysis of **1**, followed by HPLC analysis of the corresponding *o*-tolythiocarbamate derivatives, allowed the identification of the hexose residue to be D-glucose.[14] The anomeric proton at δ_H 4.40 (1H, J = 8.0 Hz) and the HMBC correlation from H-5' to C-1′ suggested the presence of a β-glucopyranose moiety. The ^1H–^1H COSY correlations allowed the assignment of four separated spin systems (Figure 1). The pregnane nucleus was confirmed by the HMBC correlations from H_3-19 to C-1, C-5, C-9, and C-10, H_3-18 to C-12, C-13, C-14, C-17, and H-6 to C-4. The D-glucopyranose residue attached at C-3 was

assigned according to an HMBC correlation from the anomeric proton (H-1′) to C-3 of the pregnane nucleus. The 4′,6′-di-acetyl groups on the sugar moiety were assigned by the HMBC correlations from both H_2-6′ and H-4′ to the carbonyl carbons. The relative configurations of **1** were determined by the correlations observed in a 2D NOESY experiment (Figure 2). The NOE correlations between H_3-18/H-20, H_3-18/H-11, H_3-19/H-11, and H_3-19/H-8 revealed the β-orientations of H_3-18, H_3-19, H-8, and the vinyl group. The multiplet splitting for H-3 [δ_H 3.56 (1H, m)] suggested that this proton was axially orientated.[19] On the basis of the above elucidation, the structure of **1** was established as shown in formula 1.

Hirsutosteroside B (**2**) has the same molecular formula as that of **1** according to its (+)-HRESIMS and ^{13}C NMR data. The ^{13}C NMR and DEPT spectra of **2** were similar to those of **1**, with small differences observed only in the hexose ring. By excluding the pregnane moiety, the remaining ten carbons comprised of a glucose residue [δ_C 101.3 (CH), 71.9 (CH), 77.5 (CH), 69.2 (CH), 74.2 (CH), 63.1 (CH_2)] and two acetyls [δ_C 172.4 (C), 172.6 (C), 20.9 (CH_3), 21.0 (CH_3)] were suggested (Table 1). A comparison of ^{1}H NMR spectroscopic data of **2** with those of **1** revealed that the two acetoxy groups should be located at C-3 and C-6 of the glucopyranose residue, as they appeared in the downfield region [δ_H 4.92, 1H, t, J = 9.0, H-3; 4.43, 1H, dd, J = 12.5, 4.5 Hz, H-6a; 4.30, 1H, dd, J = 12.5, 2.5 Hz, H-6b]. In the same manner, acid-catalyzed hydrolysis of **2** was found to liberate D-glucose as the sugar component.

The HRESIMS of cladophenol glycoside A (**3**) exhibited a pseudomolecular ion peak at *m*/*z* 619.4189 $[M+Na]^+$, consistent with a molecular formula of $C_{34}H_{60}O_8$, and requiring five degrees of unsaturation. The ^{1}H NMR spectrum of **3** showed an anomeric proton at δ_H 4.37 (1H, d, J = 8.0 Hz), which was suggested to arise from a β-xylopyranose moiety resonating at δ_C 104.4 (CH, C-1″), 74.3 (CH, C-2″), 76.3 (CH, C-3″), 69.9 (CH, C-4″), 65.6 (CH, C-5″) (Table 2),[20] and also was confirmed by the HMBC correlation from H-5″ to C-1″ and the NOE correlations of H-1″/H-3″ and H-2″/H-5″ (Figure 2). Likewise, the D-xylose residue was deduced according to the above described RP HPLC analysis of its *o*-tolythiocarbamate. Considering the molecular formula and degree of unsaturation,

the remaining 29 carbons with characteristic signals of a phenyl ring and eight methyls were suggested to be an α-tocopherylhydroquinone derivative.[21,22] The HMBC correlations from the anomeric proton (H-1″) to the sp^2 quaternary carbon (δ_C 145.8, C) led to the assignment of the xylopyranose residue at C-4 (Figure 1). A hydroxy group was assigned at C-7' of the polyprenyl side chain according to the HMBC correlations from 19'-CH_3 to C-6', C-7' (δ_C 73.0, C), and C-8'. Biogenetically, natural α-tocopherylhydroquinones are originated from reduction of the corresponding α-tocopherylquinones, which are the result of a two-electron oxidation of α-tocopherols.[23] Thus, the absolute configurations at C-3' and C-11' of **3** were suggested to be the same as those of natural (3'*R*,7'*R*,11'*R*) α-tocopherol. However, the configuration at C-7' of the side chain remained unresolved.

