

Malayenolides A–D, Novel Diterpenes from the Indonesian Sea Pen *Veretillum malayense*

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Received November 2, 1998

Malayenolides A–D (**1–4**), four new briarane diterpenes, were isolated from the sea pen *Veretillum malayense* collected in Indonesia. The new diterpenes possess benzoate and senecioate substituents, both of which are rare among marine natural products. The structures of the new compounds were determined from spectral data. Compounds **1–4** are toxic to brine shrimp.

Gorgonians and soft corals have been proven to be rich sources of diterpenes,¹ especially those with briarane and cembrane skeletons.² Earlier studies from our laboratory on several species of gorgonians have resulted in the isolation of a number of oxygenated diterpenes, many of which possess the briarane skeleton.³ As part of our ongoing search for bioactive compounds from marine organisms,⁴ we examined an extract of the sea pen *Veretillum malayense* Hickson 1916 (order Pennatulacea, family Veretillidae), collected in Indonesia, because the extract exhibited toxicity to brine shrimp. Investigation of this extract led to the discovery of four new briarane diterpenes, designated as malayenolides A–D (**1–4**), which are de-

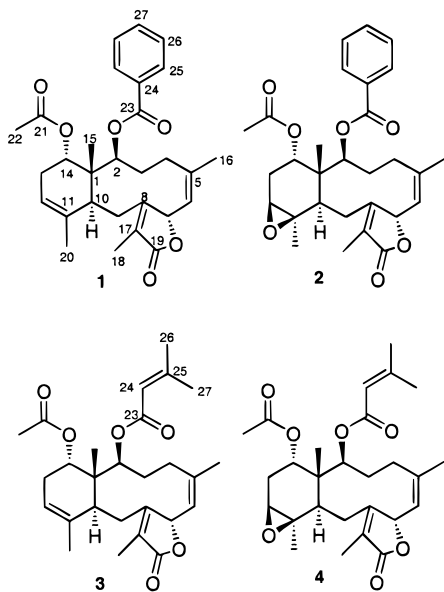
Results and Discussion

Frozen specimens of *V. malayense* were freeze-dried and then extracted twice with MeOH and twice more with MeOH–CH₂Cl₂ (1:1). The combined extracts, after evaporation of solvents, were subjected to solvent partitioning⁶ to give hexane, CH₂Cl₂, and *n*-BuOH solubles. The CH₂Cl₂ solubles were chromatographed over SiO₂, and selected fractions therefrom were rechromatographed on reversed-phase (C₁₈) HPLC to yield malayenolides A–D (**1–4**).

Malayenolide A (**1**) was obtained as an amorphous solid. The molecular formula C₂₉H₃₄O₆, established by HR-FABMS [*m/z* 479.2441 (*M* + *H*)⁺, Δ –0.7 mmu], indicated 13 degrees of unsaturation. The IR spectrum showed bands at 1760, 1731, and 1712 cm^{–1}, consistent with the presence of α,β-unsaturated γ-lactone, and ester carbonyl groups. NMR data (Tables 1 and 2) supported the existence of an acetate and an α,β-unsaturated γ-lactone. The presence of a benzoate group was evident⁷ from aromatic proton signals at δ 7.96 (2H, d, *J* = 7.2 Hz), 7.42 (2H, t, *J* = 7.2 Hz), and 7.53 (1H, t, *J* = 7.2 Hz), and ¹³C signals at δ 166.0 (s, carbonyl), 130.1 (s), 129.4 (d, 2C), 128.4 (d, 2C), and 132.9 (d). The forementioned groups in combination with four additional olefinic resonances at δ 116.7 (d), 136.3 (s), 122.8 (d), and 143.6 (s) in the ¹³C NMR spectrum accounted for 11 double-bond equivalents, indicating that malayenolide A (**1**) required two carbocycles in addition to the benzene and γ-lactone rings.

The gross structure of **1** was established by a detailed analysis of 2D NMR data. The COSY and relayed coherence transfer–COSY (RCT–COSY) allowed assignment of five different spin systems: H-2 to H-4, H-6 to H-7, H-9 to H-10, H-12 to H-14, and H-25 to H-27. The HMQC spectrum provided evidence for assignments of the protonated carbons. The HMBC data enabled assignments of the chemical shifts of quaternary carbons and correction of the different spin units separated by quaternary carbons (Figure 1). The acetate was located at C-14 based on an HMBC correlation between H-14 (δ 4.87) and the ¹³C signal at δ 170.3, which was coupled (HMBC) to the methyl singlet at δ 1.93. Hence, the benzoate was relegated to C-2, although an HMBC correlation between H-2 and C-23 was not observed.

