Cytotoxic β-Carbolines and Cyclic Peroxides from the Palauan Sponge *Plakortis nigra*

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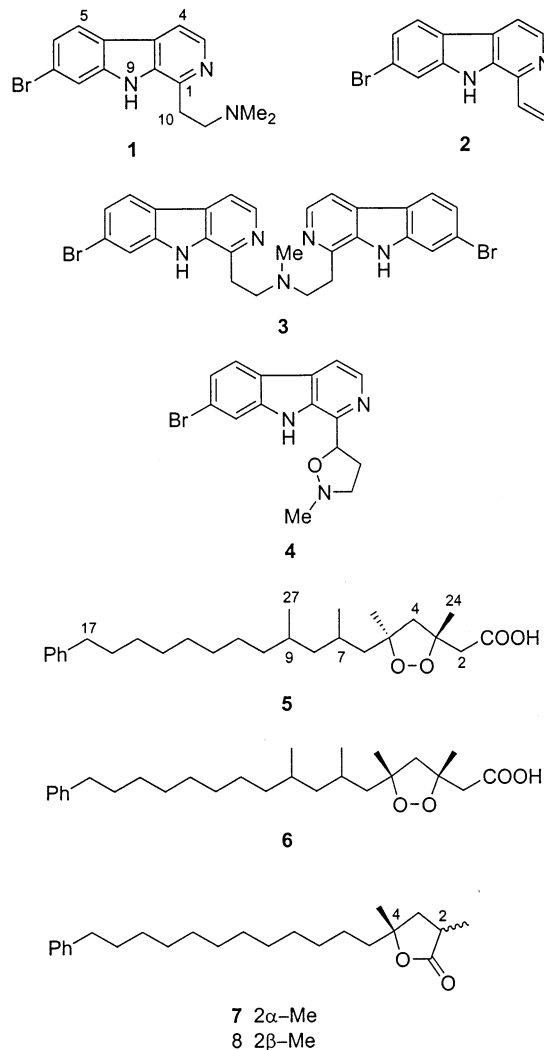
Four β-carbolines, plakortamines A–D, two cyclic peroxides, epiplakinic acids G and H, and two related γ-lactones, (2S*,4R*)- and (2R*,4R*)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactones, were isolated from the deep-water sponge *Plakortis nigra* from Palau. The structures of the eight new metabolites were elucidated by interpretation of spectroscopic data. Most of the metabolites inhibited the HCT-116 human colon tumor cell line.

Cyclic peroxides are commonly found in sponges of the genera *Plakortis* and *Plakinastrella*.[1,2] These peroxides can be subdivided into two groups: the plakinic acids that contain a five-membered peroxide ring[1] and the more common six-membered cyclic peroxides.[2] Simple β-carbolines such as those described in this paper are normally associated with the ascidians (tunicates)[3] and are exemplified by the eudistomins, which are antiviral constituents of the Caribbean tunicate *Eudistoma olivaceum*.[4] The majority of β-carbolines found in sponges belong to a much more complex group known as the manzamines,[5] although a few simple examples have been described.[6] In this paper we describe the first example of cytotoxic β-carbolines coexisting with cytotoxic cyclic peroxides and related lipids in the deep-water sponge *Plakortis nigra*.

Results and Discussion

The dark brown sponge *P. nigra* was collected by hand in Palau from a depth of 380 ft by using a mixed gas rebreathing apparatus. The lyophilized sponge was extracted with methanol, and after removal of the solvent, the resulting material was partitioned between ethyl acetate and water. The aqueous extract was made basic with sodium hydroxide solution and again extracted with ethyl acetate (peroxy acids may have inadvertently been discarded or destroyed during this step). The ethyl acetate extracts, which exhibited activity against the HCT-116 human colon tumor cell line, were combined and fractionated on Sephadex LH-20 using methanol as eluant to obtain plakortamines A (**1**, 144.4 mg, 0.61% dry wt), B (**2**, 15.0 mg, 0.063% dry wt), and C (**3**, 4.9 mg, 0.021% dry wt) and several fractions that required further purification. One of these fractions was subjected to reversed-phase medium-pressure liquid chromatography on a Diaion HP-20SS column to obtain plakortamine D (**4**, 6.5 mg, 0.027% dry wt). Some of the fractions from the Sephadex column appeared by ¹H NMR spectroscopy to consist of salts of the alkaloids and organic acids. After experiencing great difficulty in separating the acids from the plakortamines, and then suffering further losses while purifying the acids, we eventually succeeded in purifying epiplakinic acids G (**5**)

