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Note

Manadodioxans A-E, polyketide endoperoxides from the marine sponge *Plakortis bergquistae*

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Abstract: Five new polyketide endoperoxides, manadodioxans A-E, were isolated from the marine sponge *Plakortis bergquistae.* Manadodioxan E showed antimicrobial activity against *Escherichia coli* at 10 μg/disk, while its oxo congener, manadodioxan D, was inactive.

Keywords: Polyketide endoperoxides, Marine sponge, *Plakortis bergquistae*, Antimicrobial

Introduction

Marine sponges of the genus *Plakortis* are known to contain stable cycloperoxides and most of their compounds possess a 1,2-dioxane ring, substituted with an acetate moiety at C-3 and three functionalities at C-4, C-6, and C-6. Since the first study to isolate plakortin [1] from the Caribbean sponge *Plakortis halichondrioides*, a number of compounds containing characteristic cycloperoxides have been identified, including plakortolide [2], plakortic acid [3], plakortenone [4], haterumadioxin [5], manadoperoxide [6], and plakortide [7]. These compounds were found to exhibit various biological activities, i.e., antibiotic [1], cytotoxic [2-5], and antimalarial [6, 8] activities and enhanced the Ca^{2+}-pumping activity of the cardiac sarcoplasmic reticulum [7]. In our screening for the antimicrobial activities of the marine organisms, we found that the sponge *P. bergquistae*, which was collected in Indonesia, exhibited antibacterial activity. We herein reported the isolation and structure elucidation of five new polyketide endoperoxides, manadodioxans A-E (**1**-**5**), from this sponge (Fig. 1).

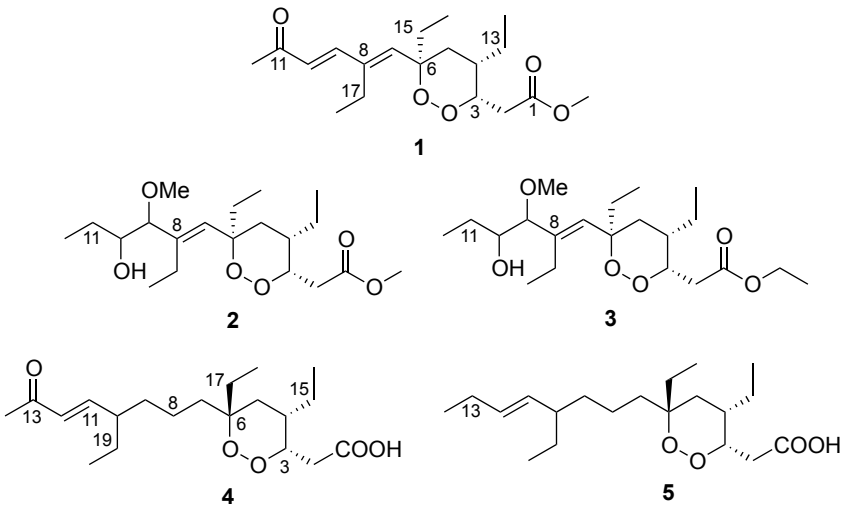

Fig. 1 Structures of **1**-**5**

Results and discussion

The marine sponge *P. bergquistae* (150 g, wet weight) was collected in Indonesia and immediately extracted with EtOH after collection. The extract was partitioned between EtOAc and water, and bioassay-guided purification from the EtOAc-soluble fraction afforded **1** (0.2 mg), **2** (0.4 mg), **3** (0.7 mg), **4** (1.3 mg), and **5** (1.8 mg).

