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NOTE

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Kabiramides J and K, Trisoxazole Macrolides from the Sponge *Pachastrissa nux*

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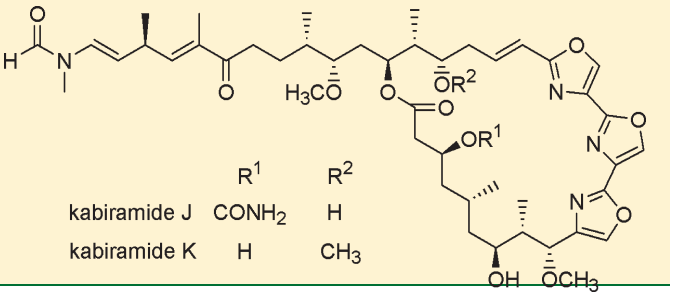
Supporting Information

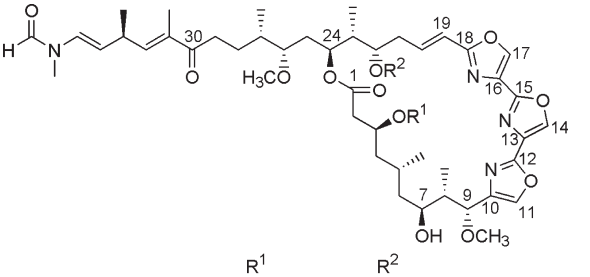
ABSTRACT: Three trisoxazole macrolides possessing a 30-α,β-enone moiety, including the known kabiramide G (**1**) and the new kabiramides J (**2**) and K (**3**), were isolated from the sponge *Pachastrissa nux*, along with the previously reported kabiramides B (**4**), C (**5**), and D (**6**). To date, the enone moiety has been found to associate solely with the trisoxazole macrolides from *P. nux*. All of the isolated macrolides showed moderate to strong antimalarial and cytotoxic activities, except for **1**, which possessed only potent cytotoxicity.



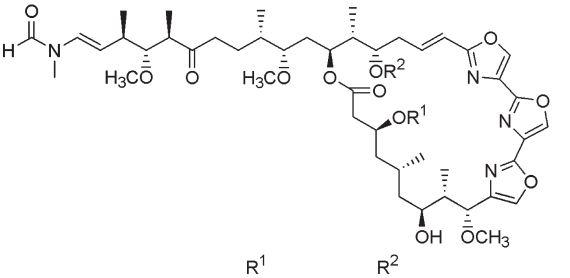
Trisoxazole macrolides are a class of exclusively marine-derived natural products containing three consecutive oxazole moieties that form part of a 25-membered lactone ring. To date, over 35 trisoxazole macrolides have been isolated from various organisms, including sponges, especially of the genera *Halichondria*, *Chondrosia*, *Mycale*, and *Jaspis*,[1] the Spanish dancer nudibranch *Hexabranchus sanguineus*,[1a,2] and the coral *Tubastrea faulkneri*.[3] Apart from their unique chemical skeletons, trisoxazole macrolides also possess strong biological activities, including cytotoxic,[1,2a,c,d] antifungal,[1a,b,g,2a,d] and proteasome-inhibiting[1h] activities. Presumably, most of the reported biological activities of trisoxazole macrolides are associated very closely with their ability to bind to actin.[4] This ability opens up the possibility of using trisoxazole macrolides as tools for cellular and molecular biology research.[5]

Recently, a series of trisoxazole macrolides, including a new 30-α,β-enone analogue, kabiramide G (**1**), were isolated from the Thai sponge *Pachastrissa nux*.[6] The sponge has now been collected from several other locations in the Gulf of Thailand, and its methanolic extracts showed potent antimalarial activity (IC_{50} 0.7 μg/mL against *Plasmodium falciparum* K1) and cytotoxicity (>80% inhibition against MCF-7 breast adenocarcinoma cell line, 10 μg/mL). Further investigation led to the isolation of additional trisoxazole macrolides, including two new 30-α,β-enone analogues, kabiramides J (**2**) and K (**3**). Herein, the isolation and structure determination of the new macrolides are presented. The antimalarial and cytotoxic activities of all of the isolated compounds are also reported.