and H (**6**) and two γ-lactones, **7** and **8**. Owing to the difficulty that we experienced in separating the acids from the bases, the yields reported for compounds **1**–**8** do not reflect their true concentrations in the crude extract as judged by its ¹H NMR spectrum, which indicated that the ratio of plakortamines to epiplakinic acids was about 1:1.

Plakortamine A (**1**) was isolated as an optically inactive pale yellow oil. The mass spectrum contained [M + H]⁺

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peaks at 318/320, indicating the presence of bromine, and the high-resolution mass measurement (m/z 318.0596, [M + H]$^+$) indicated a molecular formula of $C_{15}H_{16}BrN_3$, with 9 unsaturation equivalents. The UV spectrum contained absorptions at 295 nm (ϵ 11 700) and 243 nm (ϵ 20 000), which, together with the presence of 11 signals in the aromatic region of the ^{13}C NMR spectrum, suggested a heteroaromatic ring system. The ^1H NMR data, with signals at δ 12.52 (br s, NH), 8.28 (d, 1 H, $J = 5.5$ Hz, H-3), 7.78 (d, 1 H, $J = 5.5$ Hz, H-4), 7.95 (d, 1 H, $J = 8$ Hz, H-5), 7.34 (dd, 1 H, $J = 8$, 1.5 Hz, H-6), and 7.67 (d, 1 H, $J = 1.5$ Hz, H-8), suggested that the heteroaromatic ring system was a 1-substituted 7-bromo-β-carboline, which satisfies the requirement for 9 unsaturation equivalents. The ^{13}C NMR spectrum, which was assigned using HSQC and HMBC data, confirmed the presence of a 1-substituted 7-bromo-β-carboline moiety. The remaining signals in the ^1H NMR spectrum at δ 2.47 (s, 6 H, NMe$_2$), 2.82 (t, 2 H, $J = 5.5$ Hz, H$_2$-11), and 3.36 (t, 2 H, $J = 5.5$ Hz, H$_2$-10) were assigned to a *N,N*-dimethylethylamine side chain, the position of which was determined by the HMBC correlations from the signals at 2.28 and 3.36 to a carbon signal at 144.0 (C-1) and from 3.36 to only one additional carbon signal at 135.9 (C-9a). Thus plakortamine A (**1**) is 7-bromo-1-(*N,N*-dimethylethylamino)-β-carboline.

Plakortamine B (**2**) was isolated as an optically inactive yellow oil. The high-resolution mass measurement indicated a molecular formula of $C_{13}H_9BrN_2$ (m/z 275.0008, [M + H]$^+$), which required 10 unsaturation equivalents. The ^{13}C NMR spectra contained 13 signals in the aromatic/olefinic region, while the ^1H NMR spectrum contained signals assigned to a vinyl group at δ 5.72 (d, 1 H, $J = 11$ Hz), 6.40 (d, 1 H, $J = 17$ Hz), and 7.18 (dd, 1 H, $J = 17$, 11 Hz) together with signals due to the 7-bromo-β-carboline moiety that are very similar to those observed in plakortamine A (**1**). Plakortamine B was therefore assigned the structure 7-bromo-1-vinyl-β-carboline (**2**).

