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Bicyclic α,ω-dicarboxylic acid derivatives from a colonial tunicate of the family Polyclinidae

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ABSTRACT

In the course of our search for bioactive metabolites from a colonial tunicate of the family Polyclinidae, six new (**1**–**6**) cyclic fatty acid derivatives were isolated. Their planar structures were established on the basis of NMR and MS spectroscopic analyses. The relative configuration was determined by NOESY experiment. Compounds **1**–**6** represent a fused bicyclic skeleton possibly derived from α,ω-dicarboxylic acids such as eicosanedioic acid or docosanedioic acid via a Diels–Alder type of cyclization. Compounds **1**–**4** and **6** showed mild cytotoxicity against a panel of five human solid tumor cell lines.

Colonial tunicates are widely recognized as one of the most prolific producers of bioactive natural products in the marine environment. Many of these metabolites exhibit potent and diverse bioactivities, such as antibacterial,[1] antiviral,[2] antioxidative,[3] cytotoxic,[4] and antitumor activities.[5] Of the six marine-derived compounds that have reached clinical trials as antitumor agents, three—didemnin B, aplidine, and ecteinascidin 743—are derived from colonial tunicates.[6] In the course of our search for bioactive metabolites from a marine colonial ascidian of the family Polyclinidae, six new (**1**–**6**) α,ω-dicarboxylic acid derivatives, which represent a unique fused bicyclic skeleton, were isolated. Herein, we describe the purification,[7] structure elucidation, and biological evaluation of these compounds.

Aplidic acid A (**1**) was isolated as a yellow oil.[8] The molecular formula was established as $C_{23}H_{30}O_4$ on the basis of the HRFABMS and NMR data (Table 1). The exact mass of the $[M-H]^-$ ion at m/z 369.2085 matched well with the expected formula of $C_{23}H_{29}O_4$ (Δ +1.9 mmu). The ^{1}H NMR spectrum of **1** displayed ten olefinic signals and one methoxyl signal, in addition to methine and methylene signals resonating between δ_H 2.88 and δ_H 1.18 ppm. The ^{13}C NMR and DEPT spectra revealed that **1** contained a methoxyl, six

Table 1
^{1}H and ^{13}C NMR data of compound **1**[a]

Position	^{1}H	^{13}C
1		169.2
2	5.87 (d, 15.0)	120.2
3	7.28 (dd, 15.0, 10.0)	146.5
4	6.30 (dd, 15.0, 10.0)	130.8
5	6.22 (dd, 15.0, 9.0)	145.5
6	2.57 (dd, 9.0, 2.5)	45.8
7	1.61 (m)	43.8
8	1.23 (td, 11.5, 8.0, H8a), 1.56 (m, H8b)	27.3
9	1.65–1.60 (m, H9a), 1.70 (m, H9b)[b]	22.9
10	1.18 (m, H10a), 1.86 (m, H10b)	29.9
11	1.84 (m)	40.2
12	5.98 (br d, 10.0)	131.7
13	5.52 (dt, 10.0, 3.5)	128.8
14	2.88 (m)	46.0
15	5.65 (dd, 15.0, 7.0)	135.6
16	6.01 (dd, 15.0, 10.0)	131.9
17	6.07 (dd, 15.0, 10.0)	132.3
18	5.60 (dd, 15.0, 7.5)	133.2
19	2.13 (dd, 15.0, 7.5)	33.0
20	1.70 (m)[b]	25.8
21	2.30 (t, 7.5)	34.3
22		177.4
23	3.73 (s)	52.0

[a] ^{1}H and ^{13}C NMR data were measured at 500 MHz and 100 MHz, respectively, in CD_3OD.
[b] Overlapped signals.

