Further New Xenicanes from a Chinese Collection of the Brown Alga *Dictyota plectens*

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Four new xenicanes, namely 4α-hydroxydictyohemiacetal (1), 4α-hydroxydictyoacetocetal (2), 13,18-diacetoxy-4-hydroxydictyo-19-al (3), and 4α-hydroxyxypachylactone (8), were isolated from a Chinese collection of the brown alga *Dictyota plectens*, along with four known analogues (4–7). The structures of the new diterpenes were determined by extensive spectroscopic data analysis. All compounds were evaluated for their antiviral activities against human immunodeficiency virus type 1 (HIV-1) and the highly pathogenic avian influenza A (H5N1) virus, and inhibitory effects on lipo polysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages (PEMΦ).

Key words brown alga; *Dictyota plectens*; xenicane; antiviral property; nitric oxide (NO) inhibition

Brown algae of the genus *Dictyota* (family Dictytaceae) are significant producers of bioactive secondary metabolites, especially diterpenes. In the past four decades, about 400 diterpenes of more than 15 chemical classes have been isolated from *Dictyota* species.1) These diterpenes can be biosynthetically distributed into three groups: xenicanes, dolabellanes, and “extended sesquiterpenes.” Biological studies have confirmed that many of these dicytota diterpenes to possess significant ecological and pharmaceutical activities, such as antifouling, antifeeding, antibacterial, cytotoxic, and antiviral properties.2–10 In our previous work,11 we have reported the isolation of 27 diterpenes from the brown alga *Dictyota plectens* of the South China Sea and their anti-inflammatory and antiviral activities against human immunodeficiency virus type 1 (HIV-1) and the highly pathogenic avian influenza A (H5N1) virus. The significant biological activities, especially antiviral properties, of some of these metabolites encouraged our further chemical investigation on the same collection of this species and resulted in the isolation of another eight xenicanes including four new ones. Herein, we report the isolation, structural elucidation, and bioactivities of these xenicanes.

Results and Discussion

Further isolation of compounds from the EtOAc-soluble portion of the EtOH extract of the brown alga *Dictyota plectens* was accomplished by repeated column chromatography with the aid of 1H-NMR guided fractionation, affording three new xenicane-type diterpenes 4α-hydroxydictyohemiacetal (1), 4α-hydroxydictyoacetocetal (2), and 13,18-diacetoxy-4-hydroxydictyo-19-al (3), a new xenicane derivative, cremulide-type diterpene 4α-hydroxyxypachylactone (8), along with four known analogues (4–7). The structures of known compounds were identified as isodictyoehemiacetal (4),12 isodictyoacetocetal (5),13 (2S*,3S*,4R*,10R*,19R*)-19-deoxy-4-hydroxy-19-methoxydictyolactone (6),5) and 4-hydroxydictyolactone (7)14,15) by comparison of their 1H- and 13C-NMR spectroscopic and optical rotations with those reported in the literature (Fig. 1).

