PLA_2 Inhibitory Activity of Marine Sesterterpenoids Cladocorans, Their Diastereomers and Analogues

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Inhibition of secretory phospholipase A_2 ($sPLA_2$) by cladocorans A and B and their diastereomers almost equaled that of manoalide.

Key words secretory phospholipase A_2 ($sPLA_2$); inhibitor; cladocoran; analogue; manoalide

Numerous natural products have been isolated from various marine invertebrates, many of which possess interesting biological activity.[1] The marine sesterterpenoids manoalide, isolated from the marine sponge *Luffariella variabilis*,[2] and cacospongionolide B, isolated from the marine sponge *Fasciospongia cavernosa*,[3] were shown to possess antimicrobial, cytotoxic and anti-inflammatory activity.[4] The potent anti-inflammatory activity of the natural products was attributed to inhibition of secretory phospholipase A_2 ($sPLA_2$). Given the general role of inflammation in diseases that include bronchial asthma and rheumatoid arthritis, identifying and developing potent inhibitors of $sPLA_2$ continues to be of great importance. The biological activity of these compounds is thought to be derived from the presence of γ-hydroxybutenolide and dihydropyran moieties.[5]

Dysidiolide, isolated from the sponge *Dysidea etheria* DE LAUBENFELS,[6] and cladocorans A and B, isolated from the coral *Cladocora cespitosa*,[7] are marine sesterterpenoids that possess a γ-hydroxybutenolide moiety. Although the inhibition of protein phosphatase cdc25A by dysidiolide has been reported,[6] the biological activity of cladocorans A and B was not reported. Cladocorans might inhibit $sPLA_2$ given the structural similarity with manoalide and cacospongionolide B. The total synthesis of dysidiolide[8,9] and cladocorans A and B[10] was recently reported by the authors. In this paper, the authors wish to report on the synthesis of cladocoran's analogues, and the inhibition of $sPLA_2$ by cladocorans, their diastereomers and analogues.

Cladocorans A and B and their diastereomers were tested for their ability to inhibit $sPLA_2$. Manoalide, a potent inhibitor of $sPLA_2$, was employed as a reference compound. The inhibitory effect of each compound was determined over a range of concentration and IC_{50} values were calculated (Table 1). Several important aspects concerning the interaction of the substrates with $sPLA_2$ were noted. Cladocoran A, 15-epicladocoran A and 15-epi-18-epicladocoran A showed inhibitory activity equivalent to the IC_{50} value of manoalide. The other compounds showed $sPLA_2$ inhibition equivalent to 1/2—1/3 that of manoalide. No significant difference in inhibition was observed for the C-15 and C-18 diastereomers. Even when the hydroxy functional group of C-18 was replaced with an acetoxy group, no significant difference in the inhibition of $sPLA_2$ was observed. Because these compounds do not have dihydropyran moiety, the γ-hydroxybutenolide moiety is thought to be more important than the dihydropyran moiety for $sPLA_2$ inhibition. Consequently, analogues **1A**—**E** possessing a γ-hydroxybutenolide moiety were design and synthesized.

Analogues **1A**, **B**, **C** and **D** were synthesized from aldehydes **2A**, **B**, **C** and **D**, respectively. Treatment of aldehydes **2A**,[11] **B**,[12] **C**[13] and **D**[14] with 3-lithiofuran, prepared from 3-bromofuran and ^{n}BuLi, yielded alcohols **3A**, **B**, **C** and **D**, respectively. Photosensitized oxygenation[15] of alcohols **3A**, **B**, **C** and **D** afforded analogues **1A**, **B**, **C** and **D**, respectively. Analogue **1E** was synthesized from aldehyde **4** *via* aldehyde **2E**. Aldehyde **4**[16] was treated with Wittig reagent, prepared from $Ph_3P^+CH_2OMeCl^-$ and KHMDS, to yield the enol ether, which was subsequently hydrolyzed to afford aldehyde **2E**. Aldehyde **2E** was treated with 3-lithiofuran to yield alcohols **3Ea** and **3Eb**, which represent diastereomers at the secondary hydroxy group. The relative configuration of the secondary hydroxy group in **3Ea** and **3Eb** was not determined, and the compound with the higher *Rf* value following TLC was designated as **3Ea**, while that with the lower *Rf* value was designated as **3Eb**. Photosensitized oxygenation of alcohols **3Ea** and **3Eb** afforded analogues **1Ea** and **1Eb**, respectively.

