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Chemistry of renieramycins. Part 7: Renieramycins T and U, novel renieramycin–ecteinascidin hybrid marine natural products from Thai sponge *Xestospongia* sp. ☆

Naomi Daikuhara [a], Yumiko Tada [a], Sachiyo Yamaki [a], Kornvika Charupant [b], Surattana Amnuoypol [b], Khanit Suwanborirux [b], Naoki Saito [a,*]

[a] Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan
[b] Center for Bioactive Natural Products from Marine Organisms and Endophytic Fungi (BNPME), Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

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ABSTRACT

Two new bistetrahydroisoquinoline marine natural products, renieramycins T (**1**) and U (**2**), were isolated from the Thai blue sponge *Xestospongia* sp. and their structures were elucidated by comparing spectral data with those of renieramycin M (**3a**) and ecteinascidin 770 (**4a**). These compounds are the first reported examples of novel ecteinascidin–renieramycin hybrid natural products. Renieramycin T (**1**) showed strong cytotoxicity to several human cancer cell lines, its IC_{50} values ranging from 4.7 to 98 nM.

Renieramycins are isoquinoline marine natural products that are structurally and biologically related to other isoquinoline natural products, including saframycins, naphthyridinomycins, quinocarcins, and ecteinascidins.[2] In our ongoing search for new anticancer metabolites in Thai marine animals, we were able to identify several biologically active compounds from the tunicate *Ecteinascidia thurstoni*,[3] the blue sponge *Xestospongia* sp.,[1,4,5] and the nudibranch *Jorunna funebris*.[6] However, all of our target natural products were isolable in only trace amounts and were relatively unstable, decomposing during extraction and isolation. We solved this problem by converting original natural products having a very unstable amino alcohol functionality at C-21 into stable α-aminonitrile compounds by pretreatment with KCN. The first examples of novel marine natural products isolable in gram scale are ecteinascidin 770 (**4a**)[3,7] and renieramycin M (**3a**).[4,8]

In our continuing chemical studies on isoquinoline marine natural products, we found two novel renieramycin-related compounds, renieramycins T (**1**) and U (**2**), from the Thai blue sponge *Xestospongia* sp. Both compounds possess a highly functionalized aromatic A ring, which is the same as that of ecteinascidins, and are the first examples of ecteinascidin–renieramycin hybrids from natural sources. In this Letter, we report the structure elucidation of these two new marine natural products **1** and **2** by means of spectroscopic analyses, and their biological data (see Fig. 1).

New compound **1**,[9] named renieramycin T,[10] was confirmed to have the molecular formula $C_{31}H_{33}N_3O_8$ by HRFABMS [*m/z* 576.2347 (MH^+)], which was the same as that of renieramycin M (**3a**). The molecular formula of **1** indicated 17 degrees of unsaturation and the presence of resonance attributable to 12 olefinic carbons, three carbonyl groups, and one nitrile carbon in **1** accounted for 11 degrees of unsaturation. Thus, this compound was presumed to contain six rings. The ^1H NMR spectrum contained a characteristic pair of doublets at δ 5.85, 5.92 ppm (J = 1.5 Hz) and the ^{13}C NMR spectrum revealed a methylenedioxy carbon at δ 101.1 ppm. These data are similar to those of **4a**. The extinction coefficient at the wavelength of maximum UV absorption was reduced to half in **1** compared to that in bisquinones (such as **3a**), and only two carbonyl resonances (δ 186.1 and 182.8 ppm) of the quinone rings were observed in the ^{13}C NMR spectrum (Table 1). These data, in addition to the D_2O exchangeable proton signal at δ 4.55 ppm and one methoxy proton signal at δ 3.98 ppm, revealed that one of the quinone rings might have been reduced to form an aromatic ring in **1**. As the diagnostic homoallylic coupling (approx. 3 Hz) between 1-H and 4-Hβ through five bonds was

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* Corresponding author. Tel./fax: +81 424 95 8794.
E-mail address: naoki@my-pharm.ac.jp (N. Saito).