Compound 1 was isolated as a colorless oil. Its molecular formula was determined to be C20H32O5 based on high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) data (m/z 343.2244 [M+Na]+), implying five degrees of unsaturation. The 1H-NMR spectrum of 1 showed four methyl signals including three olefinic methyl singlets at δH 1.92 (3H, s, H-20), 1.68 (3H, s, H-16), and 1.59 (3H, s, H-15) and one methyl doublet at δH 1.06 (3H, d, J=6.6Hz, H-17) (Table 1), while the 13C-NMR spectrum exhibited 20 carbon signals including six olefinic carbons and three oxygenated carbons (Table 2). The six olefinic carbon signals at δC 145.5 (C, C-1), 134.3 (C, C-6), 131.3 (C, C-14), 128.2 (CH, C-7), 124.8 (CH, C-13), and 119.3 (CH, C-9), along with three olefinic proton signals at δH 5.60 (1H, dd, J=8.4, 3.6Hz, H-9), 5.37 (1H, dd, J=10.8, 3.6Hz, H-7), and 5.07 (1H, t, J=6.6Hz, H-13), were attributed to three trisubstituted double bonds. The presence of a five-membered ring hemiacetal was revealed by the diagnostic NMR signals for an hemiacetal methine [δc 101.3 (CH, C-18)]; δH 5.61 (1H, brs, H-18) and an oxymethylene [δc 71.7 (CH2, C-19); δH 4.41 (1H, d, J=12.0Hz, H-19a) and 4.38 (1H, d, J=12.0Hz, H-19b)], in combination with the heteronuclear multiple bond connectivity (HMBC) correlations from the hemiacetal methine protons to the oxymethylene carbon and from the hemiacetal methine and oxymethylene protons to the same two carbons at δc 145.5 (C, C-1) and 46.9 (CH, C-2) (Fig. 2). Four degrees of unsaturation, accounted for by the functional groups from five in the molecular, indicated the remaining of a cyclic structure in 1. In addition, a 6-methylhept-5-en-2-yl side chain was established by the 1H–1H correlation spectroscopy (COSY) relationships of H-13/H-12 (δH 1.93, m), H-12/H-11 (δH 1.23, m; 1.13, m), H-11/H-10 (δH 1.90, m), H-10/H-17, and H-10/H-3 (δH 2.04, brs), and the HMBC correlations from H-15 and H-16 to C-13 and C-14 and from H-17 to C-10 (δc 31.4, CH) and C-11 (δc 38.5, CH2). All of

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these NMR data are characteristic of a xenicane-type diterpene closely related to the co-occurring analogue isodictyolactone (4). The locations of two double bonds at C-6/C-7 and C-1/C-9 were confirmed by the HMBC correlations from H3-20 and the oxymethylene protons H2-19 to olefinic carbons C-6 and C-7, C-1 and C-9, as well as the 1H–1H COSY correlations between H-7/H2-8 [δH 3.17 (1H, ddd, J=15.6, 10.8, 3.6 Hz, H-8a); 2.66 (1H, ddd, J=15.6, 8.4, 3.6 Hz, H-8b)] and H2-19. Moreover, the 1H–1H COSY correlations between H2-5 [δH 2.34 (1H, dd, J=13.2, 1.8 Hz, H-5a); 2.15 (1H, dd, J=13.2, 3.6 Hz, H-5b)] and an oxymethine proton at δH 4.29 (1H, dd, J=3.6, 1.8 Hz, H-4) disclosed that C-4 (δC 73.2, CH) was hydroxylated. The relative configurations at C-2, C-3, C-10, and C-18, and the geometries of C-6/C-7 and C-1/C-9 double bonds in 1 were consistent with those of 4 as revealed by the similar weak proton–proton couplings between H-2 (δH 2.93, br s)/H-18, H-2/H-3 (δH 2.04, br s), and H-3/H-10 and significant nuclear Overhauser effect spectroscopy (NOESY) correlations between H-2/H3-20, H-3/H-7, and H-9/H2-19 (Fig. 3), while the relative configuration at C-4 was assigned to be identical to that of (2S,3S,4R,10R,19R)-19-deoxy-4-hydroxy-19-methoxydictyolactone (6) by the compatible
chemical shift of C-4 and weak coupling between H-3 and H-4. Thus, compound 1 was determined as 4α-hydroxyisodictyosidacycl

Compound 2 has a molecular formula of C$_{21}$H$_{34}$O$_3$ as determined by HR-ESI-MS data (m/z 357.2401 [M+Na]$^+$), requiring five degrees of molecular unsaturation. Detailed analysis of one- and two-dimensional (1D)- and (2D)-NMR data revealed that 2 had a structure closely related to those of I and isodictyosidacetel (5). In comparison with 1, the only difference was found by the presence of a methoxy group [δ$_{\text{H}}$ 3.31 (3H, s)], indicating a methylated derivative of 1. The methoxyl group was attached to the acetal carbon C-18 (δ$_{\text{H}}$ 107.8) as evidenced by the HMBC correlations from the methoxy protons to the acetal methine carbon C-18 and from the acetal methine proton H-18 (δ$_{\text{H}}$ 5.09, br s) to C-1 (δ$_{\text{C}}$ 146.0, C), C-2 (δ$_{\text{C}}$ 45.7, CH$_3$), C-3 (δ$_{\text{C}}$ 49.2, CH$_3$), and the methoxy carbon. The relative configuration of 2 was suggested to be identical to that of 1 based on the similar NOE relationships. Thus, compound 2 was defined as 4α-hydroxyisodictyosidacetel, which could be a methoxylated artifact of 1 formed during the isolation process.