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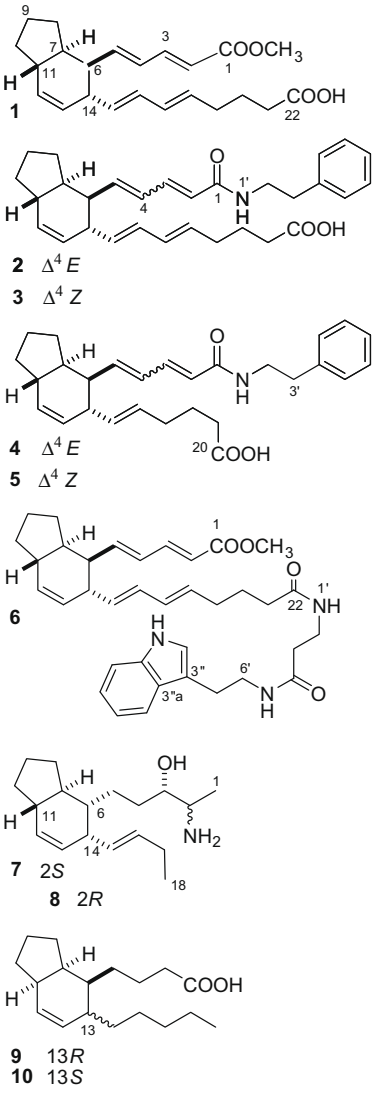
methylenes, and fourteen methines. The NMR data revealed the presence of five double bonds (δ_H/δ_C 5.60/133.2, 6.07/132.3, 6.01/131.9, 5.65/135.6, 5.52/128.8, 5.98/131.7, 6.22/145.5, 6.30/130.8. 7.28/146.5, and 5.87/120.2), which accounted for five of the nine degrees of unsaturation (including two carbonyls), thus indicating the presence of two rings in the molecule. The ^1H–^1H coupling constants of corresponding olefinic protons ($J_{2,3}$ = 15.0, $J_{4,5}$ = 15.0, $J_{12,13}$ = 10.0, $J_{15,16}$ = 15.0, $J_{17,18}$ = 15.0) suggested *E* geometry for the $\Delta^{2,4,15,17}$ and *Z* geometry for the Δ^{12} double bonds. Interpretation of the COSY and HMBC spectra indicated the presence of octa-5,7-dienoyl ($C_8H_{11}O_2$) and methyl penta-2,4-dienoate ($C_6H_7O_2$) moieties. The remaining partial structure (C_9H_{12}) was assigned as a fused bicyclic structure (2,3,3a,4,5,7a-hexahydro-4,5-disubstituted-1*H*-indene) by analysis of COSY, TOCSY, and HMBC data (Fig. 1). Scalar coupling of H-14/H-13/H-12 and H-10/H-11/H-7/H-8 could be delineated from the COSY spectrum. Long-range HMBC correlations from H-6 (δ_H 2.57) to C-7 (δ_C 43.8), C-11 (δ_C 40.2), C-13 (δ_C 128.8), and C-14 (δ_C 46.0); H-8b (δ_H 1.56) to C-7, C-9 (δ_C 22.9), C-10 (δ_C 29.9), and C-11; H-10a (δ_H 1.18) to C-7, C-9, C-11, and C-12 (δ_C 131.7); H-12 (δ_H 5.98) to C-7 and C-11; H-13 (δ_H 5.52) to C-6 (δ_C 45.8) and C-11 (δ_C 40.2); H-14 (δ_H 2.88) to C-6 (δ_C 45.8) and C-7 (δ_C 43.8), established the connectivity of the bicyclic ring (Fig. 1). COSY correlations H-6/H-5 (δ_H 6.22) and H-14/H-15 (δ_H 5.65) established the connectivity of the alkyl chains to the ring.



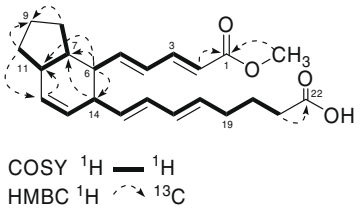


Figure 1. COSY and HMBC correlations of compound **1**.

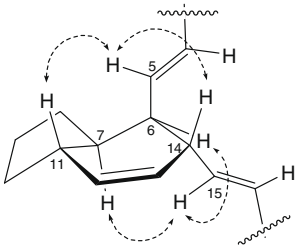
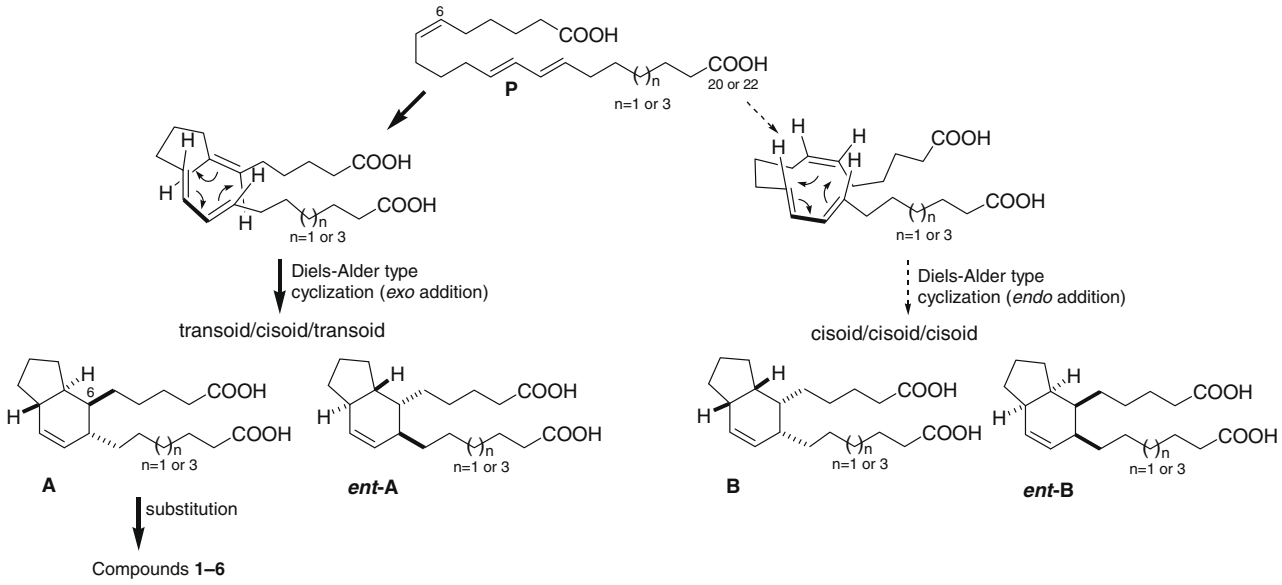