Plakortamine C (**3**) was isolated as an optically inactive pale yellow gum. The molecular formula, $C_{27}H_{23}N_5Br_2$ (m/z 578.0372, [M + H]$^+$), coupled with the presence of only 14 signals in the ^{13}C NMR spectrum, suggested that plakortamine C contained two 1-substituted 7-bromo-β-carboline groups. Assuming that each of the aromatic signals was due to 2 protons, the remaining signals in the ^1H NMR spectrum at 3.31 (t, 4 H, $J = 5.5$ Hz), 3.09 (t, 4 H, $J = 5.5$ Hz), and 2.58 (s, 3 H) were assigned to a $-CH_2-CH_2-N(Me)-CH_2-CH_2-$ chain joining the two 7-bromo-β-carboline entities.

Plakortamine D (**4**), [α]$_D$ $-2.1°$ (*c* 0.6, MeOH), was obtained as a yellow oil. The molecular formula, $C_{15}H_{14}BrN_3O$ (m/z 332.0385, [M + H]$^+$), required 10 unsaturation equivalents, nine of which were associated with a 7-bromo-β-carboline moiety. The ^1H NMR spectrum contained an *N*-methyl signal at δ 2.99 (s, 3 H) and signals at 5.90 (dd, 1 H, $J = 8.5$, 7 Hz) 3.61 (t, 1 H, $J = 8$ Hz), 3.08 (m, 1 H), 2.80 (m, 1 H), and 2.53 (m, 1 H), which were shown by the COSY spectrum to be due to a O−CH−CH$_2$−CH$_2$−N unit. To account for the remaining unsaturation equivalent, there must be an N−O bond to form a ring, which is attached at C-1 to the carbon bearing oxygen (C-10). Further support for this structure came from the HMBC experiment that showed correlations from the *N*-methyl signal to C-12, from H-10 to C-1, C-9a, and C-11, and from H-11 (2.56) to C-1, C-10, and C-12. There was insufficient material to determine the absolute configuration at C-10.

Epiplakinic acid G (**5**), [α]$_D$ $-17.2°$ (*c* 0.3, MeOH), was isolated as a colorless gum. The molecular formula,

$C_{27}H_{44}O_4$, was initially inferred from the high-resolution mass measurement of the [M + Na]$^+$ ion at m/z 455.3127. The ^{13}C NMR spectrum contained four signals at δ 143.8, 129.2 (2C), 129.0 (2C), and 126.4 that were assigned to a terminal phenyl group, a signal at 174.3 due to the carboxylic acid, two signals at 87.8 and 84.9 due to fully substituted carbon atoms bearing oxygen, and four methyl signals at 24.8, 24.1, 22.2, and 20.7. The ^1H NMR spectrum confirmed the presence of the phenyl group and the four methyl groups that gave rise to signals at δ 1.46 (s, 3 H), 1.33 (s, 3 H), 0.95 (d, 3 H, $J = 6.5$ Hz), and 0.83 (d, 3 H, $J = 7$ Hz). The signals at δ 1.46 and 1.33 were assigned to methyl groups on carbons bearing oxygen. Interpretation of the HSQC and HMBC spectra suggested the presence of a five-membered cyclic peroxide moiety. The C-2 methylene signals at δ 2.77 (d, 1 H, $J = 16$ Hz) and 2.73 (d, 1 H, $J = 16$ Hz) showed HMBC correlations to the carboxylic acid signal at 174.3 (C-1) and to signals at 84.9 (C-3), 58.3 (C-4), and 24.8 (C-24, δ_H 1.46). The Me-24 signal at δ 1.46 showed HMBC correlations to C-2, C-3, and C-4, while the Me-25 signal at 1.33 showed correlations to C-4, C-5 (87.8), and C-6 (47.5). Since C-3 and C-5 are the only carbon atoms bearing oxygen, there must be a peroxide bridge linking the two carbons in order to satisfy both the number of oxygens and the number of unsaturation equivalents in the molecule. The Me-26 signal at δ 0.95 (d, 3 H, $J = 6.5$ Hz) showed HMBC correlations to signals at 47.5 (C-6), 28.1 (C-7), and 47.9 (C-8), while the Me-27 signal at 0.83 (d, 3 H, $J = 7$ Hz) was correlated to signals at 47.9 (C-8), 30.7 (C-9), and 37.9 (C-10). These correlations established the structure of the C-1 to C-10 portion of the molecule. The H$_2$-17 signal at δ 2.59 (t, 2 H, $J = 8$ Hz) showed HMBC correlations to signals at 143.8 (C-18), 129.0 (C-19, 23), and 32.7 (C-15). The remaining methylene signals must be due to a linear chain between C-10 and C-15. The stereochemistry about the peroxide ring was established by the observation of NOEs between Me-24 and the H-4b signal at δ 2.22 (d, 1 H, $J = 13$ Hz) and between Me-25 and the H-4a signal at 2.49 (d, 1 H, $J = 13$ Hz). Unfortunately no useful NOEs were observed that could be used to assign the relative stereochemistry at C-26 or C-27.