Manadodioxan A (**1**) had the molecular formula $C_{19}H_{30}O_5$, which was determined by HRESIMS and ^{13}C NMR (Table 1) spectrometries. The ^{1}H NMR spectrum (Table 1) showed three triplet methyl signals at δ 0.87 (t, J = 7.7 Hz, H_3-16), 0.90 (t, J = 7.4 Hz, H_3-14), and 1.04 (t, J = 7.6 Hz, H_3-18), two singlet methyl signals at δ 2.29 (H_3-12) and 3.70 (1-OMe), an oxygen-bearing signal at δ 4.49 (m), three olefin signals at δ 5.93 (s, H-7), 6.16 (d, J = 16.2 Hz, H-10), and 7.05 (d, J = 16.2 Hz, H-9), and methylene/methine signals at δ 1-3. An analysis of 2D spectra, including COSY, HSQC, and HMBC spectra, revealed the presence of a 1,2-dioxane ring, substituted with four alkyl functionalities at C-3, C-4, C-6, and C-6 (Fig. 2). HMBC correlations from H_2-2 and 1-OMe to C-1 showed that a methyl ester was attached to C-2. The connections of two ethyl groups at C-4 and C-6 were identified by HMBC correlations from H_3-14 and H_2-13 to C-4 and from H_3-16 and H_2-15 to C-6. Another functional group at C-6 contained an α,β,γ,δ-unsaturated ketone and a 9*E* configuration was indicated by the coupling constant, $J_{9,10}$ = 16.2 Hz. NOE correlations, H-7/H-9 and H-10/H_3-18, indicated the geometory of double bond C-7/C-8. The relative configurations of C-3, C-4, and C-6 were established by NOE correlations, H_2-2/H-5_{ax} (δ 1.40), H-7/H-5_{eq} (δ 1.79), and H-7/H-4 (Fig. 3). Thus, the structure of **1** was determined.

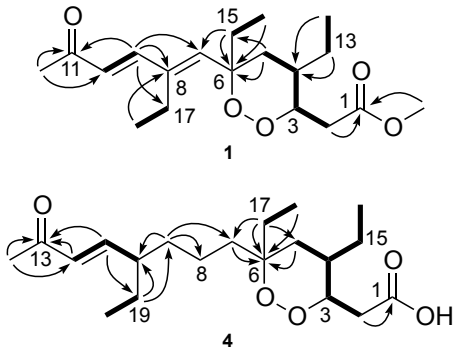

Fig. 2 COSY (bold lines) and key HMBC (arrows) correlations for **1** and **4**

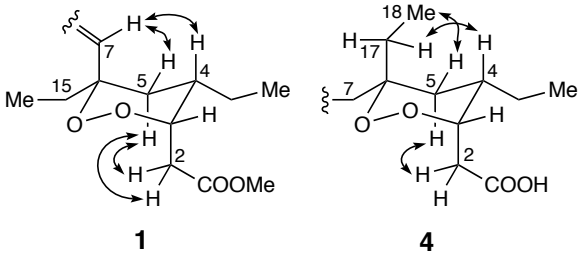

Fig. 3 Key NOE correlations for **1** and **4**

The molecular formula of manadodioxan B (**2**) was determined to be $C_{20}H_{36}O_6$ based on HRESIMS. ^1H and ^{13}C NMR spectra (Table 2) showed that **2** was a congener of **1**. An analysis of 2D spectra clearly revealed that the α,β,γ,δ-unsaturated ketone moiety in **1** was absent in **2**. The terminal singlet methyl group (H_3-12) in **1** was replaced with the triplet methyl group C-12 (δ_H 0.98 (t, J = 7.4 Hz); δ_C 10.4) in **2**, which was successively connected to the methylene C-11 (δ_H 1.36 and 1.72; δ_C 25.5), hydroxyl-bearing carbon C-10 (δ_H 3.50; δ_C 73.2), methoxy-bearing carbon C-9 (δ_H 3.46 (d, J = 6.5 Hz); δ_C 89.8), and trisubstituted double bond C-8 (δ_C 140.9)/C-7 (δ_H 5.42 (s);