The sponge specimens were collected from three separate expeditions: two from Koh-Tao, Surat-Thani Province, in April 2004 and April 2006, and another from Chumphon Islands



	R1	R2
1	$CONH_2$	CH_3
2	$CONH_2$	H
3	H	CH_3



	R1	R2
4	$CONH_2$	H
5	$CONH_2$	CH_3
6	H	CH_3

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National Park, Chumphon Province, in April 2008. The specimens from the 2004 and 2006 collections were pooled and macerated in MeOH, and the MeOH extract was further fractionated to yield hexane, CH_2Cl_2, and *n*-BuOH extracts. On the basis of the antimalarial activity, the CH_2Cl_2 extract was selected for further investigation. Successive chromatography over SiO_2, Sephadex LH20, and RP HPLC columns led to the isolation of **2**, along with the previously reported kabiramides B−D (**4**−**6**)[2b,c] and G (**1**).[6] The sponge specimen from the 2008 expedition was also macerated and fractionated in a similar fashion. The antimalarial-active CH_2Cl_2 fraction was fractionated chromatographically using SiO_2, Sephadex LH20, and RP C18 HPLC to yield **3**, together with **1**, **2**, and **4**−**6**. The structures of **1**, **4**, **5**, and **6** were elucidated on the basis of the complete spectroscopic analyses of MS, UV, IR, and NMR spectra and were confirmed by the comparisons with the published data. The absolute configurations of all four compounds as shown were based on the specific rotations, measured to be in a range comparable with that of the reported values.[2b,c,6]

Compound **2** was obtained as a white, amorphous solid. The molecular formula of **2** was proposed to be $C_{46}H_{65}N_5O_{13}$ according to both HR and LR ESIMS spectra. The NMR spectra of **2** (500 MHz for ^{1}H, C_6D_6; Table 1) indicated the presence of a trisoxazole macrolide skeleton similar to other macrolides isolated in this work, with the characteristic signals of a 1:2 rotameric mixture caused by the *N*-methyl formamide moiety. Specifically, the core skeleton of **2** closely resembles that of **1**, with the presence of the characteristic 30-enone moiety on the side chain as observed at δ_H 6.32 (dq, J = 9.3, 1.2 Hz; H-32) and at δ_C 201.0 (C-30), 136.0 (C-31), and 144.0 (C-32) in the NMR spectra. The structure of **2** was elucidated primarily based on the analysis of the ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). Compared with the parent structure of compound **1**, **2** differs from **1** in that the methoxy group at C-22 is replaced by a hydroxy group. This was confirmed by the observation of a long-range correlation from 22-O*H* (δ 4.35, br d, J = 7.7 Hz) to C-22 (δ 68.3). Compound **2** was therefore proposed to be a new member of trisoxazole macrolides possessing a 30-α,β-enone moiety, named kabiramide J.

As most trisoxazole macrolides possess strictly conserved configurations, this allowed us to propose the similarity in the configurations between **1** and **2**, also based on the direct comparison of the chemical shifts and coupling constants of the two compounds. Specific attention was paid to the geometric configurations at Δ^{31}, which was proposed as *E* according to the chemical shift of 31-CH_3 (δ_C 11.8).[6] Another asymmetric carbon of concern was C-9, which is the only position where the variations in the configurations among trisoxazole macrolides have been reported. The small coupling constant of H-9 (δ 4.95, d, J = 1.0 Hz) indicated that **2** possessed the α-orientation at C-9, similar to that of other kabiramide analogues.[2d]