Epiplakinic acid H (**6**), [α]$_D$ $+33°$ (*c* 0.07, MeOH), was isolated as a colorless gum. The molecular formula, $C_{27}H_{44}O_4$, which is the same as that of epiplakinic acid G (**5**), was inferred from the high-resolution mass measurement of the [M + Na]$^+$ ion at m/z 455.3128. The ^1H and ^{13}C NMR spectra were so similar to those of **5** that it was apparent that epiplakinic acid H (**6**) was an epimer of epiplakinic acid G (**5**) at either C-3 or C-5. This was confirmed by the observation of NOE correlations from both the Me-24 signal at δ 1.48 and the Me-25 signal at 1.37 to the H-4β signal at 2.22 in the ROESY spectrum. The H-4α signal at δ 2.47 showed correlations to the C-2 signal at 2.73 and a signal at 1.64 that was assigned to H-7. Since the ^{13}C NMR signals of Me-26 and Me-27 are nearly identical in both compounds, we consider it preferable to assign the same stereochemistry at C-5, C-7, and C-9, implying that **5** and **6** are epimeric at C-3.

Both (2*S**,4*R**)- and (2*R**,4*R**)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactones (**7** and **8**) were isolated as colorless oils. They had the same molecular formula, $C_{24}H_{38}O_2$, which was determined by high-resolution mass measurement of the [M + Na]$^+$ ions at m/z 381.2763 and 381.2770, respectively. The IR bands at 1765 cm^{-1} indicated that both compounds contained a γ-lactone ring, which, together with a phenyl ring, accounted for the six degrees of unsaturation required by the molecular

formula. The ^{1}H and ^{13}C NMR spectra of **7** and **8** were remarkably similar, especially the signals due to the monosubstituted phenyl ring and the attached linear alkyl chain, with the only notable differences being associated with the substituents about the γ-lactone ring. In the case of lactone **7**, the COSY spectrum revealed coupling from the methyl doublet at δ 1.21 ($J = 7$ Hz, Me-23) to the H-2 methine signal at 2.91, which must be adjacent to the lactone carbonyl and which was in turn coupled to the H-3 signals at 2.27 and 1.72. The methyl singlet at δ 1.34 must be adjacent to the only remaining fully substituted carbon at 86.1, which must also be attached to the lactone oxygen, the alkyl chain, and the methylene group at C-3. The HMBC data confirmed the presence of the γ-lactone ring and its substituents. The Me-23 signal showed correlations to C-1 (d 181.6), C-2 (36.3), and C-3 (42.5), while the Me-24 signal showed correlations to C-3, C-4 (86.1), and C-5 (42.8). The ROESY spectrum showed weak correlations between the Me-23 signal and the H-3a signal at δ 1.72 and between the Me-24 signal and the H-3β signal at 2.27, while the H-2 signal showed a stronger correlation to the H-3β signal than to the H-3α signal, all of which supported the 2S^*,4R^* stereochemistry. A similar analysis of the NMR data for lactone **8** revealed that its planar structure was the same as that of lactone **7**. The ROESY spectrum of **8** did not show strong correlations from the Me-23 and Me-24 signals to either of the H-3 signals, but since there are only two chiral centers in the compound, lactone **8** must have the 2R^*,4R^* stereochemistry.