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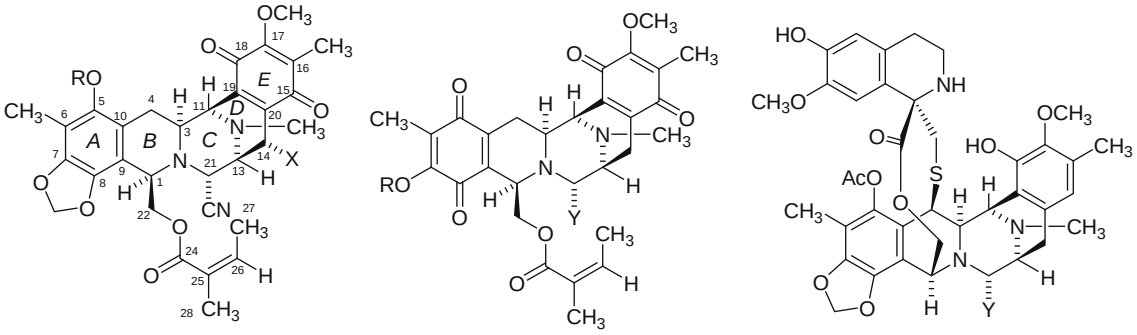

Renieramycins
T (**1**): R = X = H
U (**2**): R = H, X = OH
6: R = Ac, X = H

Renieramycins
M (**3a**): R = CH_3, Y = CN
E (**3b**): R = CH_3, Y= OH
S (**5**): R = H, Y = CN

Ecteinascidins
770 (**4a**): Y = CN
743 (**4b**): Y = OH

Figure 1. Structures of renieramycins and their related marine natural products.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for renieramycin T (**1**) in $CDCl_3$

Atom No.	δ_C	δ_H	HMBC correlation	NOE correlation
1	56.4	4.16 (dd, 4.9, 3.7)	21-H, 22-Hb	3-H, 22-Hb
3	56.2	3.24 (ddd, 11.9, 2.7, 2.4)	4-Hα, 4-Hβ, 11-H, 21-H	1-H, 3-H, 11-H
4	26.8	α 2.87 (dd, 15.0, 2.4)		4-Hβ, 3-H, 11-H
		β 1.67 (dd, 15.0, 11.9)		4-Hα, 5-OH
5	144.7		4-Hα, 4-Hβ, 6-CH_3	
6	106.2		6-CH_3	
7	144.9		6-CH_3, OCH_2O	
8	136.8		OCH_2O	
9	112.1		4-Hα, 22-Ha, 22-Hb	
10	113.1		4-Hα, 4-Hβ	
11	54.9	4.00 (dd, 2.7, 0.5)	4-Hα, 13-H, NCH_3	3-H, 4-Hα, NCH_3
13	54.8	3.37 (ddd, 7.3, 2.4, 0.5)	11-H, 14-Hα, NCH_3	14-Hα, 21-H, NCH_3
14	21.2	α 2.75 (dd, 20.8, 7.3)	13-H, 21-H	13-H, 14-Hβ
		β 2.30 (d, 20.8)		14-Hα, 21-H
15	186.1		14-Hα, 14-Hβ, 16-CH_3	
16	129.0		16-CH_3	
17	155.4		16-CH_3, 17-OCH_3	
18	182.8		11-H	
19	135.7		3-H, 11-H, 14-Hα, 14-Hβ	
20	141.8		11-H, 13-H, 14-Hα, 14-Hβ	
21	59.6	4.11 (d, 2.4)	1-H, 13-H, 14-Hα, 14-Hβ	13-H, 14-Hβ, 22-Hb
22	64.6	a 3.99 (dd, 11.3, 4.9)		22-Hb
		b 4.41 (dd, 11.3, 3.7)		1-H, 21-H, 22-Ha
24	167.1		22-Ha, 22-Hb, 25-CH_3, 26-H	
25	126.8		25-CH_3, 26-CH_3	
26	139.7	6.00 (qq, 7.3, 1.5)	25-CH_3, 26-CH_3	25-CH_3, 26-CH_3
27	15.7	1.85 (dq, 7.3, 1.5)	26-H	26-H
28	20.5	1.69 (dq, 1.5, 1.5)	26-H	26-H
6-CH_3	8.8	2.11 (s)		
16-CH_3	8.7	1.94 (s)		
17-OCH_3	60.9	3.98 (s)		
N-CH_3	41.4	2.29 (s)	11-H, 13-H	
OCH_2O	101.1	a 5.85 (d, 1.5)		OCHO
		b 5.92 (d, 1.5)		OCHO
CN	117.4		13-H, 21-H	
5-OH		4.55 (br s)		4-Hβ