Analogues **1A**—**E** were tested for their ability to inhibit

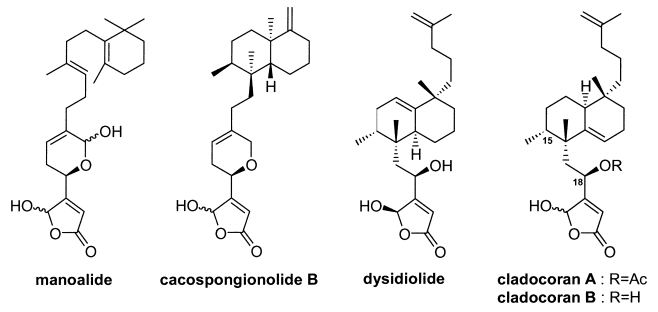

Fig. 1. Structure of Manoalide, Cacospongionolide B, Cladocorans, and Dysidiolide

Table 1. Inhibition of $sPLA_2$ by Cladocorans A and B and their Diastereomers

Compound	IC_{50} (μM)
Cladocoran A	0.78 (±0.06)
15-Epicladocoran A	0.87 (±0.05)
18-Epicladocoran A	1.37 (±0.09)
15-Epi-18-epicladocoran A	0.66 (±0.07)
Cladocoran B	1.95 (±0.08)
15-Epicladocoran B	1.23 (±0.11)
18-Epicladocoran B	1.82 (±0.10)
15-Epi-18-epicladocoran B	1.83 (±0.05)
Manoalide	0.59 (±0.20)

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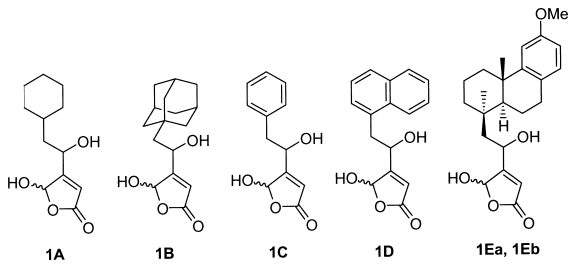
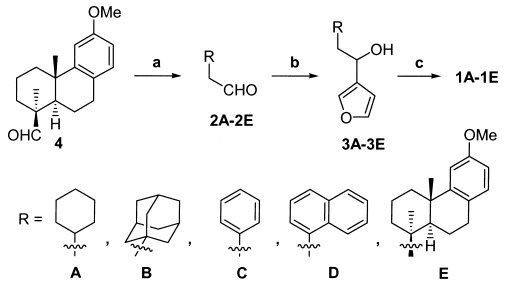


Fig. 2. Structure of Cladocoran Analogues



Reagents and conditions: (a) (i) $Ph_3P^+CH_2OMeCl^-$, KHMDS, THF, −78 to 0 °C, (ii) 1 M HCl aq., 1,4-dioxane, 40 °C; (b) 3-bromofuran, ^{n}BuLi, THF, −78 °C; (c) O_2, Rose Bengal, *hv*, ^{i}Pr$_2$NEt, CH_2Cl_2, −78 °C to r.t.

Chart 1

Table 2. Inhibition of *s*PLA$_2$ by Cladocoran Analogues

Compound	IC_{50} (μM)
1A	>20
1B	17 (±0.7)
1C	>20
1D	>20
1Ea	8.9 (±2.2)
1Eb	7.5 (±1.4)
Manoalid	0.59 (±0.20)

*s*PLA$_2$ using manoalide as the reference compound (Table 2). Analogues **1A**—**D**, possessing a relatively smaller hydrophobic substructure, showed no inhibition of *s*PLA$_2$. Analogues **1Ea** and **1Eb**, on the other hand, possessing a comparatively larger hydrophobic substructure, showed *s*PLA$_2$ inhibition equivalent to 1/15 that of manoalide.