The relative stereochemistry of malayenolide A (**1**) was assumed to be the same as that of funicolide A⁸ and brianthein W⁸ by comparison of proton–proton coupling constants and ¹H and ¹³C chemical shifts with published values. This was confirmed by observed NOESY correlations, which are shown in Figure 2. Hence, the complete structure of malayenolide A was assigned as **1**.



scribed in this paper. The new briaranes differ from previously reported ones primarily in the occurrence of benzoate and senecioate substituents in contrast to the far more common acetate and alkanolate groups. Very few chemical investigations of sea pens of the genus *Veretillum* have been reported previously, and in those studies, briarane diterpenes were isolated from the sea pen *V. cynomorium*.⁵

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Table 1. ^1H NMR Data for Compounds **1**–**4**^a

H	1	2	3	4
2	5.14 (br s)	5.08 (br s)	4.90 (br s)	4.86 (br s)
3 β	2.35 (m)	2.31 (m)	2.15 (m)	2.15 (m)
3 α	1.86 (m)	1.85 (m)	1.72 (m)	1.71 (m)
4 β	2.61 (m)	2.60 (br d, 12.6)	2.54 (br m)	2.55 (br d, 13.2)
4 α	2.33 (br d, 14.7)	2.31 (m)	2.28 (m)	2.25 (m)
6	5.19 (d, 9.6)	5.17 (d, 9.3)	5.12 (d, 8.6)	5.12 (br d, 9.9)
7	5.52 (br d)	5.52 (br d, 8.7)	5.46 (br d, 8.6)	5.49 (br d, 9.9)
9 β	2.94 (br d, 16)	3.14 (dd 16.6, 7.2)	2.87 (br d, 16.3)	3.08 (dd, 16.2, 6.3)
9 α	2.56 (dd, 16, 7.5)	2.81 (br d, 16.6)	2.48 (dd, 16.3, 17.2)	2.75 (br d, 16.2)
10	2.79 (br)	2.48 (br d, 6.9)	2.70 (br)	2.41 (br d, 5.7)
12	5.20 (br d, 6)	3.06 (d, 6.6)	5.16 (br d, 5.3)	3.04 (d, 5.7)
13 β	2.25 (br d, 18.3)	2.18 (m)	2.17 (m)	2.19 (m)
13 α	2.05 (br dd, 18.3, 3.9)	2.00 (m)	2.02 (m)	1.97 (m)
14	4.87 (br s)	4.80 (br s)	4.75 (br s)	4.71 (br s)
15	1.13 (br s)	1.07 (s)	0.96 (br s)	0.92 (s)
16	2.12 (br s)	2.17 (br s)	2.06 (br s)	2.10 (br s)
18	1.88 (s)	1.96 (s)	1.84 (s)	1.94 (s)
20	1.60 (s)	1.24 (s)	1.55 (s)	1.21 (s)
acetate	1.93 (s)	2.01 (s)	1.91 (s)	1.99 (s)
benzoate	7.96 (2H, d, 7.2)	7.95 (2H, dd, 8.4, 1.2)		
	7.42 (2H, t, 7.2)	7.43 (2H, t, 7.8)		
	7.53 (1H, t, 7.2)	7.56 (2H, t, 7.8)		
senecioate			5.61 (br s)	5.61 (br s)
			1.85 (s)	1.88 (s)
			2.10 (s)	2.11 (s)

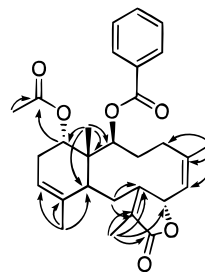
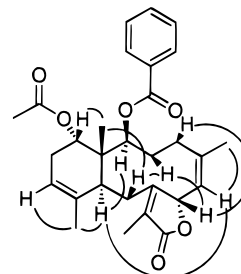
^a Spectra were recorded in CDCl_3 at 500 MHz, referenced to residual solvent CDCl_3 (δ 7.26).