Cladophenol glycoside B (**4**) had a molecular formula of $C_{38}H_{66}O_9$ as established by HRESIMS. The ^1H and ^{13}C NMR spectroscopic data of **4** were similar to those of **3**, except that an additional *n*-butyryl group was observed at the xylopyranose residue. This butyrate moiety was assigned at C-3″ of the sugar residue based on the HMBC correlation from H-3″ to the carbonyl carbon at δ_C 174.6 (C). Accordingly, **4** was deduced as a 3″-*n*-butyryl derivative of **3**.

Lobatasteroside A (**5**) had a molecular formula of $C_{34}H_{56}O_7$ with seven degrees of unsaturation. The NMR data of **5** revealed the presence of a 6-deoxyhexose, which was identified as L-fucose after acid-catalyzed hydrolysis and the subsequent analysis by the aforementioned HPLC method. The anomeric carbon was assigned the α-configuration according to the coupling constant of H-1' (J = 2.4 Hz) (see Experimental Section).[8] The ^1H NMR data showed five methyl signals [δ_H 0.92 (3H, s), 1.17 (3H, s), 1.03 (9H, d, J = 6.8 Hz)], an endocyclic double bond [5.40 (1H, d, J = 3.2 Hz), and a *gem*-disubstituted double bond [4.76 (1H, br s) and 4.70 (1H, br s)], reminiscent of crassarosterosides.[8] A comprehensive comparison of NMR spectroscopic data of **5** with those of crassarosterosides A–C revealed that **5** is the deacetyl derivative of crassarosterosides. The postulated gross structure of **5** was confirmed by detailed analysis of 2D NMR data, too.

The cytotoxicity of compounds **1–11** against a panel of four cancer cell lines was evaluated. The results are shown in Table 3. The isolates were found to exhibit cytotoxicity toward the four cancer cell lines (K562, P388, HT-29, and A549) with IC_{50} values ranging from 10.2 to 39.3μM. Compounds **1** and **5–11** were also evaluated for anti-inflammatory activity by suppressing superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB. The result revealed that **1** did not exhibit inhibition activity toward superoxide anion generation (IC_{50} > 10 μM), but significantly inhibited the fMLP/CB-induced elastase release with IC_{50} values of 4.1 ± 0.1 μM. Compound **7** showed moderate activities toward superoxide anion generation and elastase release with IC_{50} values of 18.6 ± 1.5 and 10.1 ± 0.8 μM, respectively, while compound **8** significantly inhibited superoxide anion generation (6.6 ± 0.6 μM) and elastase release (2.9 ± 0.5μM).

Conclusion

Five new glycosides, hirsutosterosides A and B (**1** and **2**), cladophenol glycosides A and B (**3** and **4**) and lobatasteroside A (**5**) along with six known steroids (**6–11**) were characterized from the soft coral *C. hirsute* and *S. nanolobata*. Among the isolates, compounds **3** and **4** were the first example of tocopheryl xyloside derivatives. α-Tocopheryl is known to possess potent *in vitro* antioxidant activity; however, its related hydroquinones are considered as potential cytotoxic agents.[24] Our present study revealed that the related hydroquinones **3** and **4** exhibited cytotoxicity toward a limited panel of

cancer cell lines, which is consistent with findings of the previous study.[24]

Experimental section

General experimental procedures

Optical rotations were recorded on a JASCO P-1020 polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. NMR spectra were recorded on a Varian 400MR NMR (400 MHz) and a Varian Unity INOVA500 FT-NMR (500 MHz) instrument. The chemical shifts were referenced to the residue solvent signal of $CDCl_3$ at δ_H 7.265 ppm and δ_C 77.0. ESIMS and HRESIMS were recorded on a Bruker APEX II mass spectrometer. Silica gel 60 (230−400 mesh, Merk) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Precoated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm, Merck) were used for TLC analyses. High-performance liquid chromatography (HPLC) for purification of compounds was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector and a semi-preparative reversed-phase column (Supelco C18 column (250 × 21.2 mm, 5 μm).

Animal material

The soft corals *Cladiella hirsuta Sinularia nanolobata* were collected by hand using SCUBA from a depth of 10 m off the coast of Sianglu Islet (23°32' N, 119°38' E) in the region of Penghu Islands, in June 2008 and at Jihui Fishing Port (23°07' N, 121°23' E), Taitung county, Taiwan, in March 2013,

respectively. They were identified by Prof. C.-F. D. (National Taiwan University, Taiwan). Two voucher specimens (PI-20080610-17 and JiH-201303) were deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and isolation