Compound **3**, also obtained as a white, amorphous solid, has a molecular formula of $C_{46}H_{66}N_4O_{12}$ as suggested by the FABMS, both LR and HR, and the NMR spectra. The ^{1}H and ^{13}C NMR spectra of **3** (Table 1) were almost identical to those of **1** and **2** and indicated that all three are closely related. The connectivity for all the carbon and proton frameworks based on the ^{1}H−^{1}H COSY and HMBC correlations was carried out in the same manner as that for **2**. The major differences between **2** and **3** were the presence of one additional methoxy group and the absence of the carbamate functionality. The methoxy group (δ_H 3.43, s) was assigned as 22-OCH_3 due to the HMBC correlation from the methoxy protons to C-22 (δ 78.6). The C-3 carbamate moiety (as respectively indicated by δ_C 158.4 and by ν 3460 and 3175 cm^{-1} in the ^{13}C NMR and IR spectra of **2**) was replaced by a hydroxy group, resonating at δ_H 4.96 (br s; 3-O*H*). Compound **3**, named kabiramide K, was proposed as a new 3-OH,22-OCH_3 kabiramide analogue, possessing a 30-α,β-enone moiety similar to that of **1** and **2**.

Whereas this could be a mere coincidence, to date only three trisoxazole macrolides with a 30-α,β-enone moiety, i.e., **1**−**3**, have been reported, all of which are from *P. nux*. The presence of the enone functionality in **1**−**3** raised the question of whether all three were genuine natural products or were otherwise a series of artifacts from their related kabiramides, also isolated from the same sponge specimens. However, no changes were detected when subjecting **5**, as a model, to various conditions that mimicked the separation protocols used throughout this investigation, e.g., overnight stirring in MeOH, in $CHCl_3$, and in CH_2Cl_2 in the presence of SiO_2. Harsher conditions, either in acidic or basic media that may facilitate the elimination, were otherwise carefully avoided throughout our isolation protocols. It is therefore reasonable to propose here that the transformation to the 30-enone moiety did not take place during the isolation process. In addition, presuming that the side chain part of the macrolide is of polyketidic origin, the encoded dehydratase domain is commonly known to yield the enone functionality, suggesting the possible biogenetic origin of the enone in the investigated sponge or even by associated microbes.

All the isolated compounds were assayed for antimalarial (against *Plasmodium falciparum* K1) and cytotoxic activities (against MCF-7 breast adenocarcinoma and normal human fibroblast), and all showed strong activities in both models, except for **1**, which showed only strong cytotoxicity against both cancer and normal cell lines (Table 2). The strong potency was not surprising because most trisoxazole macrolides are presumed to possess actin-binding activity, an ability to interrupt the functions of the primary cytoskeleton in any type of living cell, leading to such strong cytotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. UV spectra were obtained from a Genesys 10 spectrophotometer, and IR spectra were from a Jasco 810 IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on an FT-NMR Varian Unity Inova 500 spectrometer, using the solvent signals (7.15 ppm of residual C_6HD_5 for ^{1}H and 128 ppm of C_6D_6 for ^{13}C) as internal standards. Mass spectra were acquired from a Micromass LCT spectrometer for ESIMS and from a Thermo Finnigan MAT 95 XL mass spectrometer for FABMS. HPLC was performed using a Waters 600E system controller, equipped with a Rheodyne 7125 injector port, a Waters 484 tunable absorbance detector, and a Jasco 807-IT integrator.

Animal Material. The sponge specimens used in this investigation were collected by scuba diving from three different expeditions: two from the vicinity of Koh-Tao, Surat-Thani Province, in April 2004 and April 2006, and one from Chumphon Islands National Park, Chumphon Province, in April 2008, all at a depth of 10−15 m. The specimens zfrom all three expeditions were identified as *Pachastrissa nux* (family Calthropellidae) by Dr. Sumaitt Putchakarn, Institute of Marine Science, Burapha University. The voucher specimen (AP04-002-01)

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for 2 and 3 (500 MHz for ^{1}H, 125 MHz for ^{13}C; C_6D_6)[a]

