Pavidolides A–E, new cembranoids from the soft coral Sinularia pavida

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Abstract

Five new cembrane-based diterpenoids, namely pavidolides A–E (1–5) were isolated from the marine soft coral Sinularia pavida, together with sarcophytin and chatancin. The structures of new compounds were determined on the basis of extensive spectroscopic data analysis. Pavidolide B (2) possesses an unprecedented 6,5,7-tricarbocyclic nucleus, whereas pavidolide C (3) is characteristic of an unusual C-5 and C-9 conjuncted cembranoid. Pavidolides C and D showed moderate antifouling activity against the larval settlement of barnacle Balanus amphitrite, while pavidolides B and C exhibited inhibitory activity against the human leukemia cell line HL-60.

Cembranoids are the largest and the most diverse group of marine derived diterpenoids, while hundreds of cembrane-based derivatives have been isolated from marine corals. The common skeleton of cembranoids presents a 14-membered carbocyclic backbone, whereas a number of unconventional 12-membered carbon skeleton and 13-membered variants are reported. The biosynthesis of these C20 isoprenoids is assumed to start from the cyclization of geranylgeraniol pyrophosphate to form an intermediate of 3,7,11-cembratrien-15-carbonium ion, which follows oxidation and ring-rearrangement to generate diverse analogues. Cembranoids perform as the chemical defense tools of marine soft corals against their natural predators to defend the animals and their larvae against predators, such as feeding deterrents or acting by the virtue of their toxicity against settlement of microorganisms. Cembranoids are also interesting for pharmacological research due to some derivatives showing significant biological activities. Polycyclic cembranoids such as bi-, tri-, and tetracyclic diterpenoids are the uncommon patterns which are biogenetically derived from the basic cembrane skeleton by successive cyclization via enzymatic or photochemical reactions. The structural variety of cembranes is not only related to various specimens, also based on the geographic variation and environmental conditions. In the course of our search for chemical diversity from the soft corals located in the reefs of South China Sea, a chemically unexamined soft coral Sinularia pavida was collected. Repeated column chromatography including semipreparative HPLC separation resulted in the isolation of seven cembranoids, including five new compounds.
The 2D NMR (COSY, HMQC, and HMBC) and HRESIMS spectroscopic analysis revealed the structure of pavidolide A (1) to be closely related to methyl sarcoate, except for C-10 of 1 containing a methylene group instead of a ketone group. The E-geometries of D₄, D₈, and D₁₁ were deduced by the NOE interactions between H₃-18 (δH 1.64, s)/H₂-6 (δH 2.25, 2.45) and from H₂-11 (δH 2.77) to H₃-19 (δH 1.53, s) and H₂-13 (δH 3.19, 3.55), in association with the upfield shifted chemical shifts of C-18 and C-19 (<20 ppm). The absolute configuration of C-1 was determined as 1R on the basis of the positive specific rotation ([α] + 124.0 (c 0.25, CHCl₃)) and positive CE at λmax 236 (ε + 11.0) which were in accordance with the data ([α] + 167.4, ECD λmax 248 (ε + 23.0) calculated for 1R isomer by TD-DFT at B3LYP/6–31G(d) level). It is noted that the reported CD data of methyl sarcoate was similar to that of 1. Thus, the absolute configuration of natural methyl sarcoate was supposed to be 1R, which was undetermined in literature.