The frozen materials of *C. hirsuta* (3.1 kg, wet wt) were cut into pieces and extracted with EtOH (3L × 2). The extract was concentrated and subsequently partitioned between EtOAc and H_2O. The EtOAc layer was dried with anhydrous Na_2SO_4 and concentrated to yield a residue (32.8 g). Subsequently, this residue was chromatographied on silica gel, eluting with EtOAc in hexane (0%–100%, gradient) and then MeOH in EtOAc (0%–100% MeOH, gradient) to yield 25 fractions. Fraction 18 was chromatographed over a Sephadex LH-20 column using acetone as the mobile phase to afford four subfractions (subfr A1–A4). Subfr A2 was separated by reversed-phase HPLC (ACN–H_2O, 66%) to afford compound **4** (3.1 mg). Fraction 19 was fractionated over a Sephadex LH-20 column, using acetone as the mobile phase, to afford four subfractions (B1–B4). Compound **1** (6.7 mg) was obtained from subfraction B2, using reversed-phase HPLC (MeOH–H_2O, 90%). Similarly, compound **2** (4.2 mg), obtained from subfraction B1, was purified by reversed-phase HPLC (MeOH–H_2O, 90%). Fraction 23 was separated by silica gel column chromatography (EtOAc–hexane, 20% to 90%) and followed by reversed-phase HPLC (MeOH–H_2O, 86%) to afford **3** (2.3 mg).

The tissues of *S. nanolobata* (3.1 kg wet wt) were treated similarly as described above to afford a residue (27.9 g). The residue was subjected to a silica gel column using gradient elution (acetone-hexane, 2% to 100%, and then MeOH/acetone, 2% to 100%) to afford 18 fractions. Fraction 13 was chromatographied by a Sephadex LH-20 column to give seven subfractions (subfr 13a to 13g). Subfr 13c was purified by a silica gel column (acetone-hexane, gradient, 10% to 100%),

and reverse-phase HPLC (MeOH-H_2O, 90%), resulting in compounds **9** (3.8 mg), **10** (6.0 mg) and **11** (5.3 mg). Fraction 15 was fractionated by a Sephadex LH-20 column (pure acetone), a silica gel column (gradient, acetone-hexane, 20% to 100%), and followed by reverse-phase HPLC (MeOH-H_2O, 90%) to give compounds **6** (3.1 mg) and **8** (14.3 mg). Compounds **5** (6.5 mg) and **7** (4.1 mg) was obtained from fraction 18 using LH-20 chromatography (acetone) and reverse-phase HPLC (MeOH-H_2O, 80%).

Hirsutosteroside A (1). White amorphous solid; $[\alpha]_D^{25}$ –54 (*c*0.1, $CHCl_3$); IR (neat) ν_{max} 3393, 2925, 1744, 1644, 1548, 1462, 1374, 1258, and 1030 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 569 $[M + Na]^+$; HRESIMS *m/z* 569.3087 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_8Na$, 569.3090).

Hirsutosteroside B (2). White amorphous solid; $[\alpha]_D^{25}$ –40 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3445, 2924, 1739, 1644, 1516, 1462, 1372, 1245, and 1034 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 569 $[M + Na]^+$; HRESIMS *m/z* 569.3087 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_8Na$, 569.3090).

Cladophenol glycoside A (3). Colorless oil; $[\alpha]_D^{25}$ +7 (c 0.66, $CHCl_3$); IR (neat) ν_{max} 3412, 2931, 1706, 1531, 1463, 1385, and 1043 cm^{-1}; ^{13}C and ^1H NMR data, see Table 2; ESIMS *m/z* 619 $[M + Na]^+$; HRESIMS *m/z* 619.4189 $[M + Na]^+$ (calcd for $C_{34}H_{60}O_8Na$, 619.4186).

Cladophenol glycoside B (4). Colorless oil;$[\alpha]_D^{25}$ +24 (c 0.91, $CHCl_3$); IR (neat) ν_{max} 3413, 2938, 1725, 1461, 1375, 1249, 1188, and 1076 cm^{-1}; ^{13}C and ^1H NMR data, see Table 2; ESIMS *m/z*619 $[M + Na]^+$; HRESIMS *m/z*619.4189 $[M + Na]^+$ (calcd for $C_{34}H_{60}O_8Na$, 619.4186).

Lobatasteroside A (5). white amorphous solid; $[\alpha]_D^{25}$ –40 (c 1.64, $CHCl_3$); IR (KBr) vmax 3420, 2939, 1640, 1461, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.40 (1H, d, *J* = 3.2 Hz, H-6), 5.00 (1H, d, *J* = 2.4 Hz, H-1'), 4.76 (1H, br s, H-28a), 4.70 (1H, br s, H-28b), 4.40 (1H, br s, H-16), 4.04 (1H, m, H-11), 4.01 (1H, m, H-5'), 3.78 (1H, br s, H-4'), 3.75 (2H, overlapped, H-2' and H-3'), 3.48 (1H,