All of the metabolites tested exhibited activity against the HCT-116 human colon tumor cell line. The most active alkaloid was plakortamine B (**2**, IC$_{50}$ 0.62 μM), followed by plakortamines C (**3**, IC$_{50}$ 2.15 μM), A (**1**, IC$_{50}$ 3.2 μM), and D (**4**, IC$_{50}$ 15 μM). Epiplakinic acids G (**5**, IC$_{50}$ 0.16 μM) and H (**6**, IC$_{50}$ 0.39 μM) were also moderately active, and even lactone **7** (IC$_{50}$ 14.5 μM), but not lactone **8**, showed mild activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolf Autopol III polarimeter (c g/100 mL) at 589 nm. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. UV spectra were obtained using a Perkin-Elmer Lambda Bio-20 spectrophotometer. ^{1}H, COSY, HMBC, HMQC, GHSQC-TOCSY, and ROESY NMR spectra were measured on a Varian Inova 300 MHz spectrometer. ^{13}C and DEPT spectra were measured on a Varian Gemini 400 MHz spectrometer. ESIMS spectra were recorded using a Finnigan LCQ mass spectrometer. High-resolution FABMS data were obtained on a VG ZAB mass spectrometer at the U. C. Riverside Regional Facility. All solvents were distilled prior to use.

Collection, Extraction, and Purification. The dark brown sponge, identified by Dr. John Hooper as *Plakortis nigra* Levi, 1959 (Plakinidae, Homosclerophida), was hand-collected by Pat Colin from a depth of 380 ft at Palau using a mixed gas rebreathing apparatus. A voucher specimen has been deposited in the SIO Benthic Invertebrate Collection (# P1181). The diced, lyophilized sponge (61.4 g dry wt) was repeatedly extracted with MeOH at room temperature to obtain a dark brown oil (8.1 g). After the extract had been partitioned between EtOAc and H$_2$O, the aqueous extract was basified with dilute sodium hydroxide to pH 10 and again extracted with EtOAc. The combined EtOAc extracts (1.3 g), which exhibited activity against the HCT-116 cell line, were chromatographed on Sephadex LH-20 using MeOH as eluant to obtain plakortamines A (**1**, 144 mg, 0.61% yield), B (**2**, 15 mg, 0.063% yield), and C (**3**, 4.9 mg, 0.021% yield). One of the fractions from LH-20 was further purified by medium-pressure chromatography on Diaion HP-20SS using gradient elution

from 20% MeOH in H$_2$O to MeOH to obtain plakortamine D (**4**, 6.5 mg, 0.027% yield). Our first attempts to separate the most cytotoxic LH-20 fractions were unsuccessful and resulted in loss of the material, which consisted mainly of epiplakinic acids. However, several cytotoxic LH-20 fractions were combined and chromatographed on silica gel using a stepwise gradient from 20% EtOAc in hexanes to 100% EtOAc to obtain several active fractions that contained phenyl signals in their ^{1}H NMR spectra. The most active of these fractions were combined and chromatographed by HPLC on silica using 20% EtOAc in hexanes to obtain epiplakininc acid G (**5**, 4.7 mg) and epiplakinic acid H (**6**, 1 mg). The less polar fractions were combined and chromatographed by HPLC on silica using 10% EtOAc in hexanes to obtain (2S^*,4R^*)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone (**7**, 1.9 mg) and (2R^*,4R^*)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone (**8**, 0.7 mg).