δ_C 131.0), as confirmed by 2D spectra. HMBC correlations from H_3-18 and H_2-17 to C-8 showed that an ethyl group was attached to the olefin carbon C-8, which also exists in **1**. NOE correlations determined relative configurations at C-3, C-4, and C-6, the same as **1**. Relative configurations at C-9 and C-10 have not yet been determined. Manadodioxan C (**3**) had the molecular formula $C_{21}H_{38}O_6$ as established by HRESIMS, indicating one more methylene unit in **3** than in **2**. The ^1H and ^{13}C NMR spectra of **2** and **3** (Table 2) were very similar, and an analysis of 2D spectra indicated the presence of an ethoxy group (δ_H 4.15 and δ_C 60.7 (CH_2); δ_H 1.24 and δ_C 14.2 (CH_3)) placed at C-1 of **3** instead of the methoxy group in **2**. NOE correlations, H-7/H-9, H-7/9-OMe, and H-10/H_3-18, indicated the geometories of double bonds C-7/C-8 of **2** and **3**.

ESIMS of manadodioxan D (**4**) showed an ion peak at *m/z* 377.2310 $[M + Na]^+$, and the molecular formula was determined to be $C_{20}H_{34}O_5$ based on HRESIMS. An analysis of 2D spectra established the entire carbon framework possessing a 1,2-dioxane ring with four alkyl functionalities containing an α,β-unsaturated ketone. Relative configurations at C-3, C-4, and C-6 were indicated by NOE correlations, H_3-18/H-5_{eq} (δ 1.50), H-17 (δ 1.99)/H-4_{ax}, and H-2 (δ 3.00)/H-5_{ax} (δ 1.23) (Fig. 3). The long alkyl chain at C-6 was oriented in the equatorial position and the functionalities at C-3 and C-4 were oriented in the axial and equatrial positions, respectively, in **4**. Although the planer structure of **4** was identical to that of "compound 1", which was isolated from a Jamaican *Plakortis* sponge [9], the carbon chemical shifts at C-7 and C-17 in $CDCl_3$ were different; δ_C 36.3 and 24.8 in **4** and δ_C 32.2 and 29.8 in "compound 1", respectively. Although the planer structure has only been reported for "compound 1" in the literature, the difference observed in carbon chemical shifts at C-7 and C-17 indicated that the long alkyl chain at C-6 may be oriented in the axial position in

"compound 1" while those at C-3 and C-4 were the same as those in **4**. Manadodioxan E (**5**) had the molecular formula $C_{20}H_{36}O_4$ and its ^1H and ^{13}C NMR chemical shifts were superimposable on those of plakortide G [7], except for the absence of the methoxy group that existed in plakortide G. Relative configurations at C-3, C-4, and C-6 of **5** were confirmed to be the same as those of **4** based on NOE correlations. Thus, the structure of **5** was determined to be a free acid of plakortide G.

The antimicrobial activities of manadodioxans D (**4**) and E (**5**) were tested against bacteria, *Escherichia coli* and *Bacillus cereus*, a fungus, *Candida albicans*, and a yeast, *Saccharomyces cerevisiae* (Table 4) [10] using the paper disk method. Compound **5** more potently inhibited the growth of the gram-negative bacterium, *E. coli*, than that of the gram-negative bacterium, *B. cereus*, but was inactive for *C. albicans* and *S. cerevisiae* at 10 μg/disk. On the other hand, **4** was inactive for the four microorganisms tested in this study. The presence of the carbonyl group at C-13 in **4** may have sequestered antimicrobial activity.

Experimental

General

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were recorded on a PerkinElmer Frontier FT-IR spectrophotometer. UV spectra were measured on a JASCO V-550 spectrophotometer. NMR spectra were recorded on a Bruker Avance 600, Bruker Avance 500, or JEOL JNM-ECX-400 NMR spectrometer in $CDCl_3$. Chemical shifts were referenced to residual solvent peaks (δ_H 7.24 and δ_C 77.0). Mass spectra were measured on a Bruker Bio-TOF mass spectrometer.

Biological material

The marine sponge was collected at a depth of 10 m in North Sulawesi, Indonesia, in December 2006 and soaked in EtOH immediately. The sponge was identified as *Plakortis bergquistae*. A voucher specimen (RMNH POR 8528) has been deposited at the Naturalis Biodiversity Center, the Netherlands.