m, H-3), 2.56 (1H, br d, *J* = 12.4 Hz, H-1β), 2.32 (1H, m, H-4a), 2.29 (1H, m, H-12a), 2.24 (2H, m, H-4a and H-15α), 2.22 (1H, m, H-25), 2.15 (1H, m, H-23a), 1.95 (1H, m, H-8), 1.85 (1H, m, H-7a), 1.82 (1H, m, H-2a), 1.67 (1H, m, H-22a), 1.64 (2H, m, H-2b and H-20), 1.57 (1H, m, H-7b), 1.49 (1H, m, H-23b), 1.25 (3H, d, *J* = 6.8 Hz), 1.20 (1H, m, H-22b), 1.17 (3H, s, H_3-19) 1.17 (3H, m, H-1α, H-12b, and H-15β), 1.08 (1H, m, H-17), 1.03 (9H, d, *J* = 6.8 Hz, H_3-21, H_3-26, and H_3-27), 0.98 (2H, m, H-9 and H-14), 0.92 (1H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.8 (C, C-24), 140.5 (C, C-5), 121.7 (CH, C-6), 106.3 (CH_2, C-28), 97.0 (CH, C-1'), 77.8 (CH, C-3), 72.5 (CH, C-16), 71.7 (CH, C-4'), 71.6 (CH, C-2'), 69.2 (CH, C-3'), 68.8 (CH, C-11), 65.9 (CH, C-5'), 61.6 (CH, C-17), 56.8 (CH, C-9), 53.6 (CH, C-14), 51.4 (CH_2, C-12), 42.9 (C, C-13),39.2 (CH_2, C-4), 39.1 (CH_2, C-1), 38.2 (C, C-10), 36.2 (CH_2, C-15), 34.7 (CH_2, C-22), 34.0 (CH, C-25), 31.8 (CH_2, C-7), 31.2 (CH, C-8), 31.1 (CH_2, C-23), 29.7 (CH_2, C-2), 29.6 (CH, C-20), 21.9 (CH_3, C-27); 21.8 (CH_3, C-26), 19.0 (CH_3, C-19), 18.2 (CH_3, C-21), 16.1 (CH_3, C-6') 14.0 (CH_3, C-18); ESIMS *m/z* 599 $[M + Na]^+$; HRESIMS *m/z* 599.3921 $[M + Na]^+$ (calcd for $C_{34}H_{56}O_7Na$, 599.3918).

Determination of sugar configuration

The absolute configuration of the sugar moiety was determined following a modified HPLC method.[14] Each compound (0.4 mg) was hydrolyzed in 1N HCl at 90 °C in a sealed tube for 5 h. The aqueous layer was neutralized with Amberlite IRA400 (OH), and dried in vaccum. The residue was treated with L-cysteine methyl ester in pyridine and heated at 60 °C for 1h. After cooling, the reaction mixture was added *o*-tolylisothiocyanate at room tempterature for additional 1h to yield the corresponding *o*-tolythiocarbamate derivatives, which was analyzed by HPLC (PurospherSTAR RP-8e column; 5 μm, 250 × 4.6 mm) and detected at 250 nm. Authentic D-glucose, L-glucose,

D-xylose, L-xylose, D-fucose, and L-fucose were found to elute at 43.3, 41.3, 45.1, 44.4, 45.1, and 50.0 min, respectively, while the sugar residues for compounds **1**–**4** were determined as D-glucose for both **1** (43.2 min) and **2** (43.2 min), D-xylose for both **3** (45.0 min) and **4** (45.1 min), and L-fucose for **5** (49.9 min) by comparing authentic samples.

Cytotoxicity testing

Cytotoxicity assays were performed according to the published protocol, Alamar Blue assay.[25,26] Cell lines (K562, P388, HT-29, and A549) were obtained from the American Type Culture Collection (ATCC). Cancer cells were plated into a 96-well microtiter plate with clear flat bottoms (Thermo Scientific Nunc MicroWell plate) at densities of $5 \times 10^3 - 1 \times 10^4$ cells per well and were incubated in a humidified, 5% CO_2 atmosphere incubator at 37˚C. After a 15h culture, the test compounds in DMSO solutions were added. After 3 days in culture, attached cells were incubated with Alamar Blue (10 μL/well, 4 h). The absorbency at 595 nm was then measured using a microplate reader. The IC_{50} values represent the concentration of tested compounds that reduced cell growth by 50% under the experimental conditions.

In vitro anti-inflammatory assay

The human neutrophils were isolated using a standard method of dextran sedimentation and Ficoll centrifugation.[27,28] As previously described procedures, the assay of superoxide anion generation was

conducted according to the SOD-inhibitable reduction of ferricytochrome *C*. The elastase release experiment was performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the enzyme substrate. LY294002, a phosphatidylinositol-3-kinase inhibitor, was used as a positive control for inhibition of superoxide anion generation and elastase release with IC_{50} 0.6 ± 0.1 and 1.2 ± 0.3 μg/mL.[29]

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Notes and references

(1) J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro and M. R. Prinsep, *Nat. Prod. Rep.*, 2015, **32**, 116.