Pavidolide B (2) has a molecular formula of C₂₀H₂₆O₄ as determined by HRESIMS (m/z 331.1901 [M+H]+) and NMR data, indicating 8 unsaturation. APT spectrum resolved 20 carbon resonances (Table 1) to two ketones, an ester carbonyl, two olefinic carbons, an oxymethine, and four methyls, in addition to 10 other aliphatic carbons. Apart from the four sites of unsaturation accounted for the above functional groups, 2 was assumed to possess a tetracyclic nucleus. In ¹H–¹H COSY, three spin systems were observed from H-1 (δH 1.90, ddd) to H₂-2 (δH 2.18, 2.49) and an isopropyl methine H-15 (δH 1.69), from H-5 (δH 2.57) to H₂-7 (δH 2.33, 2.46) and H-11 (δH 3.42, d), and from H-12 (δH 3.72, d) to H-14 (δH 5.00, br d) (Fig. 1). The connectivity of the subunits was achieved by HMBC interactions. The key HMBC relationships from H₃-18 (δH 0.82, s) to the ketone C-3 (δC 211.5), quaternary carbon C-4 (δC 54.4), and two methines C-5 (δC 48.2) and C-13 (δC 53.8), and from H₂-13 (δH 2.96, dd, J = 7.0, 9.0 Hz) to C-3 and C-11 (δC 59.2, CH) in addition to the COSY spin system revealed a perhydroindanone moiety, in which the positions of a methyl...
group at C-4 and a ketone at C-3 were clarified. Moreover, the observation of HMBC interactions from H2-19 (δH 1.97, s) to C-7 (δC 33.8, CH2), C-9 (δC 128.4, CH) and from the olefinic proton H-9 (δH 5.91, s) to C-7, C-19, C-10 (δC 199.3), and C-11, in association with the proton spin system observed in COSY, ascertained a 8-membered lactone to be fused to the perhydroindanone across C-5 and C-11. Thus, a 6,5,7-membered tricyclic nucleus was established. Further HMBC interactions from a carbonyl carbon (δC 197.9, C-20) to H-11, H-12, H-13, and H-14, indicated a γ-lactone to be located across C-12 and C-14.

The relative configuration of 2 was determined on the basis of NOE relationships (Fig. 1). The NOE interaction observed between H3-18 and H-2a (12.0 Hz) was indicative of the cis-ring fusion within the perhydroindanone unit, while the cis-conjunction of cycloheptene to the cyclopentane ring was evident from the NOE correlation between H-5 and H-11. The opposite orientation of H-5 toward H3-18 was recognized by the NOE correlation between H3-18 and H2-6 in association with the absence of H-5 and H3-18 interaction. Additional NOE correlations from H2-18 to H-12 and H-1, and between H-12 and H-14, allowed H-1, H-12, H-13, and H-14 to be oriented in the same face as H3-18. The olefinic geometry was deduced as 8β by the NOE between H-9 and H3-19, in association with the chemical shift of C-19 (δC 27.7 > 20 ppm).

In order to determine the absolute configurations of 2, the computational ECD data for 2 and its enantiomer (Fig. 2) were calculated at the B3LYP/6-311++G(2d, p) level performing in the Gaussian 03 package. Comparison of the experimental CD data with those of calculated data revealed the absolute configurations of 2 to be in agreement with 1S, 4R, 5S, 11R, 12R, 13S, and 14R.

Pavidolide C (3) has a molecular formula of C21H30O5 as determined by HRRESIMS (m/z 385.2016 [M+Na]+, calcd 385.1991) and NMR data, requiring 7 unsaturation. Its IR absorptions at 1739, 1712, and 1689 cm⁻¹ suggested the presence of three carbonyl groups. APT spectrum exhibited a total of 21 carbon resonances (Table 1) which were classified into two ketones, one ester carbonyl, two olefinic carbons, one oxygen-bearing quaternary sp³ carbon, and 15 aliphatic resonances. These data featured a cembrane-type derivative. The 1H–1H COSY cross-peaks allowed to establish the building blocks from C-1 to C-2 (A), C-5 to C-7 (B), and C-11 to C-13 (C), while an isopropyl group was positioned at C-1. The HMBC cross-peaks from H2-18 (δH 1.67, s) to the olefinic carbons C-3 (δC 143.2, qC) and C-4 (δC 109.2, qC) and methine C-5 (δC 41.4, CH) connected the units of A and B via a double bond, while the corrections of H3-19 (δH 1.55) to C-7 (δC 39.5, CH3), C-8 (δC 85.1, qC), and C-9 (δC 58.0, CH); and from H-9 and H2-11 to C-10 (δC 212.2) led to the linkage of B and C units via ketone C-10. The connectivity of C-1 to C-13 via a ketone C-14 (δC 214.8) was evident from the correlations of H2-13 and H-1 to C-14 (Fig. 3). These findings enabled to construct a 14-membered carboyclic ring, which was positioned by a double bond at C-3/C-4, two methyl groups at C-4 and C-8, and two ketone groups at C-10 and C-14. The COSY relationship between H-5 (δH 3.25, dd, J = 3.5, 4.0 Hz) and H-9 (δH 2.8, d, J = 3.5 Hz) led to the formation of a cyclopentane ring across C-5 and C-9, which was further supported by the HMBC interactions from H-9 to C-5 and C-4. Moreover, a methyl ester was deduced to be substituted at C-12 on the basis of the HMBC interactions from C-20 (δC 174.3) to the methoxy proton (δH 3.72, s), H2-11 (δH 2.45, 3.09), and H-12 (δH 2.76, 2.83). Since the above functional groups were accounted for the six sites of unsaturation, the remaining site must be attributed to an ether bridge to connect C-3 and C-8. The chemical shift C-3 (δC 143.2) and the significant upfield shifted C-4 (δC 109.2) also featured C-3 to be oxygenated. The relative configurations of 3 were determined by the assistance of the NOESY experiments (Fig. 4) and coupling constants. The NOE interactions from H-9 to H-5 and H-12 indicated the cyclopentane ring to be cis-fused, while H2-19 was oriented in the same face as H-9. Thus, the ether oxygen atom was oriented in the opposite face toward H-9. The calculable J values such as JH3-19H2-11 (12.0 Hz) and JH5-H12 (12.0 Hz) for axial–axial coupling, and JH3-19H12 (5.0 Hz) and JH11a-H12 (3.0 Hz) for axial–equatorial coupling, reflected the inflexible conformation of cyclopentane ring, which was in agreement with the MM2 energy-minimized conformation (Fig. 4). The NOE interactions from H2-18 to H-2a (δH 2.43), H-5 and H-1, and between H-5/H-9, H-9/H-11b (δH 2.45), H2-19/H11a (δH 3.09) (Fig. 3), in association with JH3-19H2-11 (12.0 Hz) for the trans-orientation of H-12 and H-11a, allowed to assign the same faces of H-1, H-5, H-9, and H-12.