negligible in **1**, **1** might have a *p*-quinone functionality only at the E ring, because this coupling was confirmed in the case of renieramycin M that has a *p*-quinone functionality at the A ring in our previous paper.[11] The quinone carbonyl at C-15 was assigned on the basis of long-range ^{1}H–^{13}C correlations between C-15 and three protons (H-14α, H-14β, and 16-CH_3), the other quinone carbonyl at C-18 was assigned on the basis of C-18 and 11-H long-range ^{1}H–^{13}C correlations. These also supported the hypothesis that the *p*-quinone ring might be the E ring. Then, the substituents on the aromatic A ring were assigned as follows: The chemical shifts of C-5 (δ 144.7 ppm), C-7 (δ 144.9 ppm), and C-8 (δ 136.8 ppm) indicate oxygen substitution, and long-range ^{1}H–^{13}C correlations observed between methylenedioxy protons and C-7 and C-8 indicate that the methylenedioxy functionality is on the A ring. The long-range ^{1}H–^{13}C correlations of 6-CH_3 protons (δ 2.11 ppm) to C-5, C-6, and C-7 also revealed the positions of these two carbons.

The acetylation of **1** with acetic anhydride in pyridine at 0 °C for 1 h afforded acetate **6**[12] in 74% yield. An observable NOE between the acetyl protons (δ 2.31 ppm) and 6-CH_3 (δ 2.02 ppm) revealed that the OAc group must exist at C-5 in **6**.

New compound **2**,[9] named renieramycin U,[13] was confirmed to have the molecular formula $C_{31}H_{33}N_3O_9$ by HRFABMS [*m*/*z* 592.2294 (MH^+)], and it had 16 mass units more than **1**. In the

Table 2
Cytotoxicity of renieramycins to various cancer cell lines (IC_{50} μM)[a]

Compound	HCT116	QG56	AsPC1	T47D
Renieramycin T (**1**)	0.039	0.077	0.098	0.0047
Acetylrenieramycin T (**6**)	0.034	0.079	0.120	0.010
Renieramycin M (**3a**)	0.0087	0.014	0.020	0.00084

[a] HCT116 = human colon carcinoma; QG56 = human lung carcinoma; AsPC1 = human pancreatic adenocarcinoma; T47D = human ductal breast epithelial tumor.

^{1}H NMR spectrum of **2**, the absence of coupling between 13-H (δ 3.39 ppm) and 14-H (δ 4.37 ppm) indicated a dihedral angle of 80–90°. An observable NOE between 14-H and 21-H (δ 4.22 ppm) revealed the relative stereochemistry at C-21. These data, in addition to the almost identical chemical shifts and coupling constants of the remaining signals as well as characteristic carbon signals including C-14 (δ 62.2 ppm), enabled the elucidation of the structure of **2** as 14α-hydroxyrenieramycin T.

The cytotoxicity to four cancer cell lines of renieramycin T (**1**) and its acetate (**6**) is summarized in Table 2.[14] Both compounds were much less cytotoxic than renieramycin M **3a**.

In conclusion, novel ecteinascidin structures of two marine natural products, renieramycins T and U, isolated from *Xestospongia* sp., were elucidated by spectroscopic analysis. Since the discovery by Corey and Schreiber's group that phthalascidin, a synthetic antitumor agent, exhibits comparable biological activity to **4b**,[15] a number of synthetic chemists have reported the synthesis of phthalascidin analogs.[16] We believe that these two compounds are the first reported examples of naturally occurring ecteinascidin–renieramycin hybrids from a marine organism. *Xestospongia* sp. produces relatively small quantities of metabolites resembling those of *Ecteinascidia* sp., and it is very interesting that those metabolites are produced by a symbiotic microorganism.