(2) H. N. Kamel and M. Slattery, Pharm. Biol., 2005, **43**, 253.

(3) Y. J. Tseng, K. P. Shen, H. L. Lin, C. Y. Huang, C. F. Dai and J. H. Sheu, *Mar. Drugs*, 2012, **10**, 1572.

(4) J. H. Su, C. Y. Huang, P. J. Li, Y. Lu, Z. H. Wen, Y. H. Kao and J. H. Sheu, *Arch. Pharm. Res.*, 2012, **35**, 779.

(5) C. H. Chao, K. J. Chou, C. Y. Huang, Z. H. Wen, C. H. Hsu, Y. C. Wu, C. F. Dai and J. H. Sheu, *Mar. Drugs*, 2011, **9**, 1955.

(6) K. H. Chen, C. F. Dai, T. L. Hwang, C. Y. Chen, J. J. Li, J. J. Chen, Y. C. Wu, J. H. Sheu, W. H. Wang and P. J. Sung, *Mar. Drugs*, 2014, **12**, 385.

(7) C. Y. Huang, C. C. Liaw, B. W. Chen, P. C. Chen, J. H. Su, P. J. Sung, C. F. Dai, M. Y.

Chiang and J. H. Sheu, *J. Nat. Prod.*, 2013, **76**, 1902.

(8) C. H. Chao, K. J. Chou, C. Y. Huang, Z. H. Wen, C. H. Hsu, Y. C. Wu, C. F. Dai and J. H. Sheu, *Mar. Drugs*, 2012, **10**, 439.

(9) T. Z. Huang, B. W. Chen, C. Y. Huang, T. L. Hwang, C. F. Dai and J. H. Sheu, *Mar. Drugs* 2014, **12**, 2446.

(10) A. F. Ahmed, M. H. Wu, G. H. Wang, Y. C. Wu, J. H. Sheu, *J. Nat. Prod.*, 2005, **68**, 1051.

(11) Y. N. Lee, C.J. Tai, T. L. Hwang and J. H. Sheu, *Mar. Drugs*, 2014, **12**, 1148.

(12) T. H. Chen, M. C. Lu, Y. C. Chang, Y. D. Su, Y. H. Chen , N. C. Lin, L. S. Fang, Y. C. Wu and P. J. Sung, *Mar. Drugs*, 2013, **11**, 4585.

(13) M. Kobayashi, F. Kanda, S. R. Damarla, D. V. Rao and C. B. Rao, *Chem. Pharm. Bull.* 1990, **38**, 2400.

(14) D. Y. Shen, C. H. Chao, H. H. Chan, G. J. Huang,; T. L. Hwang, C. Y. Lai, K. H. Lee, T. D. Thang and T. S. Wu, *Phytochemistry*, 2012, **82**, 110.

(15) V. Anjaneyulu, K. N. Rao, J. S. Babu and M. Kobayashi, *Indian J. Chem. Sect B*., 1994, **33B**, 144.

(16) M. Vanisree, G. V. Subbaraju and C. B. Rao, *Indian J.Chem. Sect B.*, 1999, **38B**, 394.

(17) J. H. Su, Y. J. Tseng, H. H. Huang, A. F. Ahmed, C. K. Lu, Y. C. Wu and J. H. Sheu, *J. Nat. Prod*., 2006, **69**, 850.

(18) F. De Riccardis, L. Minale, M. Iorizzi, C. Debitus and C Lévi, *J. Nat. Prod*., 1993, **56**, 282.

(19) C. Y Kuo, Y. S. Juan, M. C. Lu, M. Y. N. Chiang, C. F. Dai, Y. C. Wu and P. J. Sung, *Int. J. Mol. Sci.*, 2014, **15**, 10136.

(20) M. U. Roslund, E. Säwén, J. Landström, J. Rönnols, K. H. M. Jonsson, M. Lundborg, M. V. Svensson and G. Widmalm, *Carbohydr. Res.*, 2011, **346**, 1311.

(21) S. Y. Cheng, S. T. Lin, S. K. Wang and C. Y. Duh, *Bull. Chem. Soc. Jpn.*, 2011, **84**, 783.

(22) E. P. Stout, J. Prudhomme, K. L. Roch, C. R. Fairchild, S. G. Franzblau, W. Aalbersberg, M. E. Hay and J. Kubanek, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5662.

(23) P. Alaupović, B. C. Johnson, Q. Crider, H. N. Bhagavan and B. J. Johnson, *Am. J. Clin. Nutr.*, 1961, **9**, 76.