The NMR spectroscopic data analysis revealed the gross structure of 4 to be the same as a known 3,7-cyclized cembrane, whose stereochemistry was established by X-ray diffraction.
closely similar NMR and NOE data indicated 4 having the same relative configurations as those of the known analogue. However, 4 showed the specific rotation ([α]D -90) to be in the opposite phase to that of the known compound ([α]D +93), indicating an enantiomer.

The NMR data of 5 closely resembled those of 4, while 2D NMR data analysis revealed both 5 and 4 having the same gross structure. The difference was due to the significant upfield shifted H-3 (δH 2.04, dd, J = 10.0, 10.5 Hz) in comparison with that (δH 2.57, dd, J = 10.0, 11.0 Hz) of 4 in addition to the downfield shifted C-18 (δC 26.2), suggesting 5 to be a C-4 epimer of 4. The presence of NOE interaction between H-3 and H3-18 as observed in ROESY of 4 further supported the structural assignment.

Compounds 6 and 7 were identical to sarcophytin13 and chataninc14,15 respectively, based on the comparison of their NMR and MS data with the data reported in literature.

All compounds were tested against a panel of tumor cell lines including HL-60, HCT-8, HePG2, BGC-823, A549, and A375. Compounds 2 and 3 showed selective inhibition against human promyelocytic leukemia cell line HL-60 with IC50 of 2.7 and 5.3 µg/mL, respectively, but exhibited weak inhibition toward the other tumor cell lines (IC50 >10 µg/mL). The rest of compounds showed weak inhibition against the panel of tumor cell lines with IC50 >10 µg/mL. In addition, compounds 3 and 4 exhibited moderate inhibition against the larval settlement of barnacle Balanus amphitrite with ED50 of 4.32 and 2.12 µg/mL and low cytotoxicity (LD50 >50 µg/mL).

In summary, this work reports an unprecedented 6,5,7-tricarbocyclic cembranoid (2) as a new skeleton, while C-5/C-9 cyclized cembranoid (3) is rarely found from nature. Compound 1 is depicted to be a precursor to derive polycyclic cembranoids. These findings implied Sinularia species continuing to produce structurally unique cembranoids to enrich the cembrane family, which may supply for lead compound hitting. The low toxic compounds 3 and 4 showing inhibitory activity against barnacle suggested them to be possible for the development of environmentally friendly antifouling agents without biocidal properties.

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Supplementary data

Supplementary data (1D and 2D NMR, IR, MS spectra of new compounds, and experimental section) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.08.049.

References and notes