Plakortamine A (1): pale yellow oil; UV (MeOH) 243 nm (ϵ 20 000), 295 nm (ϵ 11 700); IR (film) 3200 (br), 1620, 1570 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 12.52 (br s, 1 H, $-N$H), 8.28 (d, 1 H, $J = 5.5$ Hz, H-3), 7.95 (d, 1 H, $J = 8$ Hz, H-5), 7.78 (d, 1 H, $J = 5.5$ Hz, H-4), 7.67 (d, 1 H, $J = 1.5$ Hz, H-8), 7.34 (dd, 1 H, $J = 8$, 1.5 Hz, H-6), 3.36 (t, 2 H, $J = 5.5$ Hz, H-10), 2.82 (t, 2 H, $J = 5.5$ Hz, H-11), 2.47 (s, 6 H, NMe$_2$); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 144.0 (C-1), 143.1 (C-8a), 138.7 (C-3), 135.9 (C-9a), 129.8 (C-4a), 124.0 (C-5), 124.0 (C-6), 123.0 (C-7), 121.5 (C-4b), 115.7 (C-8), 114.5 (C-4), 58.5 (C-11), 44.7 (NMe$_2$), 30.9 (C-10); HREIMS [M + H]$^+$ m/z 318.0596 (calcd for C$_{15}$H$_{17}$^{79}BrN$_3$, 318.0606).

Plakortamine B (2): yellow oil; UV (MeOH) 214 nm (ϵ 32 000); IR (film) 3200 (br), 1620, 1560, 1235 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 8.61 (br s, 1 H, $-N$H), 8.48 (d, 1 H, $J = 5.5$ Hz, H-3), 7.97 (d, 1 H, $J = 8$ Hz, H-5), 7.84 (d, 1 H, $J = 5.5$ Hz, H-4), 7.69 (d, 1 H, $J = 1.5$ Hz, H-8), 7.41 (dd, 1 H, $J = 8$, 1.5 Hz, H-6), 7.18 (dd, 1 H, $J = 17$, 11 Hz, H-10), 6.40 (d, 1 H, $J = 17$ Hz, H-11), 5.72 (d, 1 H, $J = 11$ Hz, H-11); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 143.2 (C-8a), 141.0 (C-1), 139.0 (C-3), 135.1 (C-9a), 132.8 (C-10), 130.6 (C-4a), 124.1 (C-5), 123.9 (C-6), 123.0 (C-7), 121.3 (C-4b), 119.9 (C-11), 115.7 (C-8), 115.1 (C-4); HREIMS [M + H]$^+$ m/z 275.0008 (calcd for C$_{13}$H$_{10}$^{81}BrN$_2$, 275.0007).

Plakortamine C (3): pale yellow oil; UV (MeOH) 243 nm (ϵ 57 400), 296 nm (ϵ 29 000); IR (film) 3200 (br), 1620, 1570 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 8.14 (d, 2 H, $J = 5.5$ Hz, H-3), 7.96 (d, 2 H, $J = 8$ Hz, H-5), 7.77 (d, 2 H, $J = 5.5$ Hz, H-4), 7.53 (d, 2 H, $J = 1.5$ Hz, H-8), 7.32 (dd, 2 H, $J = 8$, 1.5 Hz, H-6), 3.31 (t, 4 H, $J = 5.5$ Hz, H-10), 3.09 (t, 4 H, $J = 5.5$ Hz, H-11), 2.58 (s, 3 H, NMe$_2$); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 143.9 (C-1,1′), 142.9 (C-8a,8a′), 138.5 (C-3,3′), 135.8 (C-9a,9a′), 129.6 (C-4a,4a′), 124.0 (C-5,5′), 123.9 (C-6,6′), 122.9 (C-7,7′), 121.4 (C-4b,4b′), 115.7 (C-8,8′), 114.3 (C-4,4′), 56.3 (C-11,11′), 41.7 (NMe), 30.4 (C-10,10′); HREIMS [M + H]$^+$ m/z 578.0372 (calcd for C$_{27}$H$_{24}$^{79}Br^{81}BrN$_5$, 578.0379).