In conclusion, cladocorans A and B and their diastereomers were capable of inhibiting the activity of *s*PLA$_2$ to an extent that almost equaled that of manoalide. Analogues **1Ea** and **1Eb**, possessing the hydrophilic substructure represented by the γ-hydroxybutenolide moiety and a comparatively large hydrophobic substructure, showed weak inhibition of *s*PLA$_2$. These results suggest that in order for a compound possessing a γ-hydroxybutenolide moiety to show inhibitory activity against *s*PLA$_2$, it must have an appropriately-sized hydrophobic substructure.

Experimental

Melting points (mp) were measured using the Yazawa melting point apparatus BY-2 and are uncorrected. Optical rotations were measured using a JASCO DIP-360 polarimeter. IR spectra were recorded using a JASCO FT-IR/620 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX-400 or DRX-500 spectrometer. Chemical shifts are given on the δ (ppm) scale using tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). EI-MS spectra were obtained using a Thermo Quest TSQ 700 spectrometer and high resolution EI-MS (HR-EI-MS) spectra were obtained using a VG Auto Spec E spectrometer. ESI-MS and high resolution ESI-MS (HR-ESI-MS) spectra were obtained using a Micromass LCT spectrometer. Flash column chromatography was carried out on Kanto Chemical Silica Gel 60N (spherical, neutral) 40—50 μm.

(6-Methoxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl)acetaldehyde (2E) To a suspension of $Ph_3P^+CH_2OMeCl^-$ (88.0 mg, 257 mmol) in THF (210 μl) was added dropwise KHMDS (0.5 M in toluene, 510 μl, 257 μmol) at −78 °C. Following stirring for 30 min, a solution of aldehyde **4** (12.7 mg, 46.6 μmol) in THF (300 μl) was added and the reaction mixture was warmed to 0 °C. Following stirring for another 30 min, the reaction mixture was diluted with Et_2O and then washed with water and saturated aqueous NaCl. The organic layer was then dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : Et_2O=20 : 1) to yield a mixture of the *E*- and *Z*-enol ethers (12.1 mg, 86% yield, *E* : *Z*=5 : 9) as a colorless oil.

To a solution of the above mixture in 1,4-dioxane (400 μl) was added aqueous HCl (1 M, 15.0 μl). Following stirring for 30 min at 40 °C, the reaction mixture was diluted with Et_2O and then washed with water and saturated aqueous NaCl. The organic layer was then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : EtOAc=20 : 1) to yield aldehyde **2E** (10.8 mg, 84% yield) as a colorless oil: $[\alpha]_D^{31}$ +46.6° (*c*=0.52, $CHCl_3$); IR (neat) cm^{-1}: 2926, 2849, 1717; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.88 (1H, t, *J*=3.3 Hz), 6.97 (1H, d, *J*=8.4 Hz), 6.81 (1H, d, *J*=2.6 Hz), 6.68 (1H, dd, *J*=2.6, 8.4 Hz), 3.78 (3H, s), 2.91 (1H, m), 2.81 (1H, m), 2.60 (1H, dd, *J*=2.9, 14.0 Hz), 2.39 (1H, ddd, *J*=1.2, 3.6, 14.0 Hz), 2.31 (1H, m), 1.84—1.94 (2H, m), 1.80 (1H, tt, *J*=3.2, 13.5 Hz), 1.61—1.74 (2H, m), 1.46 (1H, dt, *J*=3.8 13.0 Hz), 1.37 (1H, dd, *J*=2.0, 12.4 Hz), 1.26 (1H, m), 1.22 (3H, s), 1.18 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 204.0, 157.7, 150.7, 129.7, 126.8, 110.9, 110.1, 55.2, 52.2, 47.5, 38.7, 38.5, 37.9, 36.7, 29.6, 29.3, 25.0, 19.3, 18.8; ESI-MS *m/z*: 287 (M^++H, 27), 243 (100); HR-ESI-MS *m/z*: 287.2032 (Calcd for $C_{19}H_{27}O_2$: M^++H, 287.2011).