(24) J. Neužil, P. K. Witting and R. Stocker, *Proc. Natl. Acad. Sci.*, 1997, **94**, 7885.

(25) J. O'Brien, I. Wilson, T. Orton and F. Pognan, *Eur. J. Biochem.*, 2000, **267**, 5421.

(26) G. R. Nakayama, M. C. Caton, M. P. Nova and Z. Parandoosh, J. Immunol. Methods, 1997,

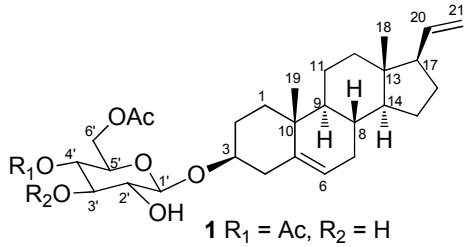
204, 205.

(27) T. L. Hwang, C. C. Wang, Y. H. Kuo, H. C. Huang, Y. C. Wu, L. M. Kuo and Y. H. Wu, *Biochem. Pharmacol.* 2010, **80**, 1190.

(28) T. L. Hwang, Y. L. Leu, S. H. Kao, M. C. Tang and H. L. Chang, *Free Radical Biol. Med.*, 2006, **41**, 1433.

(29) Y. N. Lee, C. J Tai, T. L. Huang and J. H. Sheu, *Mar. Drugs*, 2014, **12**, 1148.

Chart 1.



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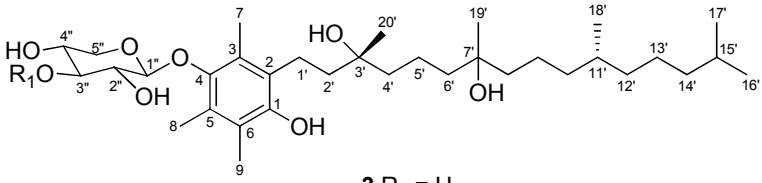
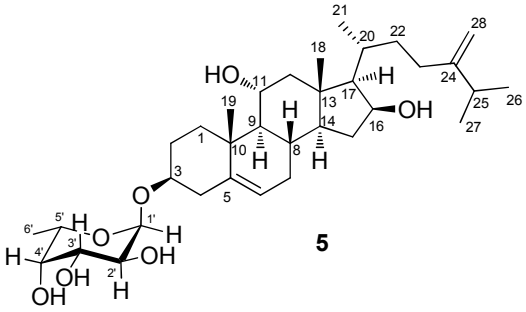
2 R_1 = H, R_2 = Ac



3 R_1 = H
4 R_1 = *n*-butyryl

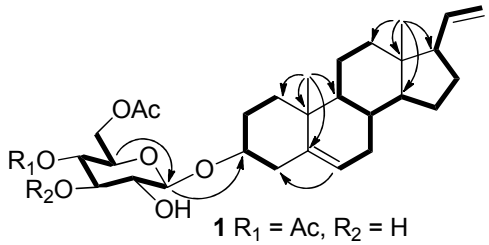


5

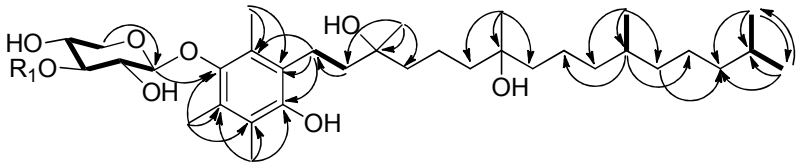
Figure captions

Fig. 1 ^{1}H-^{1}H COSY (▬) and HMBC (→) correlations for **1**–**4**.

Fig. 2 Selected NOESY Correlations for **1**and β-xylopyranose residue of **3**.

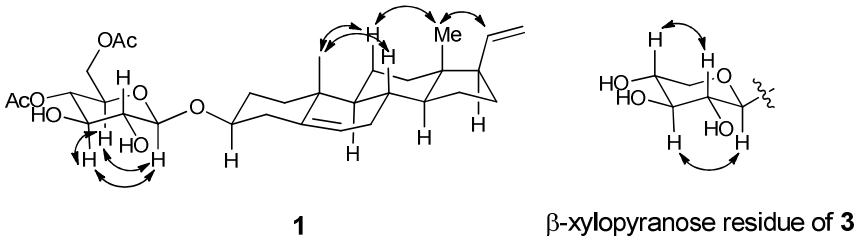


1 R_1 = Ac, R_2 = H
2 R_1 = H, R_2 = Ac



3 R_1 = H
4 R_1 = *n*-butyryl

Fig.1 ^{1}H-^{1}H COSY (▬) and HMBC (→) correlations for **1**–**4**.