Extraction and isolation

The marine sponge (150 g, wet weight) was extracted with MeOH. The concentrated aqueous residue was successively extracted with EtOAc. The EtOAc fraction (1.1 g) was subjected to silica gel column chromatography with *n*-hexane/EtOAc to afford fraction A (130 mg) eluted with *n*-hexane/EtOAc (1:1) and fraction B (405 mg) eluted with *n*-hexane/EtOAc (9:1). Fraction A was purified by ODS column chromatography with MeOH/H_2O to afford **4** (1.3 mg, 75% MeOH-H_2O) and **5** (1.8 mg, 90% MeOH-H_2O). Fraction B was purified by ODS and C30 HPLC to afford **1** (0.2 mg), **2** (0.4 mg), and **3** (0.7 mg).

Manadodioxan A (**1**): an amorphous solid. $[\alpha]_D^{21}$ -100° (*c* = 0.22, MeOH). IR ν_{max} (film) cm^{-1}: 3453, 2926, 2855, 1738, 1667, 1592, 1460, 1363, 1257, 1164 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 204 (4.65), 278 (4.78). ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 1. HRESIMS $[M + Na]^+$ *m/z* 361.1997 (calcd for $C_{19}H_{30}O_5Na$, 361.1989).

Manadodioxan B (**2**): an amorphous solid. $[\alpha]_D^{21}$ -97° (*c* = 0.33, MeOH). IR ν_{max} (film) cm^{-1}: 3453, 2925, 1739 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 206 (4.60). ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 2. Key NOE correlations: H_2-2/H-5_{ax} (δ 1.32), H-7/H-5_{eq} (δ 1.75), and H-7/H-4. HRESITOFMS $[M + Na]^+$ *m/z* 395.2399 (calcd for $C_{20}H_{36}O_6Na$, 395.2408).

Manadodioxan C (**3**): an amorphous solid. $[\alpha]_D^{21}$ -110° (*c* = 0.77, MeOH). IR ν_{max}

(film) cm^{-1}: 3453, 2928, 2856, 1729, 1460, 1379, 1274, 1113 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 218 (5.34). ^{1}H- (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 2. Key NOE correlations: H_2-2/H-5_{ax} (δ 1.31), H-7/H-5_{eq} (δ 1.75), and H-7/H-4. HRESIMS $[M + Na]^+$ *m*/*z* 409.2557 (calcd for $C_{21}H_{38}O_6Na$, 409.2564).

Manadodioxan D (**4**): an amorphous solid. $[\alpha]_D^{21}$ +43° (*c* = 0.68, MeOH). IR ν_{max} (film) cm^{-1}: 3450, 2928, 1714, 1460 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 204 (4.53, sh), 224 (4.68). ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) spectroscopic data, Table 3. HRESITOFMS *m*/*z* 377.2310 $[M + Na]^+$ (Calcd for $C_{20}H_{34}O_5Na$, 377.2302).

Manadodioxan E (**5**): an amorphous solid. $[\alpha]_D^{21}$ +54° (*c* = 2.2, MeOH). IR ν_{max} (film) cm^{-1}: 3352, 2926, 2841, 1653, 1450, 1114, 1016, 662. UV λ_{max} (MeOH) nm (log ε): 204 (4.27). ^{1}H- (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 3. NOE correlations: H_3-18/H-5_{eq} (δ 1.50), H-17 (δ 2.01)/H-4_{ax}, and H-2 (δ 3.03)/H-5_{ax} (δ 1.22). HRESITOFMS *m*/*z*: 363.2460 $[M + Na]^+$ (Calcd for $C_{20}H_{36}O_4Na$: 363.2509).

Antifungal assay

Growth inhibitory activity was determined by the paper disk method. Paper disks (ϕ6 mm), impregnated with 10 μg of **4** or **5**, were incubated on agar plates containing *E. coli* or *B. cereus* at 37 °C and *C. albicans* or *S. cerevisiae* at 27 °C overnight.

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10. The antimicrobial activities of **1**-**3** were not tested due to their limited amounts.