Typical Procedure for the Synthesis of Alcohols 3A—E ^{n}BuLi (1.56 M in hexane, 360 μl, 563 μmol) was added to a solution of 3-bromofuran (60.7 μl, 675 μmol) in THF (625 μl) at −78 °C. After 30 min, the yellow solution was treated with a solution of aldehyde **2A** (12.5 mg, 99.0 μmol) in THF (500 μl). Following stirring for 30 min, saturated aqueous NH_4Cl was added, the mixture was warmed to room temperature (r.t.) and then Et_2O was added. The organic layer was washed with saturated aqueous NH_4Cl, water and then saturated aqueous NaCl. The mixture was then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : EtOAc=6 : 1) to yield alcohol **3A** (12.9 mg, 67% yield) as colorless crystals.

3A: mp 53—55 °C; IR (KBr) cm^{-1}: 3254, 2924; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37 (2H, m), 6.40 (1H, dd, *J*=0.9, 1.3 Hz), 4.75 (1H, dd, *J*=5.5, 8.4 Hz), 1.80 (2H, m), 1.62—1.72 (4H, m), 1.53 (1H, ddd, *J*=5.5, 7.6, 13.7 Hz), 1.42 (1H, m), 1.10—1.29 (3H, m), 0.94 (2H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 143.2, 138.8, 129.6, 108.5, 64.4, 45.5, 34.0, 33.8, 32.9, 26.5, 26.2, 26.1; EI-MS *m/z*: 194 (M^+, 16), 150 (100); HR-EI-MS *m/z*: 194.1315 (Calcd for $C_{12}H_{18}O_2$: M^+, 194.1307).

3B: 78% yield as colorless crystals; mp 60—63 °C; IR (KBr) cm^{-1}: 3274, 2905; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.36 (2H, m), 6.39 (1H, m), 4.88 (1H, dd, *J*=3.9, 8.1 Hz), 1.96 (3H, br s), 1.56—1.73 (13H, m), 1.50 (1H, dd, *J*=3.9, 14.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 143.3, 138.5, 130.9, 108.6, 63.2, 52.5, 43.0, 37.0, 32.3, 28.7; EI-MS *m/z*: 246 (M^+, 25), 228 (M^+-H_2O, 15); HR-EI-MS *m/z*: 246.1645 (Calcd for $C_{16}H_{22}O_2$: M^+, 246.1620).

3C: 65% yield as a colorless oil; IR (neat) cm^{-1}: 3376, 2921; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.40 (1H, t, *J*=1.7 Hz), 7.36 (1H, dd, *J*=0.6, 0.7 Hz), 7.32 (2H, m), 7.21—7.25 (3H, m), 6.42 (1H, m), 4.90 (1H, m), 3.06 (1H, dd, *J*=5.1, 13.6 Hz), 3.00 (1H, dd, *J*=8.2, 13.6 Hz), 1.80 (1H, d, *J*=3.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 143.2, 139.1, 137.7, 129.5, 128.5, 128.3, 126.6, 108.5, 67.9, 44.6; EI-MS *m/z*: 188 (M^+, 100); HR-EI-MS *m/z*: 188.0824 (Calcd for $C_{12}H_{12}O_2$: M^+, 188.0837).

3D: 75% yield as colorless crystals; mp 44—47 °C; IR (KBr) cm^{-1}: 3387, 2927; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10 (1H, d, *J*=8.1 Hz), 7.91 (1H, d, *J*=7.5 Hz), 7.80 (1H, d, *J*=8.1 Hz), 7.56 (2H, m), 7.35—7.45 (4H, m), 6.50 (1H, s), 5.03 (1H, dd, *J*=4.7, 8.5 Hz), 3.55 (1H, dd, *J*=4.7, 14.0 Hz), 3.43 (1H, dd, *J*=8.5, 14.0 Hz), 2.11 (1H, br s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 143.2, 139.0, 133.9, 133.8, 132.0, 128.8, 128.4, 127.8, 127.4, 126.0, 125.6, 125.3, 123.6, 108.5, 67.0, 41.6; EI-MS *m/z*: 238 (M^+, 20), 142 (100); HR-EI-MS *m/z*: 238.0978 (Calcd for $C_{16}H_{14}O_2$: M^+, 238.0994).