	2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	171.6, C		172.7, C	
2	43.4, CH_2	2.71, dd (16.1, 6.6); 2.42, dd (16.1, 1.2)	45.5, CH_2	2.65, dd (14.3, 3.2); 2.35, br d (14.3)
3	71.3, CH	5.31, br dd (12.1, 6.6)	67.5, CH	4.73, br dd (10.6, 10.6)
4	45.7, CH_2	2.07, m; 1.11, m	44.0, CH_2	2.30, m; 1.00, br dd (10.6, 10.6)
5	25.6, CH	1.92 (overlap)	25.0, CH	2.76, m
6	43.4, CH_2	1.71, dd (6.7, 6.1)	45.1, CH_2	1.67, ddd (13.0, 11.0, 3.2); 1.55, m
7	73.3, CH	3.90, br dd (6.1, 5.5)[b]	71.2 [71.3], CH	4.14 (overlap)
8	38.6, CH	2.19, m	43.1, CH	2.25 (overlap)
9	78.3, CH	4.95, d (1.0)	80.1 [80.09], CH	4.12 (overlap)
10	142.8, C		140.2, C	
11	135.6, CH	7.13, s	137.0, CH	6.90, s
12	155.7, C		155.8, C	
13	131.4, C		131.2, C[c]	
14	137.0, CH	7.30, s	137.0, CH	7.20, s
15	156.5, C		156.7, C	
16	130.0, C		131.1, C[c]	
17	136.7, CH	7.17, s	137.0, CH	7.16, s
18	164.1, C		163.1, C	
19	114.9 [114.8], CH	6.06 [6.03], dd (16.1, 1.0)	116.0, CH	5.95, dd (15.5, 1.7)
20	147.0, CH	7.53, ddd (16.1, 9.4, 3.4)	142.6, CH	7.43, ddd (15.5, 10.7, 4.6)
21	38.4, CH_2	2.48, ddd (14.8, 9.4, 8.9); 1.97 (overlap)	34.1, CH_2	2.55 (overlap); 2.15 (overlap)
22	68.3, CH	4.44, br dd (9.0, 7.7)[b]	78.6, CH	3.84, m
23	43.39, CH	1.60 (overlap)	40.6, CH	1.79 (overlap)
24	74.2, CH	5.71, td (10.3, <1.0)	72.7, CH	5.76, td (9.7, 1.0)
25	35.1, CH_2	2.00 (overlap); 1.57 (overlap)	33.6, CH_2	1.82 (overlap); 1.45, m
26	82.5 [82.4], CH	3.30, m	82.11 [82.10], CH	3.11 [3.09], m
27	35.2, CH	1.85 (overlap)	35.0, CH	1.75 (overlap)
28	27.4 [27.3], CH_2	2.09 (overlap); 1.62 (overlap)	27.2 [27.1], CH_2	2.02 [1.99] (overlap); 1.51 [1.44] (overlap)
29	35.7, CH_2	2.67, m; 2.64, m	35.7, CH_2	2.60, m; 2.63, m
30	201.0, C		200.8, C	
31	136.0, C		135.9 [136.0], C	
32	144.0, CH	6.32 [6.35], dq (9.3, 1.2)	143.8 [142.5], CH	6.27 [6.30], dq (9.5, 1.2)
33	34.5 [34.7], CH	2.93 [3.02], m	34.7 [34.5], CH	2.88 [3.00] (overlap)
34	111.7 [113.8], CH	4.49 [4.63], dd (14.0, 7.3)	111.6 [113.7], CH	4.45 [4.59], dd (14.0, 7.3)
35	128.6 [124.8], CH	5.86 [7.38], d (14.0)	130.2 [124.8], CH	5.80 [7.36], d (14.0)
3-$OCONH_2$	158.4, C	7.78, br s; 6.85, br s		
3-OH				4.96, br sd
5-CH_3	19.3, CH_3	0.73, d (6.6)	21.5, CH_3	1.08 [1.07], d (6.6)
7-OH		2.82, d (6.5)		5.84, br sd
8-CH_3	10.7, CH_3	0.98, d (7.8)	13.6 [13.5], CH_3	0.70 [0.71], d (6.8)
9-OCH_3	57.5 [57.51], CH_3	3.04 [3.05], s	56.8 [56.9], CH_3	2.90 [2.91], s
22-OH		4.35, br d (7.7)		
22-OCH_3			57.4 [57.3], CH_3	3.43 [3.42], s
23-CH_3	9.9, CH_3	1.04, d (6.8)	9.5, CH_3	0.97, d (7.1)
26-OCH_3	57.6 [57.63], CH_3	3.39 [3.38], s	57.7 [57.8], CH_3	3.29, s
27-CH_3	16.0 [15.9], CH_3	0.93 [0.92], d (7.1)	15.9 [15.8], CH_3	0.77 [0.75], d (7.1)