Table 1. ^{1}H and ^{13}C NMR data for compound **1**

position	δ_C	δ_H (*J* in Hz)	HMBC [a]
1	172.0		
2	31.2	3.01 dd 15.8, 9.4	1, 3, 4
		2.40 dd 15.8, 3.6	1, 3
3	78.8	4.49 m	5
4	35.7	2.04 m	2, 3, 5, 13
5	35.5	1.79 dd 13.4, 4.0	3, 4, 15
		1.40 t 13.4	3, 4, 6, 7, 15
6	84.5		
7	142.9	5.93 s	5, 6, 8, 9, 15, 17
8	141.1		
9	147.8	7.05 d 16.2	7, 8, 10, 11, 17
10	126.2	6.16 d 16.2	8, 11, 12
11	198.9		
12	27.2	2.29 (3H) s	10, 11
13	25.0	1.20 dq 13.6, 7.4	3, 4, 5, 14
		1.16 dq 13.6, 7.4	3, 4, 5, 14
14	11.0	0.90 (3H) t 7.4	4, 13
15	32.6	1.66 (2H) q 7.7	6, 7
16	7.7	0.87 (3H) t 7.7	6, 15
17	19.8	2.46 dq 14.3, 7.6	7, 8, 9, 18
		2.44 dq 14.3, 7.6	7, 8, 9, 18
18	12.9	1.04 (3H) t 7.6	8, 17
1-OCH_3	51.9	3.70 (3H) s	1

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

Table 2. NMR data for **2** and **3** in $CDCl_3$.

Position	**2**			**3**		
	δ_C	δ_H (*J* in Hz)	HMBC[a]	δ_C	δ_H (*J* in Hz)	HMBC[a]
1	172.1			171.6		
2	31.3	3.01 dd 15.7, 9.3	1, 3, 4	31.7	2.99 dd 15.8, 9.1	1, 3
		2.38 dd 15.7, 3.5	1, 3		2.38 dd 15.8, 3.4	1
3	79.0	4.47 m	4, 5	78.9	4.48 m	
4	35.67[b]	2.04 m	3, 5, 13	35.72[b]	2.03 m	2, 3, 13, 14
5	35.67[b]	1.75 dd 13.1, 3.8	3, 4, 15	35.65[b]	1.75 dd 13.1, 4.0	3, 4
		1.32 t 13.1	4, 6, 7, 13, 15		1.31 t 13.1	
6	83.9			83.9		
7	131.0	5.42 s	5, 6, 8, 17	131.1	5.41 s	5, 6, 8, 9, 17
8	140.9			140.8		
9	89.8	3.46 d 6.5	7, 8, 10, 11, 17, 9-OCH_3	89.8	3.45 d 5.1	7, 8, 10, 11, 17, 9-OCH_3
10	73.2	3.50 m	12	73.2	3.49 m	
11	25.5	1.72 m	10, 12	25.5	1.70 m	12
		1.36 m	9, 10, 12		1.35 m	10, 12
12	10.4	0.98 (3H) t 7.4	10, 11	10.4	0.98 (3H) t 7.4	10, 11
13	25.0	1.21 m	3, 4, 14	25.0	1.22 m	3, 4, 5, 14
		1.14 m	3, 4, 14		1.13 m	3, 4, 5, 14
14	11.0	0.89 (3H) t 7.5	4, 13	11.1	0.89 (3H) t 7.4	4, 13
15	32.7	1.70 dq 13.7, 7.5	5, 6, 7, 16	32.7	1.70 m	5, 6, 7, 16
		1.60 dq 13.7, 7.5	5, 6, 7, 16		1.60 m	5, 6, 7, 16
16	7.8	0.89 (3H) t 7.5	6, 15	7.8	0.89 (3H) t 7.4	6, 15
17	20.9	2.34 dq 13.7, 7.4	7, 8, 9, 18	20.9	2.34 dq 13.4, 7.5	7, 8, 9, 18
		2.19 dq 13.7, 7.4	7, 8, 9, 18		2.18 dq 13.4, 7.5	7, 8, 18
18	13.1	1.07 (3H) t 7.4	8, 17	13.1	1.08 (3H) t 7.5	8
1-OCH_3	51.9	3.69 (3H) s	1			
1-O$\underline{CH_2}CH_3$				60.7	4.15 (2H) m	1
1-$OCH_2\underline{CH_3}$				14.2	1.24 (3H) t 7.2	1-O$\underline{C}H_2CH_3$
9-OCH_3	57.1	3.28 (3H) s	9	57.1	3.28 (3H) s	9