3Ea: 49% yield as a colorless oil (*Rf* 0.21; hexane : EtOAc=5 : 1); $[\alpha]_D^{24}$ +19.2° (*c*=1.30, $CHCl_3$); IR (neat) cm^{-1}: 3443, 2926; ^{1}H-NMR (400 MHz,

$CDCl_3$) δ: 7.40 (2H, m), 6.96 (1H, d, J=8.4 Hz), 6.83 (1H, d, J=2.6 Hz), 6.67 (1H, dd, J=2.6, 8.4 Hz), 6.43 (1H, dd, J=1.2, 1.2 Hz), 4.84 (1H, dd, J=2.1, 8.3 Hz), 3.78 (3H, s) 2.88 (1H, m), 2.78 (1H, ddd, J=7.1, 11.6, 16.6 Hz), 2.30 (1H, m), 2.12—2.19 (2H, m), 1.80—1.94 (2H, m), 1.59—1.74 (3H, m), 1.45 (1H, dt, J=3.7, 13.1 Hz), 1.36 (1H, dd, J=2.0, 12.3 Hz), 1.25 (3H, s), 1.10 (1H, m), 1.06 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 157.7, 151.3, 143.3, 138.4, 131.3, 129.7, 127.2, 110.8, 110.3, 108.5, 65.1, 55.2, 52.9, 40.4, 39.1, 38.5, 38.2, 36.2, 30.0, 28.9, 25.6, 19.2, 19.1; ESI-MS m/z: 337 (M^++H−H_2O, 100); HR-ESI-MS m/z: 337.2141 (Calcd for $C_{23}H_{29}O_2$: M^++H−H_2O, 337.2168).

3Eb: 45% yield as colorless crystals (Rf 0.16; hexane : EtOAc=5 : 1); mp 104—108 °C; $[\alpha]_D^{25}$ +68.3° (c=1.39, $CHCl_3$); IR (KBr) cm^{-1}: 3498, 2925; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.40 (2H, m), 6.96 (1H, d, J=8.4 Hz), 6.79 (1H, d, J=2.6 Hz), 6.67 (1H, dd, J=2.6, 8.4 Hz), 6.44 (1H, dd, J=1.1, 1.1 Hz), 4.81 (1H, dd, J=5.0, 6.9 Hz), 3.77 (3H, s) 2.90 (1H, m), 2.80 (1H, ddd, J=7.3, 11.4, 16.8 Hz), 2.24 (1H, m), 2.16 (1H, dd, J=4.7, 14.6 Hz), 1.93 (1H, m), 1.69—1.80 (2H, m), 1.48—1.62 (3H, m), 1.42 (1H, dt, J=4.3, 12.6 Hz), 1.34 (1H, dd, J=2.0, 12.3 Hz), 1.21 (3H, s), 1.13 (3H, s), 1.01 (1H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 157.7, 151.3, 143.5, 138.7, 130.9, 129.7, 127.3, 110.8, 110.2, 108.4, 64.3, 55.2, 52.8, 40.4, 38.9, 38.1, 37.2, 36.0, 29.9, 29.6, 25.5, 19.0, 18.9; ESI-MS m/z: 337 (M^++H−H_2O, 100), 279 (70); HR-ESI-MS m/z: 337.2141 (Calcd for $C_{23}H_{29}O_2$: M^++H−H_2O, 337.2168).