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Table 1. Continued

	2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
31-CH_3	11.8 [11.7], CH_3	1.88 [1.83], d (1.3)	11.8 [11.7], CH_3	1.83 [1.77], d (1.2)
33-CH_3	20.9 [20.7], CH_3	0.94 [0.95], d (7.2)	20.9 [20.7], CH_3	0.91 [0.92], d (6.8)
35-NCH_3	26.9 [31.9], CH_3	2.60 [2.11], s	26.8 [31.8], CH_3	2.56 [2.07], s
35-NCHO	161.7 [160.5], CH	7.86 [7.57], s	161.5 [160.4], CH	7.81 [7.54], s

[a] Chemical shifts of the minor conformers are presented in brackets. [b] Coupling constants were calculated according to the D_2O-exchanged spectrum. [c,d] Chemical shifts are interchangeable.

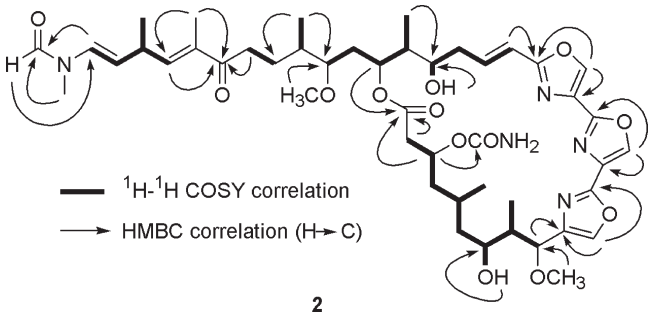

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations of **2**.

Table 2. Antimalarial and Cytotoxic Activities of the Isolated Compounds

		cytotoxicity (IC_{50}; μM)[b]	
compound	antimalarial (IC_{50}; μM)[a]	MCF-7	fibroblast
1	n.a.[c]	0.02	2.37
2	0.31	0.02	n.d.[d]
3	0.39	0.07	n.d.[d]
4	1.67	0.45	0.95
5	4.79	0.47	7.59
6	1.87	0.02	0.50

[a] Referencing dihydroartemisinin (IC_{50} 3.8−4.4 nM). [b] Referencing camptothecin (IC_{50} against MCF-7 and human fibroblast 1.6 and 459.3 nM, respectively). [c] Compound showed no calculable inhibition against targeted protozoan at the highest concentration of 10 mg/mL. [d] Activity was not determined.

was deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

Extraction and Isolation. The sponge specimens from the 2004 and 2006 expeditions were pooled (517 g dry weight) and exhaustively macerated in MeOH. The MeOH extract, once dried, was partitioned to yield hexane, CH_2Cl_2, and *n*-BuOH extracts. The antimalarial-active CH_2Cl_2 extract was fractionated over SiO_2 columns (5% MeOH in CH_2Cl_2; then gradient 2.5:1:96.5 to 30:1:69 MeOH/hexane/CH_2Cl_2) to afford two major fractions. The first fraction was chromatographed on a Sephadex LH20 column (MeOH), then an RP-HPLC column (C8, 10 μm, 250 × 10 mm, 40% H_2O in MeCN), to yield **1**, **2**, **4**, and **5** (25, 3, 23, and 62 mg, respectively). The second fraction was separated over an RP-HPLC column (C18, 10 μm, 250 × 10 mm, 35% H_2O in MeCN) to afford **6** (3 mg).