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

[b] May be interchangeable.

Table 3. NMR data for **4** and **5** in $CDCl_3$.

Position	**4**			**5**		
	δ_C	δ_H (*J* in Hz)	HMBC[a]	δ_C	δ_H (*J* in Hz)	HMBC[a]
1	174.3			176.9		
2	30.9	3.00 dd 15.7, 9.8	1, 3, 4	31.2	3.03 dd 16.4, 10.8	1, 3
		2.38 dd 15.7, 3.5	1, 3		2.38 dd 16.4, 3.6	1, 3
3	78.4	4.44 m	1, 2, 4, 5	78.4	4.43 m	2
4	34.3	2.15 m	3, 16	34.3	2.14 m	2, 3, 6, 15, 16
5	32.8	1.50 m	3, 4, 6, 7	33.0	1.50 m	3, 4, 6, 7
		1.23 m	3, 4, 7, 15, 17		1.22 m	6, 17
6	82.6			82.6		
7	36.3	1.40 m	5, 6, 8, 9	35.5	1.27 m	8, 17
		1.25 m	5, 6, 8, 17		1.13 m	8
8	20.3	1.24 (2H) m		20.1	1.28 m	6, 7
					1.14 m	7, 10
9	34.6	1.43 m	8	36.2	1.42 m	
		1.31 m	7, 8, 10		1.16 m	8, 10, 11
10	44.4	2.04 m		44.2	1.74 m	
11	152.4	6.52 dd 16.0, 9.2	9, 10, 13, 19	133.2	5.03 dd 15.1, 9.2	9, 10, 13, 19
12	131.3	6.03 d 16.0	10, 13, 14	132.3	5.36 m	10, 13, 14
13	198.7			25.7	1.98 (2H) m	11, 12, 14
14	27.1	2.24 (3H) s	12, 13	14.3	0.94 (3H) t 7.3	12, 13
15	25.1	1.21 m	3, 4, 5, 16	25.1	1.22 m	3, 16
		1.15 m	3, 4, 5, 16		1.14 m	3, 16
16	11.0	0.90 (3H) q 7.4	4, 15	11.0	0.89 (3H) t 7.8	15
17	24.8	1.99 m	5, 6, 7, 18	24.6	2.02 m	5, 6, 7, 18
		1.54 m	5, 6, 7, 18		1.47 m	6, 7, 18
18	7.6	0.83 (3H) t 7.4	6, 17	7.5	0.83 (3H) t 7.4	6, 17
19	27.3	1.47 m	9, 10, 11, 20	28.3	1.34 m	10, 11, 20
		1.33 m	9, 11, 20		1.14 m	
20	11.7	0.84 (3H) t 7.7	10, 19	11.7	0.80 (3H) t 7.4	19

[a] HMBC correlations are from proton(s) stated for the indicated carbon(s).

Table 4. Growth inhibitory activities of **4** and **5** against *E. coli, B. cereus*, *C. albicans*, and *S. cerevisiae.*

	inhibitory zone (mm)[a]			
	E. coli	*B. cereus*	*C. albicans*	*S. cerevisiae*
4	inactive	inactive	inactive	inactive
5	16	9	inactive	inactive

[a]Paper disks (ϕ6 mm), impregnated with 10 μg of **4** or **5**, were incubated on agar plates containing *E. coli* or *B. cereus* at 37 °C and *C. albicans* or *S. cerevisiae* at 27 °C overnight.