Typical Procedure for the Synthesis of γ-Hydroxybutenolides 1A—E Rose Bengal (0.7 mg) was added to a solution of alcohol **3A** (15.0 mg, 77.2 μmol) and ^{i}Pr_2NEt (29.0 μl, 166 μmol) in CH_2Cl_2 (9.65 ml) at r.t. The suspension was cooled to −78 °C, saturated with anhydrous O_2 and then irradiated for 4 h using a 270 W tungsten filament lamp under an atmosphere of O_2. The resultant pink solution was warmed to r.t., and saturated aqueous oxalic acid (676 μl) was added. Following 30 min of vigorous stirring, the mixture was diluted with Et_2O and then washed with water and saturated aqueous NaCl. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : EtOAc=1 : 1) to yield γ-hydroxybutenolide **1A** (10.0 mg, 57% yield) as colorless crystals.

1A: mp 95—96 °C; IR (KBr) cm^{-1}: 3424, 2924, 1778; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.11 (1H, br s), 5.99 (1H, s), 4.59 (1H, br s), 1.90 (1H, br d, J=12.8 Hz), 1.60—1.76 (5H, m), 1.53 (2H, m), 1.16—1.36 (3H, m), 1.03 (1H, m), 0.92 (1H, m); ^{13}C-NMR (125 MHz, CD_3OD) δ: 175.9, 173.1, 117.2, 99.6, 66.7, 44.2, 35.4, 35.0, 33.2, 27.7, 27.5, 27.2; ESI-MS m/z: 191 (M^++H−2H_2O, 70), 173 (100); HR-ESI-MS m/z: 191.1056 (Calcd for $C_{12}H_{15}O_2$: M^++H−2H_2O, 191.1072).

1B: 49% yield as colorless crystals; mp 146—148 °C; IR (KBr) cm^{-1}: 3424, 2903, 1753; ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.12 (1H, br s), 5.97 (1H, s), 4.69 (1H, br s), 1.97 (3H, br s), 1.63—1.78 (12H, m), 1.42 (2H, m); ^{13}C-NMR (100 MHz, CD_3OD) δ: 177.0, 173.1, 117.0, 99.5, 65.7, 50.8, 44.0, 38.1, 33.7, 30.2; ESI-MS m/z: 279 (M^++H, 45), 261 (M^++H−H_2O, 100); HR-ESI-MS m/z: 279.1606 (Calcd for $C_{16}H_{23}O_4$: M^++H, 279.1596).

1C: 27% yield as a colorless oil; IR (neat) cm^{-1}: 3376, 2924, 1745; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.22—7.36 (5H, m), 6.07 (1H, br s), 6.00 (1H, s), 4.82 (1H, ddd, J=1.4, 4.8, 8.6 Hz), 3.11 (1H, dd, J=4.8, 13.7 Hz), 2.96 (1H, dd, J=8.6, 13.7 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.7, 169.2, 136.0, 129.5, 128.9, 127.3, 118.3, 98.0, 68.9, 42.1; ESI-MS m/z: 203 (M^++H−H_2O, 65), 185 (100), 157 (75); HR-ESI-MS m/z: 203.0708 (Calcd for $C_{12}H_{11}O_3$: M^++H−H_2O, 203.0708).

1D: 57% yield as colorless crystals; mp 123—126 °C; IR (KBr) cm^{-1}: 3358, 2929, 1739; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.15 (1H, d, J=8.4 Hz), 7.88 (1H, d, J=8.1 Hz), 7.79 (1H, m), 7.51 (2H, m), 7.42 (2H, m), 6.28 (1H, br s), 6.04 (1H, br s), 4.90 (1H, br s), 3.63 (1H, br s), 3.27 (1H, br s); ^{13}C-NMR (100 MHz, CD_3OD) δ: 174.3, 173.0, 135.5, 134.8, 133.4, 129.9, 129.3, 128.6, 127.1, 126.6, 126.4, 124.7, 118.4, 100.0, 69.5, 40.5; ESI-MS m/z: 253 (M^++H−H_2O, 95), 235 (45), 207 (100), 179 (65); HR-ESI-MS m/z: 253.0844 (Calcd for $C_{16}H_{13}O_3$: M^++H−H_2O, 253.0865).