The molecular formula of compound 3 was determined to be C$_{23}$H$_{36}$O$_7$ by HR-ESI-MS data (m/z 443.2406 [M+Na]$^+$), indicating seven degrees of unsaturation. The 1H- and 13C-NMR spectra of 3 showed signals for one conjugated aldehyde (δ$_{\text{C}}$ 196.2, C-19); δ$_{\text{H}}$ 9.52 (1H, s), two acetylts (δ$_{\text{C}}$ 170.7, C, 170.1 (C), 21.2 (CH$_3$), 21.0 (CH$_3$); δ$_{\text{H}}$ 2.04 (3H, s), 1.99 (3H, s), and three olefinic double bonds including a terminal [δ$_{\text{C}}$ 142.7 (C, C-14), 112.7 (CH$_3$, C-15); δ$_{\text{H}}$ 4.92 (1H, brs, H-15a), 4.87 (1H, brs, H-15b)] and two trisubstituted [δ$_{\text{C}}$ 157.3 (C, C-9), 149.7 (C, C-1), 138.1 (C, C-6), 124.9 (CH, C-7); δ$_{\text{H}}$ 6.84 (1H, dd, J=7.8, 3.0Hz, H-9), 5.24 (1H, brd, J=11.4Hz, H-7)] ones. Thus, a monocyclic structure could be assigned for 3 according to the remaining one degree of unsaturation. In addition, two oxymethines [δ$_{\text{C}}$ 77.4 (CH, C-13), 74.9 (CH, C-4); δ$_{\text{H}}$ 5.06 (1H, t, J=6.6Hz, H-13), 4.24 (1H, dd, J=3.6, 1.8Hz, H-4)] and one oxymethylene [δ$_{\text{C}}$ 63.8 (CH$_2$, C-18); δ$_{\text{H}}$ 4.62 (1H, dd, J=10.2, 6.6Hz, H-18a), 4.49 (1H, dd, J=10.2, 8.4Hz, H-18b)] were present. These NMR data were very compatible with those of the co-occurring xenicane-type diterpene 18-acetoxy-4-hydroxyisodictyo-19-al (11, 12, 16). The difference was attributed to the presence of an additional acetoxy and a terminal double bond as described above, but the absence of an olefinic methyl in comparison with the known analogue. Further detailed analysis of HMBC and 1H-1H COSY data revealed that 3 shared the same partial structure of 18-acetoxy-4-hydroxydictyo-19-al with the exception of the side chain (Fig. 2). The HMBC correlations from the olefinic methyl protons H$_3$-16 (δ$_{\text{H}}$ 1.68, s) to an oxymethine carbon C-13 and the two carbon atoms of a terminal double bond (C-14 and C-15) and from the oxymethine proton H-13 to a carbonyl carbon at δ$_{\text{C}}$ 170.1 disclosed the presence of an isopropenyl at the end of the side chain and the attachment of an acetoxy to C-13 in 3. The relative configurations at C-2, C-3, C-4, and C-10, as well as the geometries of C-6/C-7 and C-1/C-9 double bonds were defined to be in agreement with those of 18-acetoxy-4-hydroxydictyo-19-al by the similar NOE relationships (Fig. 3) in association with the chemical shifts and coupling constants, while the configuration at C-13 remained to be determined. Thus, 3 was elucidated as 13,18-diacetoxy-4-hydroxyisodictyo-19-al.