1 β-xylopyranose residue of **3**

Fig.2 Selected NOESY Correlations for **1**and β-xylopyranose residue of **3**.

Table 1 ^{1}H and ^{13}C NMR spectroscopic data of **1** and **2**

Position	1		2	
	δ_H (J in Hz)[a]	δ_C (mult.)[b]	δ_H (J in Hz)[c]	δ_C (mult.)[d]
1	1.71 m	37.32, CH_2	1.72 m	37.2, CH_2
	1.07 m		1.07 m	
2	1.95 m	29.6, CH_2	1.94 m	29.6, CH_2
	1.62 m		1.62 m	
3	3.56 m	79.6, CH	3.57 m	79.7, CH
4	2.36 m	38.8, CH_2	2.38 m	38.6, CH_2
	2.29 m		2.29 m	
5		140.3, C		140.2, C
6	5.37br s	122.1, CH	5.38 br s	122.2, CH
7	2.02 m	31.9, CH_2	2.02 m	32,0, CH_2
	1.56 m		1.55 m	
8	1.47 m	32.0, CH	1.46 m	32.0, CH
9	0.96 m	50.4, CH	0.96 m	50.4, CH
10		36.9, C		36.8, C
11	1.52 m	20.7 CH_2	1.52 m	20.7 CH_2
	1.47 m		1.47 m	
12	1.88 m	37.26, CH_2	1.88 m	37.2, CH_2
	1.08 m		1.07 m	
13		43.4, C		43.3, C
14	1.02 m	55.9, CH	1.02 m	55.9, CH
15	1.68 m	24.9, CH_2	1.68 m	24.9, CH_2
	1.18 m		1.18 m	
16	1.79 m	27.2, CH_2	1.80 m	27.2, CH_2
	1.56 m		1.54 m	
17	1.97 m	55.3, CH	1.96 m	55.3, CH
18	0.62 s	12.7, CH_3	0.62 s	12.7, CH_3
19	1.03 s	19.4, CH_3	1.02 s	19.4, CH_3
20	5.75 m	139.7, CH	5.76 m	139.8, CH
21	4.98, br d (9.2)	114.6, CH_2	4.98, br d (9.0)	114.5, CH_2
	4.97 br d (18.8)		4.97 br d (19.0)	
1′	4.40 d (7.6)	100.9, CH	4.44 d (7.5)	101.3, CH
2′	3.45 dd (9.2, 7.6)	74.2, CH	3.48 dd (9.0, 7.5)	71.9, CH
3′	3.69 dd (10.0, 9.2)	74.6, CH	4.93 t (10.0, 9.0)	77.5, CH
4′	4.92 dd (10.0, 9.6)	70.6, CH	3.53 t (10.0, 8.5)	69.2, CH
5′	3.63 m	71.9, CH	3.50 m	74.2, CH
6′	4.27 dd (12.4, 4.8)	62.5 CH_2	4.46 dd (12.5, 4.5)	63.1 CH_2
	4.10 brd (12.4)		4.30 dd (12.5, 1.5)	
OAc		170.6, C		172.4, C
	2.12 s	20.8, CH_3	2.17 s	20.9, CH_3
OAc		170.8, C		171.6, C
	2.07	20.9, CH_3	2.12 s	21.0, CH_3

[a]Spectra recorded in $CDCl_3$ at 400 MHz. [b]Spectra recorded in $CDCl_3$ at 100 MHz. [c]Spectra recorded in $CDCl_3$ at 500 MHz. [d]Spectra recorded in $CDCl_3$ at 125MHz.