The specimen from the 2008 expedition (790 g dry weight) was extracted and fractionated in a similar fashion to that described above. The CH_2Cl_2 extract was chromatographed on SiO_2 columns (5% MeOH in CH_2Cl_2, then 0.5% MeOH in EtOAc), then Sephadex LH20 (MeOH) and RP-HPLC columns (C8, 10 μm, 250 × 10 mm, 40% H_2O in MeCN), to yield two major fractions. The first one was further purified on an RP-HPLC column (C18, 5 μm, 150 × 4.6 mm, 25% H_2O in MeOH) to afford **2** (12 mg). The second fraction was also subjected to an RP-HPLC column (C18, 5 μm, 150 × 4.6 mm, 22% H_2O in MeOH), and **3** was obtained (6 mg).

Kabiramide J (***2***): white, amorphous solid; $[\alpha]^{20}_D$ +6 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 248 (4.55) nm; IR (thin film) ν_{max} 3600−3250 (br), 3460, 3375, 3175, 2960, 2925, 1735, 1720, 1690, 1659 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; ESIMS *m*/*z* (% relative intensity) 918.5 ($[M + Na]^+$, 100); HRESIMS *m*/*z* 918.4463 (calcd for $C_{46}H_{65}N_5O_{13}Na$, 918.4476).

Kabiramide K (***3***): white, amorphous solid; $[\alpha]^{20}_D$ +9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 246 (4.55) nm; IR (thin film) ν_{max} 3600−3250 (br), 3175, 2960, 2925, 1735, 1690, 1659 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; FABMS *m*/*z* (% relative intensity) 867.5 ($[MH]^+$, 3), 563.5 (2), 282.3 (100), 256.3 (18); HRFABMS *m*/*z* 867.4713 (calcd for $C_{46}H_{67}N_4O_{12}$, 867.4755).

Kabiramide G (***1***): $[\alpha]^{20}_D$ +27 (*c* 0.3, $CHCl_3$); lit. $[\alpha]^{20}_D$ +38 (*c* 0.4, $CHCl_3$).[6]

Kabiramide B (***4***): $[\alpha]^{20}_D$ +4 (*c* 0.6, $CHCl_3$); lit. $[\alpha]^{20}_D$ +8 (*c* 0.1, $CHCl_3$).[2c]

Kabiramide C (***5***): $[\alpha]^{20}_D$ +10 (*c* 0.6, $CHCl_3$); lit. $[\alpha]^{20}_D$ +20 (*c* 0.1, $CHCl_3$).[2b]

Kabiramide D (***6***): $[\alpha]^{20}_D$ −11 (*c* 0.2, $CHCl_3$); lit. $[\alpha]^{20}_D$ −5 (*c* 0.1, $CHCl_3$).[2c]

Antimalarial Assay. The antimalarial activity determination was carried out by the Central Bioassay Lab, BIOTEC, Thailand, and was based on the microdilution radioisotope technique.[7] The targeted parasite was *P. falciparum* K1 (multidrug-resistant) strain. The activity was compared to that of dihydroartemisinin as the standard drug (IC_{50} 3.8−4.4 nM).

Cytotoxic Assay. The cytotoxicity was determined using a microplate sulforhodamine B assay,[8] targeting MCF-7 breast adenocarcinoma and human fibroblasts as cancer and normal cell lines, respectively. All the resulting activities used camptothecin as the standard reference (IC_{50}'s against cancer and normal cell lines 1.6 and 459.3 nM, respectively).

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of **2** and **3** and spectroscopic data of **1** and **4**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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