Compound 8 was assigned a molecular formula of C$_{20}$H$_{30}$O$_3$ according to the HR-ESI-MS data (m/z 341.2084 [M+Na]$^+$), implying six degrees of unsaturation. The presence of an α,β-unsaturated γ-lactone was indicated by the NMR signals for an ester carbonyl (δ$_{\text{C}}$ 176.1, C-18), two olefinic carbons at δ$_{\text{C}}$ 163.0 (C, C-1) and 132.2 (C, C-2), and one oxymethylene [δ$_{\text{C}}$ 72.2 (CH$_2$, C-19); δ$_{\text{H}}$ 4.77 (1H, d, J=18.0Hz, H-19a), 4.55
(1H, d, J=18.0Hz, H-19b)], in association with the IR absorption bands at 1758 and 1662cm⁻¹. A cyclopropane moiety was recognized by the diagnostic upfield proton signal at δ_H 0.17 (1H, dd, J=10.8, 5.4Hz, H-8b). In addition, two olefinic carbons [δ_C 131.3 (C, C-14), 124.4 (CH, C-13)], two olefinic methyl singlets [δ_H 1.66 (3H, s, H-3-16), 1.57 (3H, s, H-3-15)], two methyl doublets [δ_H 1.02 (3H, d, J=6.6Hz, H-3-20), 1.01 (3H, d, J=6.6Hz, H-3-17)], and a secondary alcohol [δ_C 70.2 (CH, C-4); δ_H 4.30 (1H, brt, J=3.0Hz, H-4)] were present in the 13C- and 1H-NMR spectra. A 6-methylhept-5-en-2-yl side chain was further established on the basis of the 1H–1H COSY and HMBC correlations similar to those depicted in 1. All the above data are consistent with a crenulide-type diterpene, represented by pachylactone 17) and the co-occurring analogue isoacetoxycrenulatin.11,17) Finally, the gross structure of 8 was established as 4-hydroxypachylactone by the HMBC correlations from the oxymethylene protons H-3 to an olefinic carbon C-2 and an aliphatic methine carbon C-6 (δ_C 28.7), and from the methyl protons H-3-20 to a methylene carbon C-5 (δ_C 46.7) and two methine carbons C-7 (δ_C 26.6) and C-6, in combination with the 1H–1H COSY correlations between the oxymethylene proton H-4 and H-3-20 [δ_H 1.88 (1H, m, H-3a), 1.70 (1H, m, H-3b)] (Fig. 2). The relative configurations of the stereogenic centers in 8 were suggested to be in agreement with those of isoacetoxycrenulatin based on the similar NMR data including the weak coupling between H-3 and H-4, the relatively large coupling constant (11.4Hz) between H-3 and H-10, and NOE relationships of H-3/H-9 (δ_H 2.83, d, J=11.4Hz) to the carbonyl carbon C-18, from the oxymethylene proton H-4 to an olefinic carbon C-2 and an aliphatic methine carbon C-6 (δ_C 28.7), and from the methyl proton H-3-20 to a methylene carbon C-5 (δ_C 46.7) and two methine carbons C-7 (δ_C 26.6) and C-6, in combination with the 1H–1H COSY correlations between the oxymethylene proton H-4 and H-3-20 [δ_H 1.88 (1H, m, H-3a), 1.70 (1H, m, H-3b)] (Fig. 2). The relative configurations of the stereogenic centers in 8 were suggested to be in agreement with those of isoacetoxycrenulatin based on the similar NMR data including the weak coupling between H-3 and H-4, the relatively large coupling constant (11.4Hz) between H-3 and H-10, and NOE relationships of H-3/H-9 (δ_H 1.70, m), H-3/H-5a, H-5a/H-7 (δ_H 1.08, m), H-7/H-9, H-8b/H-19b, and H-4/H-11 (Fig. 3). Thus, 8 was elucidated as 4α-hydroxypachylactone.