Table 2 ^{1}H and ^{13}C NMR spectroscopic data of **3** and **4**

Position	3		4	
	δ_H (J in Hz)[a]	δ_C (mult.)[b]	δ_H (J in Hz)[c]	δ_C (mult.)[d]
1		149.0, C		149.0, C
2		125.8, C		125.8, C
3		126.9, C		126.9, C
4		145.8, C		145.8, C
5		121.9,C		121.8,C
6		128.3, C		128.4, C
7	2.23, s	13.4, CH_3	2.23, s	13.2, CH_3
8	2.20, s	13.8, CH_3	2.21, s	13.9, CH_3
9	2.15, s	12.4, CH_3	2.16, s	12.5, CH_3
1'	2.69, t (7.0)	20.7, CH_2	2.70, t (7.2)	20.7, CH_2
2'	1.68, m	39.9, CH_2	1.68, m	40.0, CH_2
3'		73.9, C		73.9, C
4'	1.48, m	42.4, CH_2	1.48, m	42.4, CH_2
5'	1.30–1.40, overlapped	21.7, CH_2	1.30–1.40, overlapped	21.6, CH_2
6'	1.40, m	42.1, CH_2	1.40, m	42.1, CH_2
7'		73.0, C		73.0, C
8'	1.40, m	42.1, CH_2	1.40, m	42.1, CH_2
9'	1.30–1.40, overlapped	21.2, CH_2	1.30–1.40, overlapped	21.2, CH_2
10'	1.10, m	37.4, CH_2	1.10, m	37.4, CH_2
11'	1.40, m	32.7, CH_2	1.40, m	32.7, CH_2
12'	1.27, m	37.5, CH_2	1.27, m	37.5, CH_2
13'	1.30–1.40, overlapped	21.7, CH_2	1.30–1.40, overlapped	21.6, CH_2
14'	1.16, m	39.5, CH_2	1.16, m	39.5, CH_2
15'	1.53, m	27.9, CH	1.54, m	27.9, CH
16' and 17'	0.88, d (6.5)	22.6, CH_3	0.88, d (6.4)	22.6, CH_3
18'	0.86, d (7.0)	19.7, CH_3	0.87, d (7.0)	19.7, CH_3
19'	1.16, s	26.9, CH_3	1.16, s	26.9, CH_3
20'	1.25, s	26.9, CH_3	1.25, s	26.9, CH_3
1"	4.53, d (7.5)	104.4, CH	4.60, d (7.2)	104.2, CH
2"	3.69, dd (8.5, 7.5)	74.3, CH	3.84, dd (9.2, 7.2)	72.5, CH
3"	3.58, dd (9.0, 8.5)	76.3, CH	4.89, dd (9.2, 8.8)	78.2, CH
4"	3.77, m	69.9, CH	3.82, m	69.2, CH
5"	3.93, dd (11.0, 5.0) 3.13, dd (11.0, 11.0)	65.6, CH_2	3.98, dd (12.0, 5.6) 3.17, dd (12.0, 9.6)	65.9, CH_2
4-butyryl				175.5, C
			2.44, t (7.6)	36.2, CH_2
			1.73, m	18.5, CH_2
			1.00, t (7.2)	13.5, CH_3

[a]Spectra recorded in $CDCl_3$ at 500 MHz. [b]Spectra recorded in $CDCl_3$ at 125 MHz. [c]Spectra recorded in $CDCl_3$ at 400 MHz. [d] Spectra recorded in $CDCl_3$ at 100MHz.

Table 3 Cytotoxicity (IC_{50}) of compounds **1**–**11**

compound	K562	P388	HT-29	A-549
1[c]	27.6	(–)[a]	(–)	32.2
2[c]	39.3	10.2	29.1	(–)
3[c]	18.5	21.1	20.3	N.T.[b]
4[c]	12.6	15.0	13.2	N.T.
5[d]	(–)	(–)	(–)	N.T.
6[d]	(–)	(–)	(–)	N.T.
7[d]	(–)	(–)	(–)	N.T.
8[d]	15.8	15.5	12.6	N.T.
9[d]	(–)	(–)	(–)	N.T.
10[d]	(–)	(–)	(–)	N.T.
11[d]	(–)	(–)	(–)	N.T.
5-Fluorouracil[c]	24.6	8.5	20.8	38.5
Doxorubicin hydrochloride[d]	0.88	0.84	0.83	N.T.

[a] IC_{50}> 40 μM. [b] Not tested. [c]Samples were assayed in the same batch and 5-fluorouracil was used as a positive control; [d]Samples were assayed in the same batch and doxorubicin hydrochloride was used as a positive control.

Table 4 Effect of **1** and **5–11** on superoxide anion generation and elastase release in fMLP/CB induced human neutrophils

compound	Superoxide anion	Elastase release
	IC_{50} (μM)[a]	IC_{50} (μM)[a]
1	> 10**	4.1 ± 0.1***
5	> 10**	> 10*
6	> 10**	> 10**
7	18.6 ± 1.5**	10.1 ± 0.8***
8	6.6 ± 0.6***	2.9 ± 0.5***
9	> 10*	> 10**
10	> 10**	> 10
11	> 10	> 10

[a] Concentration necessary for 50% inhibition (IC_{50}). Results are presented as mean ± SEM (n = 3 or 4). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control value.

Graphical abstract

Steroidal and α-Tocopherylhydroquinone Glycosides from two Soft Corals *Cladiella hirsuta and Sinularia nanolobata*

Chih-Hua Chao, Tzu-Zin Huang, Chia-Yun Wu, Bo-Wei Chen, Chiung-Yao Huang, Tsong-Long Hwang, Chang-Feng Dai, and Jyh-Horng Sheu*

Cytotoxic and anti-inflammatory glycosides were isolated from two soft corals, *Cladiella hirsuta* and *Sinularia nanolobata*. Their structures were elucidated by extensive spectroscopic analysis. Compounds **3** and **4** represent the first example of tocopheryl xyloside derivatives.