Figure 2. Key NOESY correlations of compound **1**.

The relative configuration of **1** was defined by a NOESY experiment. The NOESY correlations H-6/H-15, H-7/H-15, H-11/H-5, and H-14/H-5 indicated a transoid/cisoid/transoid arrangement of H-11/H-7/H-6/H-14 of the fused bicyclic ring system (Fig. 2). The small coupling constant (2.5 Hz) between H-6 and H-7 also supported a cisoid arrangement of H-7 and H-6. Biogenetically, compound **1** may be derived by an intramolecular Diels–Alder type cyclization of properly unsaturated C_{20} or C_{22} α,ω-dicarboxylic acids such as eicosanedioic acid or docosanedioic acid (Scheme 1). A pair of transoid/cisoid/transoid products (types **A** and *ent*-**A**) are expected by non-stereospecific cyclization of (6*Z*,11*E*,13*E*)-dicarboxylic acid precursor (**P**) via exo addition, while all-cisoid isomers (types **B** and *ent*-**B**) would be expected via endo addition. The absolute configuration of the compound was analyzed with an exciton chirality method.[9] According to the similar NMR data and the same sign of specific rotation, compounds **1**–**6** were assumed to share the same absolute configuration. As a typical example, the CD spectrum of **3** was examined (Fig. 3). The CD spectrum of **3** showed a rather weak but clear bisignate Cotton effect at λ 207 nm ($\Delta\varepsilon$ +0.14) and 241 nm ($\Delta\varepsilon$ −0.27). This Cotton effect was attributed to an exciton coupling between the electric dipole transition moments of the diene chromophores (λ_{max} 222.5 nm). The negative exciton chirality of **3** suggested that the two diene chromophores are oriented in a counterclockwise manner (Fig. 3). In an energy-minimized structure of **3**, the counterclockwise orientation of the two diene chromophores was clear for the (6*S*,7*S*,11*S*,14*R*) configuration (type **A** in Scheme 1).

Aplidic acid B (**2**) was isolated as a yellow, amorphous powder. The molecular formula was established as $C_{30}H_{37}NO_3$ on the basis of the HRFABMS and NMR data. The exact mass of the $[M+H]^+$ ion at *m*/*z* 460.2835 matched well with the expected formula of $C_{30}H_{38}NO_3$ (Δ −1.7 mmu). The structure of **2** was similar to **1**, with the primary difference of a methyl ester being replaced by a phenethylamide. Two triplets at δ_H 3.48 (H-2′) and 2.84 (H-3′), and a spin system comprised of signals at δ_H 7.23 (H-5′, H-9′), 7.29 (H-6′, 8′), and 7.20 (H-7′), indicated the presence of a 2-phenethylamine moiety. A long-range HMBC correlation from H-2′ (δ_H 3.48) to C-1 (δ_C 169.0) established connectivity via an amide bond.

4*Z*-Aplidic acid B (**3**) was isolated as a red, amorphous powder. The molecular formula was established as $C_{30}H_{37}NO_3$ on the basis of the HRFABMS and NMR data. The exact mass of the $[M+H]^+$ ion at *m*/*z* 460.2835 matched well with the expected formula of $C_{30}H_{38}NO_3$ (Δ −1.7 mmu). A notable difference in spectral data



Scheme 1. Hypothetical biogenesis of compounds **1**–**6**.