All compounds were assayed for the in vitro anti-HIV-1 replication activity, the result showed that 1 and 6 were active against the replication of wild-type HIV-1 virus with inhibitory concentration 50% (IC₅₀) of 28.1 and 25.4µM,
respectively (the positive control nevirapine, IC₅₀ 0.05 μM), while the other compounds were inactive at concentration of 30.0 μM. In addition, all compounds were assessed for their inhibitory activities against the hemagglutinin (HA)-mediated highly pathogenic H5N1 (A/Viet Nam/1203/2004) infection using an HIV-based pseudotyping system. In the primary assay, 7 showed specific inhibition against HA-mediated viral entry with an inhibition rate of 66.8% at 30.0 μM, however the other compounds were inactive (inhibition rate <50%) at this concentration. Moreover, all compounds were tested for the in vitro anti-inflammatory activity. The bioassay result revealed that 7 and 8 could effectively inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages (PEMФ) with inhibition rates of 76.0% and 53.2%, respectively, at 10.0 μM, whereas the other compounds showed weak activity (inhibition rate <50%) at this concentration.

Experimental

General Experimental Procedures IR spectra were recorded on a Bruker Equinox 55 spectrometer. Optical rotations were obtained on a PolAAR 3005 digital polarimeter. 1D- and 2D-NMR spectra were acquired on a Bruker Avance-600 FT NMR spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were expressed in parts per million (ppm), and coupling constants (J) were reported in Hertz (Hz). HR-ESI-MS data were determined by a Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemistry Co., Ltd.), Sephadex LH-20 (GE Healthcare Biosciences AB), and octadecyl silica (ODS) (50 μm, YMC) were used for column chromatography. TLC analysis was carried out using Precoated silica gel plates (Merck, kieselgel-60F₂₅₄, 0.25 mm). Semipreparative HPLC was performed on an Agilent 1100 series instrument equipped with a YMC-Pack C₁₈ (10 μm, 250×10 mm) column. 293T cells were obtained from ATCC. Vescular stomatitis virus glycoprotein (VSVG)-pseudotyped HIV-1 vector NL4-3-luc was supplied by Basic Sciences, Chinese Academy of Medical Sciences and Peking Union College. A codon-optimized HA gene from A/Viet Nam/1203/2004 (H5N1) was cloned into pcDNA3. Vesicular stomatitis virus glycoprotein (VSV-G) plasmid was cotransfected with env-deficient HIV-1 vector (PNL4-3.Iuc.R) into human embryonic kidney 293T cells based on a modified Ca₃(PO₄)₂ method. Sixteen hours post-transfection, plates were washed by PBS, and fresh medium (DMEM with 10% FBS) was added into the plates. Forty-eight hours post-transfection, supernatant containing VSVG/HIV-1 virions was collected and centrifuged at 1500 rpm for 10 min to obtain a 1 ml supernatant.
harvested and filtered through a 0.45 μm filter. Viral solution was quantified in terms of p24 concentrations, which were detected by ELISA, then diluted to 0.2 ng p24/mL for direct use or storage at −80°C.

For the infection assay, 293T cells were plated on 24-well plates at the density of 6×10^4 cells per well, one day prior to infection. Compounds, dissolved in DMSO, were added into target cells and incubated for 15 min prior to adding VSVG/HIV-1. The same amount of solvent was used as blank control, while nevirapine was employed as positive control. Forty-eight hours post-infection, infected cells were lysed in 50 μL of Cell Lysis Reagent. Luciferase activity of the cell lysate was determined by a Sirius luminometer according to the manufacturer’s instructions.

**Assay for Inhibition of H5N1 Entry** A previously established protocol\(^1\) was followed. Hemagglutinin envelope expression plasmid was co-transfected with NA and Env-deficient HIV vector, PNL4-3.luc.R\(^-\) expression plasmid was co-transfected with NA and Env-deficient HIV vector, PNL4-3.luc.R\(^-\), with the same amount of solvent was used as blank control, and each test compound (30 mM in DMSO) was diluted to 1–30 mM range at r.t. before the experiment.

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**Conflict of Interest** The authors declare no conflict of interest.

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