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7. Ecteinascidin 770 is the stable form of ecteinascidin 743, a compound first reported by Reinhart et al. in 1990. See: (a) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Kieffer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512–4515; (b) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. F.; McConnel, O. J. *J. Org. Chem.* **1990**, *55*, 4508–4512.
8. Renieramycin M is the stable form of renieramycin E, a compound that was isolated in a minute amount marine natural product from the methanol extract of the blue sponge *Reniera* sp. inhabiting a small marine lake in Urukhapel Island, Palau. See: He, H.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 5822–5824.
9. The sponge *Xestospongia* sp. was collected by SCUBA in the vicinity of Sichang Island at depths of 3–5 m in November 2006 and frozen until used. The collected sponge (18 kg, wet weight) was homogenized and phosphate buffer was added to the resulting solution (18 L) to adjust the pH to 7. Then, 10% KCN solution (120 mL) was added slowly to the suspension and the mixture was stirred for 5 h. The mixture was macerated with MeOH (10 L × 3) and filtered, and the filtrate was concentrated under reduced pressure. The aqueous MeOH solution was partitioned with EtOAc (1 L × 4) and the solvent was removed to give a residue. The residue was subjected to trituration with MeOH to yield an orange precipitate of crude renieramycin M[4] (**3a**, 3.70 g), the recrystallization of which from EtOAc gave pure **3a** (3.26 g). The mother liquor was concentrated in vacuo to give a residue (1.03 g), which was subjected to flash silica gel column chromatography (hexane/EtOAc gradient) to furnish two new compounds, renieramycins T (**1**, 12.5 mg) and U (**2**, 2.0 mg), along with renieramycin S[5] (**5**, 29.5 mg).
10. Renieramycin T (**1**): amorphous powder, ^{1}H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 268 (3.80), 370 (2.72); FABMS *m/z* 576 (MH^+); HRFABMS *m/z* 576.2347 (MH^+, calcd for $C_{31}H_{34}N_3O_8$, 576.2346); IR (KBr) 3455, 1713, 1653, 1616, 1235 cm^{-1}; $[\alpha]_D^{26}$ −28.6 (*c*, 0.60, $CHCl_3$); CD (*c* 0.016 mmol/L, MeOH, 24 °C) −3.91 (346), 0 (290), +6.41 (264), +0.47 (234), +0.77 (229), +0.60 (224), +4.99 (214).
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12. *Acetylrenieramycin T* (**6**): amorphous powder, ^{1}H NMR δ 1.62 (1H, dd, *J* = 15.0, 12.2 Hz, 4Hβ), 1.66 (3H, dq, *J* = 1.5, 1.5 Hz, 25-CH_3), 1.86 (3H, dq, *J* = 7.3, 1.5 Hz, 26-CH_3), 1.92 (3H, s, 16-CH_3), 2.02 (3H, s, 6-CH_3), 2.27 (3H, s, NCH_3), 2.30 (1H, d, *J* = 21.2 Hz, 14-Hβ), 2.31 (3H, s, $COCH_3$), 2.52 (1H, dd, *J* = 15.0, 2.1 Hz, 4-Hα), 2.74 (1H, dd, *J* = 21.1, 7.3 Hz, 14-Hα), 3.22 (1H, ddd, *J* = 12.2, 2.7, 2.1 Hz, 3-H), 3.37 (1H, ddd, *J* = 7.3, 2.4, 1.0 Hz, 13-H), 3.97 (3H, s, OCH_3), 3.98 (1H, dd, *J* = 2.7, 1.0 Hz, 11-H), 4.03 (1H, dd, *J* = 11.6, 4.1 Hz, 22-Ha), 4.12 (1H, d, *J* = 2.4 Hz, 21-H), 4.16 (1H, dd, *J* = 4.1, 2.4 Hz, 1-H), 4.83 (1H, dd, *J* = 11.6, 2.4 Hz, 22-Hb), 5.93 (1H, d, *J* = 1.5 Hz, OCHO), 5.98 (1H, qq, *J* = 7.3, 1.5 Hz, 26-H), 6.00 (1H, d, *J* = 1.5 Hz, OCHO); ^{13}C NMR δ 8.6 (16-CH_3), 9.4 (6-CH_3), 15.8 (26-CH_3), 20.2 ($COCH_3$), 20.5 (25-CH_3), 21.0 (C-14), 27.9 (C-4), 41.4 (NCH_3), 54.7 (C-11), 54.8 (C-13), 55.4 (C-3), 56.5 (C-1), 59.0 (C-21), 60.6 (OCH_3), 63.2 (C-22), 101.7 (OCH_2O), 112.0 (C-6), 112.3 (C-9), 117.3 (CN), 120.1 (C-10), 126.7 (C-25), 128.8 (C-16), 135.9 (C-19), 140.2 (C-26), 140.4 (C-8), 140.7 (C-5), 141.7 (C-20), 144.9 (C-7), 155.5 (C-17), 167.0 (OCO), 168.7 (CH_3CO), 182.8 (C-18), 186.0 (C-15); UV (MeOH) λ_{max} nm (log ε) 268 (3.79), 370 (2.71); FABMS *m/z* 618 (MH^+); HRFABMS *m/z* 618.2457 (MH^+, calcd for $C_{33}H_{36}N_3O_9$, 618.2451); IR (KBr) 2228, 1763, 1717, 1653, 1616, 1250 cm^{-1}; $[\alpha]_D^{26}$ + 15.3 (*c*, 0.31, $CHCl_3$); CD (*c* 0.016 mmol/L, MeOH, 24 °C) −2.73 (356), −1.06 (301), −1.36 (292), 0 (285), +6.56 (264), 0 (239), −4.55 (223), 0 (216), +7.10 (211).
13. Renieramycin U (**2**): amorphous powder, ^{1}H NMR δ 1.54 (1H, dd, *J* = 15.3, 11.6 Hz, 4Hβ), 1.69 (3H, dq, *J* = 1.5, 1.5 Hz, 25-CH_3), 1.85 (3H, dq, *J* = 7.3, 1.5 Hz, 26-CH_3), 1.95 (3H, s, 16-CH_3), 2.10 (3H, s, 6-CH_3), 2.46 (3H, s, NCH_3), 2.86 (1H, dd, *J* = 15.3, 2.4 Hz, 4-Hα), 3.19 (1H, ddd, *J* = 11.6, 2.4, 2.4 Hz, 3-H), 3.39 (1H, dd, *J* = 2.7, 0.5 Hz, 13-H), 3.49 (1H, d, *J* = 2.1 Hz, 14-OH), 3.96 (1H, dd, *J* = 11.3, 5.2 Hz, 22-Ha), 4.04 (3H, s, OCH_3), 4.08 (1H, dd, *J* = 2.4, 0.5 Hz, 11-H), 4.14 (1H, dd, *J* = 5.2, 3.4 Hz, 1-H), 4.22 (1H, d, *J* = 2.7 Hz, 21-H), 4.26 (1H, br s, OH), 4.37 (1H, d, *J* = 1.2 Hz, 14-H), 4.41 (1H, dd, *J* = 11.3, 3.4 Hz, 22-Hb), 5.89 (1H, d, *J* = 1.2 Hz, OCHO), 5.92 (1H, d, *J* = 1.2 Hz, OCHO), 6.02 (1H, qq, *J* = 7.3, 1.5 Hz, 26-H); ^{13}C NMR δ 8.5 (16-CH_3), 8.7 (6-CH_3), 15.7 (26-CH_3), 20.5 (25-CH_3), 26.7 (C-4), 42.4 (NCH_3), 55.6 (C-3), 55.8 (C-11), 56.6 (C-1), 57.7 (C-21), 61.0 (OCH_3), 62.2 (C-14), 62.7 (C-13), 64.7 (C-22), 101.1 (OCH_2O), 112.0 (C-6), 112.0 (C-9), 116.9 (CN), 119.4 (C-10), 126.7 (C-25), 128.9 (C-16), 136.0 (C-19), 139.9 (C-26), 140.3 (C-8), 140.7 (C-5), 140.7 (C-20), 144.7 (C-7), 155.8 (C-17), 167.1 (OCO), 183.0 (C-18), 188.1 (C-15); UV (MeOH) λ_{max} nm (log ε) 268 (3.81), 370 (2.71); FABMS *m/z* 592 (MH^+); HRFABMS *m/z* 592.2294 (MH^+, calcd for $C_{31}H_{34}N_3O_9$, 592.2295); IR (KBr) 3451, 2232, 1713, 1655, 1616, 1261, 1234 cm^{-1}; $[\alpha]_D^{23}$ −51.3 (*c*, 0.07, $CHCl_3$); CD (*c* 0.034 mmol/L, MeOH, 24 °C) −1.74 (343), 0 (270), +0.43 (261), +0.15 (246), +6.87 (212).
14. A single-cell suspension of each cell line (2×10^3 cells/well) was added to the serially diluted test compounds in a microplate. Then, the cells were cultured for 4 d. Cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with the untreated control.
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