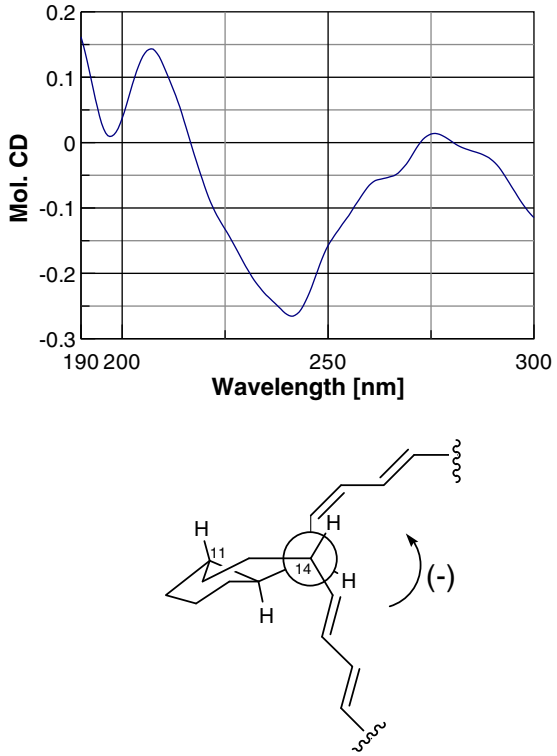


Figure 3. CD spectrum and a negative chirality of the diene chromophores of compound **3**.

from those of **2** was the coupling constant between H-4 and H-5 ($J_{4,5}$ = 11.0 Hz vs 15.0 Hz of **2**), along with different ^{1}H and ^{13}C NMR data of the following atoms: H-6/C-6 (δ_H 3.05/δ_C 44.1 vs δ_H 2.55/δ_C 45.8 of **2**); H-5/C-5 (δ_H 5.73/δ_C 143.1 vs δ_H 6.13/δ_C 143.4 of **2**); H-4/C-4 (δ_H 6.17/δ_C 127.4 vs δ_H 6.25/δ_C 131.0 of **2**); H-3/C-3 (δ_H 7.55/δ_C 136.9 vs δ_H 7.12/δ_C 142.0 of **2**). Thus, compound **3** was defined as a geometric isomer of **2** with 4*Z* configuration.[10]

Aplidic acid C (**4**) was isolated as a yellow, amorphous powder. The molecular formula was established as $C_{28}H_{35}NO_3$ on the basis of the HRFABMS and NMR data. The exact mass of the $[M+H]^+$ ion at *m*/*z* 434.2698 matched well with the expected formula of $C_{28}H_{36}NO_3$ (Δ +0.3 mmu). The main difference from compound **2** was that the octa-5,7-dienoyl ($C_8H_{11}O_2$) moiety of **2** was replaced by a hex-5-enoyl ($C_6H_9O_2$) moiety.

Table 2
Cytotoxicity of compounds **1**–**4** and **6**[a]

Compounds	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	25.2	15.2	11.6	19.5	12.4
2	28.4	13.5	11.4	21.6	13.5
3	37.7	16.4	12.2	27.3	12.2
4	12.3	14.7	14.5	14.0	12.0
6	17.5	17.2	11.7	15.6	13.5
Doxorubicin	0.001	0.003	0.001	0.029	0.071

[a] Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

4*Z*-Aplidic acid C (**5**) was isolated as a red, amorphous powder. The molecular formula was established as $C_{28}H_{35}NO_3$ on the basis of the HRFABMS and NMR data. The exact mass of the $[M+H]^+$ ion at *m*/*z* 434.2690 matched well with the expected formula of $C_{28}H_{36}NO_3$ (Δ −0.5 mmu). Compound **5** was assigned the same molecular formula as **4**, but was a geometric isomer with the 4*Z* configuration, as indicated by the coupling constant between H-4 and H-5 ($J_{4,5}$ = 12.0 Hz vs 15.0 Hz of **4**).