Table 2. ^{13}C NMR Data for Compounds **1**–**4**^a

C	1	2	3	4
1(s)	41.7	41.6	41.4	41.3
2(d)	74.9	74.9	73.0	73.1
3(t)	33.6	33.6	33.7	33.7
4(t)	29.5	29.5	29.6	29.4
5(s)	143.6	143.7	143.8	143.9
6(d)	122.8	122.9	122.5	122.6
7(d)	80.9	80.8	80.9	80.9
8(s)	159.9	160.1	160.1	160.4
9(t)	29.5	28.5	29.6	28.5
10(d)	37.4	35.7	37.5	35.7
11(s)	136.3	63.0	136.4	63.1
12(d)	116.7	60.7	116.6	60.8
13(t)	25.8	25.8	25.8	25.7
14(d)	72.7	72.6	72.8	72.6
15(q)	14.8	13.9	14.6	13.7
16(q)	27.7	27.7	27.6	27.7
17(s)	124.6	125.3	124.4	125.1
18(q)	9.6	9.5	9.5	9.4
19(s)	174.0	173.9	174.1	174.0
20(q)	21.4	23.0	21.5	23.0
21(s)	170.3	169.9	170.5	170.1
22(q)	21.1	21.0	21.1	21.1
23(s)	166.0	166.0	165.8	165.8
24	130.1(s)	129.9(s)	115.7(d)	115.6(d)
25	129.4(d)(2C)	129.5(d)(2C)	156.7(s)	157.4(s)
26	128.4(d)(2C)	128.5(d)(2C)	27.3(q)	27.4(q)
27	132.9(d)	133.1(d)	20.1(q)	20.3(q)

^a Spectra were recorded in CDCl_3 at 125 MHz, referenced to CDCl_3 (δ 77).

Malayenolide B (**2**) was assigned the molecular formula $\text{C}_{29}\text{H}_{34}\text{O}_7$ based on HRFABMS and NMR data, the latter being unambiguously assigned by COSY, RCT–COSY, HMQC, and HMBC experiments. The ^1H and ^{13}C NMR data of **2** were essentially identical to those of **1** except that the NMR signals for the C-11 (12) double bond were missing in the NMR spectra of **2**, and instead, resonances for an epoxide were observed in the ^1H NMR (δ 3.06, H-12) and ^{13}C NMR [δ 63.0 (s); 60.7 (d)] spectra. Therefore, malayenolide B was assigned structure **2**. No HMBC correlation was observed between H-2 or H-14 and either ester carbonyl carbon and, hence, the relative position of acetoxy vs. benzyloxy is by analogy with **1**. The stereo-

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

chemistry of the epoxide was based on NOESY correlations observed between H-20 (δ 1.24, Me) and H-12 (δ 3.06) and between H-20 and H-10 (δ 2.48). This conclusion was consistent with the ^{13}C chemical shifts of C-11 and C-12, which were virtually identical to those of related 11,12 β -epoxybriaranes.^{3b}

Malayenolides C (**3**) and D (**4**) had molecular formulas of $\text{C}_{27}\text{H}_{36}\text{O}_6$ and $\text{C}_{27}\text{H}_{36}\text{O}_7$, respectively, based on their HRFABMS and NMR data (Tables 1 and 2). The presence of a senecioate group in both **3** and **4** was indicated by olefinic proton signals at δ 5.61 (br s) and two vinyl methyl singlets at δ 1.85 and 2.10, and ^{13}C NMR signals at δ 165.8 (s), 115.7 (d), 156.7 (s), 27.3 (q), and 20.1 (q). The NMR data was unambiguously assigned by COSY, HMQC, and HMBC experiments, as in the case of **1** and **2**, thus confirming the same diterpene skeleton. The acetoxy substituent in **3** was assigned to C-14 on the basis of an HMBC correlation between H-14 and the acetoxy carbonyl,

which was identified by HMBC correlation with the adjacent methyl signal. Hence, the senecioate group must be at C-2. The positions of the ester groups in **4** were assigned by analogy with **1** and **3** because no HMBC correlation between H-2 or H-14 and either ester carbonyl was observed. The relative stereochemistry of **4** was established by the same types of NOEs as observed for **2**. The relative stereochemistry of **3** was assigned by analogy with **1**, **2**, and **4** and the similarity of chemical shifts and coupling constants for **1** and **3**. Therefore, malayenolides C and D were assigned structures **3** and **4**, respectively.

Benzoate- and senecioate-containing compounds are uncommon in marine natural products, although many benzoate-containing metabolites have been isolated from terrestrial plants. Prior to this work, few para-substituted benzoate-containing compounds had been isolated from marine organisms. These include bretonin A and isobretonin A, two unique glycerols esterified by *p*-hydroxybenzoic acid, which were isolated from a demosponge;⁹ kelleltinins I and II, two polyols esterified by *p*-hydroxybenzoic acid, isolated from the marine mollusk *Kellettia kelletii*;¹⁰ tubastraine, a *p*-bromobenzoic acid derivative, isolated from the stony coral *Tubastraea micrantha*;¹¹ and insulicolide A, a *p*-nitrobenzoate-containing metabolite from the marine fungus *Aspergillus insulicola*.¹²

Malayenolides A–D (**1**–**4**) showed toxicity to brine shrimp,¹³ LC₅₀ 100 µg/mL, <2 µg/mL, 20 µg/mL, and 20 µg/mL, respectively.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230–240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C₁₈ column. IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument, UV spectra on a Hewlett–Packard spectrophotometer. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3-mm ¹H–¹³C switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer, and optical rotations on a Rudolph Autopol III automatic polarimeter.