Plakortamine D (4): pale yellow oil; [α]$_D$ $-2.1°$ (c 0.6, MeOH); UV (MeOH) 243 nm (ϵ 26 600), 296 nm (ϵ 14 700); IR (film) 3400 (br), 1625, 1570, 1420 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 11.37 (br s, 1 H, $-N$H), 8.29 (d, 1 H, $J = 5.5$ Hz, H-3), 7.99 (d, 1 H, $J = 8$ Hz, H-5), 7.95 (d, 1 H, $J = 5.5$ Hz, H-4), 7.78 (d, 1 H, $J = 1.5$ Hz, H-8), 7.41 (dd, 1 H, $J = 8$, 1.5 Hz, H-6), 5.90 (dd, 1 H, $J = 8.5$, 7 Hz, H-10) 3.61 (t, 1 H, $J = 8$ Hz, H-12), 3.08 (m, 1 H, H-11), 2.99 (s, 3 H, NMe), 2.80 (m, 1 H, H-12), 2.53 (m, 1 H, H-11); ^{13}C NMR (CDCl$_3$, 400 MHz) δ 145.3 (C-1), 141.8 (C-8a), 134.5 (C-3), 132.0 (C-9a), 130.4 (C-4a), 123.6 (C-5), 123.1 (C-7), 122.9 (C-6), 119.9 (C-4b), 115.2 (C-8), 114.5 (C-4), 79.1 (C-10), 58.2 (C-12), 45.0 (NMe), 37.0 (C-11); HREIMS [M + H]$^+$ m/z 332.0385 (calcd for C$_{15}$H$_{15}$^{79}BrN$_3$O, 332.0399).

Epiplakinic acid G (5): colorless oil; [α]$_D$ $-17.2°$ (c 0.3, MeOH); UV (MeOH) 255 nm (ϵ 253), 260 nm (ϵ 276), 269 nm (ϵ 218); IR (film) 3300 (br), 1705, 1450 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 7.24 (t, 2 H, $J = 7.5$ Hz), 7.18 (m, 3 H), 2.77 (d, 1 H, $J = 16$ Hz, H-2), 2.73 (d, 1 H, $J = 16$ Hz, H-2), 2.59 (t, 2 H, $J = 8$ Hz, H$_2$-17), 2.49 (d, 1 H, $J = 13$ Hz, H-4), 2.22 (d, 1 H, $J = 13$ Hz, H-4), 1.46 (s, 3 H, Me-24), 1.33 (s, 3 H, Me-25), 0.95 (d, 3 H, $J = 6.5$ Hz, Me-26), 0.83 (d, 3 H, $J = 7$ Hz, Me-

27); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 174.3 (C-1), 143.8 (C-18), 129.2 (C-20,22), 129.0 (C-19,23), 126.4 (C-21), 87.8 (C-5), 84.9 (C-3), 58.3 (C-4), 47.9 (C-8), 47.5 (C-6), 45.3 (C-2), 37.9 (C-10), 36.9 (C-17), 32.7 (C-15), 31.1, 31.0, 30.7 (C-9), 30.6, 30.3, 28.1 (C-7), 27.9 (C-16), 24.8 (C-24), 24.1 (C-25), 22.2 (C-26), 20.7 (C-27); ESIMS (+ve) m/z 455 [M + Na]$^+$, (−ve) 431 [M − H]$^-$; HRMALDIMS [M + Na]$^+$ m/z 455.3127 (calcd for C$_{27}$H$_{44}$O$_4$Na, 455.3132).