Aplidamide A (**6**) was isolated as a yellow, amorphous powder. The molecular formula was established as $C_{36}H_{45}N_3O_4$ on the basis of the HRFABMS and NMR data. The exact mass of the $[M+Na]^+$ ion at *m*/*z* 606.3287 matched well with the expected formula of $C_{36}H_{45}N_3O_4Na$ (Δ −2.0 mmu). Most of the structure of **6** was similar to **1**, but the main difference was that an *N*-[2-(1*H*-indol-3-yl)ethyl]-3-aminopropanamide moiety was connected to C-22 by an amide bond. A singlet at δ_H 7.08 (H-2″) and a spin system comprised of signals at δ_H 7.57 (H-4″), 7.01 (H-5″), 7.09 (H-6″), and 7.34 (H-7″) indicated the presence of an indol-3-yl moiety. Long-range HMBC correlations from H-2′ (δ_H 3.41) to C-22 (δ_C 175.9), and from H-6′ (δ_H 3.50) to C-4′ (δ_C 173.4) and C-3″ (δ_C 113.0) established the connectivities of the partial structures.

A relevant bicyclic fatty acids, cyclopinolenic acids 1 and 2 (**9** and **10,** respectively), were reported as an artifact from tall oil. It is putatively produced from pinolenic acid (via Diels–Alder cyclization) under alkaline conditions of sulfate pulping and heating

during tall oil distillation.[11] Analogously, compounds **1**–**6** may be suspected as artifacts produced from C_{20} or C_{22} polyunsaturated dicarboxylic acids. However, our attempt to detect precursor acids was unfruitful. The absence of appropriate dicarboxylic acid precursors and alternative stereoisomeric products (types **B** and *ent*-**B**, Scheme 1) in the MeOH extract argues against the possibility that compounds **1**–**6** may be artifacts. In fact, metabolites with similar bicyclic skeletons (**7** and **8**) were previously reported from a tunicate of the family Polyclinidae.[4]

Compounds **1**–**4** and **6** showed weak cytotoxicity against a panel of five human solid tumor cell lines (Table 2). Compounds **4** and **6** exhibited higher cytotoxicity to A549 (human lung cancer) and XF498 (human CNS cancer) than other analogues. Compound **1** was also evaluated for antibacterial activity against 20 clinically isolated methicillin-resistant strains, and for anti-inflammatory activity by measuring their inhibitory effects on the production of pro-inflammatory mediators (NO, IL-6, and TNF-α) in RAW 264.7 murine macrophage cells.[12,13] However, no antibacterial activity or anti-inflammatory activity were observed up to 100 μg/mL.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.094.

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7. The ascidians were collected by hand with scuba (20 m depth) in October 2004, off the coast of Jeju Island, Korea. The colonies were thin, hard, and encrusting with a rough, leathery surface. The color was dark olive brown, and the dimension of the colony was around 15 × 10 × 0.5 cm. The fresh specimen was immediately frozen and kept at −20 °C until chemical investigation. A voucher specimen (J04J-13) was deposited at the Natural History Museum, Hannam University. The frozen ascidians (0.5 kg) were chopped into small pieces and extracted with MeOH at room temperature. The MeOH extract did not show significant toxicity to brine shrimp larvae (LD_{50} 923.4 μg/mL). The MeOH extract was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH fraction was subjected to reverse phase MPLC column chromatography with a step gradient solvent system of 20% to 100% MeOH/H_2O to afford 17 fractions. Fraction 10 (0.09 g), one of the bioactive fractions (LD_{50} 41.1 μg/mL, brine shrimp assay), was subjected to a reversed-phase HPLC (Shodex C18 M10E column) and eluted with 85% CH_3CN containing 0.05% formic acid to afford compounds **1** (15.0 mg) and **6** (2.6 mg). Compounds **2** (2.1 mg) and **3** (2.7 mg) were obtained by separation of fraction 8 (0.05 g, LD_{50} >1000 μg/mL, brine shrimp assay) on a reversed-phase HPLC (Shodex C18 M10E column) and eluted with 75% CH_3CN containing 0.05% formic acid. Fraction 7 (0.09 g, LD_{50} >1000 μg/mL, brine shrimp assay) was subjected to a Sephadex LH-20 with a step gradient solvent system of 60% to 100% MeOH/H_2O to afford 8 subfractions. Sub-fraction 6 (14.0 mg) was subjected to a reverse phase HPLC (Shodex C18 M10E column) and eluted with 75% CH_3CN containing 0.05% formic acid to afford compounds **4** (1.2 mg) and **5** (0.5 mg).
8. *Aplidic acid A* (**1**): yellow oil; $[\alpha]_D^{25}$ −93 (*c* 0.73, MeOH); IR (film) ν_{max} 3018, 2952, 2872, 1716, 1638, 1436, 1307, 1264, 1139, 1001, 756 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 217.5 (3.94) nm; LRFABMS *m*/*z* 369 $[M-H]^-$; HRFABMS *m*/*z* 369.2085 (calcd for $C_{23}H_{29}O_4$, 369.2066); ^1H and ^{13}C NMR data, see Table 1.
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