1Ea: 63% yield as a colorless oil; $[\alpha]_D^{26}$ +37.0° (c=0.27, $CHCl_3$); IR (neat) cm^{-1}: 3376, 2925, 1746; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.96 (1H, d, J=8.4 Hz), 6.80 (1H, d, J=2.6 Hz), 6.67 (1H, dd, J=2.6, 8.4 Hz), 6.19 (1H, s), 6.06 (1H, s), 4.76 (1H, d, J=9.4 Hz), 3.77 (3H, s), 2.89 (1H, dd, J=5.5, 16.6 Hz), 2.79 (1H, ddd, J=7.1, 11.5, 16.6 Hz), 2.29 (1H, br d, J=12.6 Hz), 2.03—2.12 (2H, m), 1.76—1.88 (2H, m), 1.57—1.72 (3H, m), 1.44 (1H, dt, J=3.7, 13.2 Hz), 1.41 (1H, dd, J=1.9, 12.4 Hz), 1.22 (3H, s), 1.14 (1H, m), 1.10 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.5, 170.3, 157.7, 150.9, 129.7, 127.0, 117.4, 111.0, 110.3, 97.6, 66.7, 55.3, 52.7, 38.9, 38.6, 38.2, 38.2, 36.5, 29.9, 28.8, 25.6, 19.2, 19.0; ESI-MS m/z: 387 (M^++H, 25), 351 (30), 243 (70), 161 (100); HR-ESI-MS m/z: 387.2178 (Calcd for $C_{23}H_{31}O_5$: M^++H, 387.2171).

1Eb: 63% yield as colorless oil; $[\alpha]_D^{26}$ +16.7° (c=0.18, $CHCl_3$); IR (neat) cm^{-1}: 3376, 2925, 1746; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.96 (1H, d, J=8.4 Hz), 6.79 (1H, d, J=2.6 Hz), 6.67 (1H, dd, J=2.6, 8.4 Hz), 6.17 (1H, br s), 6.07 (1H, br s), 4.73 (1H, br d, J=7.9 Hz), 3.92 (1H, br s), 3.77 (3H, s) 2.91 (1H, dd, J=6.5, 16.5 Hz), 2.81 (1H, ddd, J=7.3, 11.4, 16.5 Hz), 2.28 (1H, br d, J=11.7 Hz), 2.07 (1H, m), 2.05 (1H, br s), 1.92 (1H, m), 1.83 (1H, m), 1.58—1.75 (3H, m), 1.47 (1H, m), 1.40 (1H, dd, J=1.9, 12.4 Hz), 1.23 (1H, m), 1.20 (3H, s), 1.16 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 171.9, 169.9, 157.8, 150.9, 129.8, 127.1, 117.5, 111.0, 110.2, 97.1, 65.8, 55.3, 52.4, 38.7, 38.6, 38.0, 37.2, 36.3, 29.9, 29.7, 25.4, 19.0, 18.9; ESI-MS m/z: 387 (M^++H, 20), 351 (25), 243 (100), 161 (60); HR-ESI-MS m/z: 387.2195 (Calcd for $C_{23}H_{31}O_5$: M^++H, 387.2171).

Assay for the Inhibition of Bee Venom *s*PLA$_2$ IC_{50} values for cladocorans A and B, their diastereomers and analogues **1A**—**E** were determined using a *s*PLA$_2$ Assay Kit (Cat. No. 765001) obtained from Cayman Chemical. UV data were recorded using a TECAN Austria Gmb, Safire microplate reader. Manoalide was obtained from Wako Pure Chemical Industries, Ltd. Following execution of the assays, the initial rate of hydrolysis for the series of substrates with final concentration ranging from 400—1.0 μM (400, 200, 100, 50, 25, 5.0, 1.0 μM) were obtained and the percent inhibition was calculated relative to the control. The percent inhibition was plotted against log[inhibitor] and the IC_{50} value was extrapolated from the resulting curve. The values reported represent the average of measurements with errors of plus and minus one standard deviation unit.

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