Epiplakinic acid H (6): colorless oil; [α]$_D$ +33° (*c* 0.07, MeOH); UV (MeOH) 254 nm (ε 269), 259 nm (ε 270), 268 nm (ε 212); IR (film) 3300 (br), 1705, 1455 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.22 (t, 2 H, J = 7.5 Hz), 7.16 (m, 3 H), 2.76 (d, 1 H, J = 16 Hz, H-2), 2.73 (d, 1 H, J = 16 Hz, H-2), 2.59 (t, 2 H, J = 8 Hz, H$_2$-17), 2.47 (d, 1 H, J = 13 Hz, H-4), 2.22 (d, 1 H, J = 13 Hz, H-4), 1.48 (s, 3 H, Me-24), 1.37 (s, 3 H, Me-25), 0.91 (d, 3 H, J = 6.5 Hz, Me-26), 0.83 (d, 3 H, J = 7 Hz, Me-27); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 175.2 (C-1), 143.8 (C-18), 129.2 (C-20,22), 129.0 (C-19,23), 126.4 (C-21), 87.9 (C-5), 84.9 (C-3), 58.3 (C-4), 47.7 (C-8), 47.2 (C-6), 46.4 (C-2), 37.8 (C-10), 36.9 (C-17), 32.8 (C-15), 31.2, 31.0, 30.7 (C-9), 30.6, 30.3, 28.2 (C-7), 27.9 (C-16), 24.8 (C-24), 24.4 (C-25), 22.1 (C-26), 20.7 (C-27); HRMALDIMS [M + Na]$^+$ m/z 455.3128 (calcd for C$_{27}$H$_{44}$O$_4$Na, 455.3132).

(2S*,4R*)-2,4-Dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone (7): colorless oil; [α]$_D$ −7.1° (*c* 0.13, MeOH); UV (MeOH) 254 nm (ε 196), 261 nm (ε 215) 268 nm (ε 170); IR (film) 1765, 1450 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.22 (t, 2 H, J = 7.5 Hz), 7.16 (m, 3 H), 2.91 (m, 1 H), 2.58 (t, 2 H, J = 8 Hz), 2.27 (dd, 1 H, J = 12.5, 9 Hz), 1.72 (dd, 1 H, J = 12.5, 11.5 Hz), 1.67 (m, 2 H), 1.59 (m, 2 H), 1.34 (s, 3 H), 1.31−1.28 (m, 18 H), 1.21 (d, 3 H, J = 7 Hz); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 181.6 (C-1), 143.8 (C-17), 129.2 (C-19,21), 129.0 (C-18,22), 126.4 (C-20), 86.1 (C-4), 42.8 (C-5), 42.5 (C-3), 36.9 (C-16), 36.3 (C-2), 32.8 (C-14), 31.0, 30.6−30.7 (6C), 30.3 (C-15), 24.9 (C-24), 24.9 (C-6), 15.7 (C-23); HRMALDIMS [M + Na]$^+$ m/z 381.2763 (calcd for C$_{24}$H$_{38}$O$_2$Na, 381.2764).

(2R*,4R*)-2,4-Dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone (8): colorless oil; [α]$_D$ +19.3° (*c* 0.05, MeOH); UV (MeOH) 252 nm (ε 326), 258 nm (ε 296) 268 nm (ε 235); IR (film) 1765, 1455 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.22 (t, 2 H, J = 7.5 Hz), 7,16 (m, 3 H), 2.86 (m, 1 H), 2.58 (t, 2 H, J = 8 Hz), 2.43 (dd, 1 H, J = 12.5, 9 Hz), 1.66 (dd, 1 H, J = 12.5, 11.5 Hz), 1.59 (m, 4 H), 1.39 (s, 3 H), 1.31−1.28 (m, 18 H), 1.22 (d, 3 H, J = 7 Hz); ^{13}C NMR (MeOH-d_4, 400 MHz) δ 181.6 (C-1), 143.8 (C-17), 129.2 (C-19,21), 129.0 (C-18,22), 126.4 (C-20), 86.3 (C-4), 42.6 (C-3), 41.1 (C-5), 36.9 (C-16), 36.8 (C-2), 32.8 (C-14), 31.0, 30.6−30.7 (6C), 30.3 (C-15), 27.2 (C-24), 25.3 (C-6), 16.3 (C-23); HRMALDIMS [M + Na]$^+$ m/z 381.2770 (calcd for C$_{24}$H$_{38}$O$_2$Na, 381.2764).

References and Notes

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