Animal Material. The sea pen *Veretillum malayense* was collected in August 1993, near Manado, Sulawesi, Indonesia (1° 43.97', 124° 58.03' E), and frozen shortly after collection. Seven of the 18 described species of *Veretillum* are considered as possibly valid by Williams.¹⁴ Of these, only one, *Veretillum cynomorium* Pallas 1766, ranging from southern Europe to western Africa, is easily identifiable and has been the subject of numerous physiological, anatomical, and behavioral ecological studies. The other species, all from the Indo-West Pacific, are difficult to separate using the existing literature, and the genus is in need of revision. The material used in the present study most closely resembles Hickson's description of *Veretillum malayense* (type locality: Sumbawa, Indonesia), especially in the form and distribution of sclerites. *Veretillum manillense* Kolliker 18723 (type locality: Philippines) differs from *V. malayense* by a lack of platelike sclerites in the rachis, and *Veretillum vanderbilti* Boone 1938 (type locality: Philippines) differs from *V. malayense* by having nodular sclerites in the rachis. Voucher specimens have been deposited at the University of Oklahoma (9-IN-93) and the California Academy of Sciences (CAS no. 115169).

Extraction and Isolation. Frozen specimens of the sea pen *V. malayense* were freeze-dried, and the dried specimens (36 g dry wt) were extracted twice by MeOH and then twice by MeOH–CH₂Cl₂ (1:1). The extracts were concentrated, combined, and partitioned between aqueous MeOH and or-

ganic solvents as described previously.⁶ This gave, after evaporation of solvents under reduced pressure, hexane (1.55 g), CH₂Cl₂ (1.09 g), and *n*-BuOH (2.48 g) solubles. The CH₂Cl₂-soluble portion showed toxicity to brine shrimp and was, therefore, fractionated on a Si gel open column using increasing amounts of EtOAc in hexane as eluent (5% EtOAc–hexane to EtOAc). Four fractions therefrom contained mainly compounds **1**–**4**, respectively. Final purification of each of the four fractions by reversed-phase HPLC using a mixture of MeOH–H₂O yielded malayenolide C (22.9 mg, using 20% H₂O–MeOH as eluent), malayenolide A (15.8 mg, 20% H₂O–MeOH), malayenolide D (10.4 mg, 22% H₂O–MeOH), and malayenolide B (9.0 mg, 30% H₂O–MeOH), respectively, in the order of polarity.

Malayenolide A (1): amorphous solid; [α]_D +158.1° (c 1.05, CHCl₃); UV (EtOH) λ_{max} 228 (ε 21 950) nm; IR (neat) ν_{max} 1760, 1731, and 1712 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* [M + H]⁺ 479.2441, calcd for C₂₉H₃₅O₆ 479.2434.

Malayenolide B (2): amorphous solid; [α]_D +85.0° (c 0.60, CHCl₃); UV (EtOH) λ_{max} 226 (ε 21 850) nm; IR (neat) ν_{max} 1750 (br), 1715, and 1668 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* [M + H]⁺ 495.2367, calcd for C₂₉H₃₅O₇ 495.2383.

Malayenolide C (3): amorphous solid; [α]_D +69.9° (c 1.53, CHCl₃); UV (EtOH) λ_{max} 222 (ε 25 900) nm; IR (neat) ν_{max} 1750, 1730, 1715, and 1652 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* [M + H]⁺ 457.2618, calcd for C₂₇H₃₇O₆ 457.2590.

Malayenolide D (4): amorphous solid; [α]_D +29.0° (c 0.69, CHCl₃); UV (EtOH) λ_{max} 220 (ε 26 200) nm; IR (neat) ν_{max} 1750, 1730, 1715, and 1652 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* [M + H]⁺ 473.2531, calcd for C₂₇H₃₇O₇ 473.2540.

Acknowledgment. This work was supported by National Cancer Institute Grant CA 52955. We thank the Coral Reef Research Foundation for providing material for this study and the government of Indonesia for permission to collect specimens from their waters through the National Cancer Institute marine collection program.

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NP980497E