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CHEMISTRY OF RENIERAMYCINS. 16. STRUCTURE OF 7-DEMETHYLRENIERAMYCIN O (= 14α-HYDROXYRENIERAMYCIN S) FROM BLUE SPONGE, *XESTOSPONGIA* sp.

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This paper is dedicated to Professor Dr. Masakatsu Shibasaki on the occasion of his 70th birthday.

Abstract – A new renieramycin-type marine natural product was isolated from the blue sponge *Xestospongia* sp. found in the Philippines and Thailand, and its structure was elucidated to be 7-demethylrenieramycin O (= 14α-hydroxy-renieramycin S, **1**) by comparing its spectral data with those of renieramycins O and S. Exposure of a dichloromethane solution of renieramycin O to sunlight gave compound **1** along with renieramycin U. Compound **1** showed weak cytotoxicity to several human cancer cell lines.

Renieramycins are isoquinoline marine natural products that are structurally and biologically related to other isoquinoline natural products, including saframycins, naphthyridinomycins, quinocarcins, and ecteinascidins.[1,2] In our ongoing search for new anticancer metabolites in the blue sponge, we were able to isolate and elucidate the structures of renieramycins M-O (**2m-o**) and Q-V (**2q-v**) from Thai *Xestospongia* sp.,[3-6] and renieramycins W-Y (**2w-y**) from Philippine *Xestospongia* sp.,[7] after stabilization of the sponge homogenized in phosphate buffer solution by the addition of potassium cyanide. In our

continuing chemical studies on marine natural products, we found a new renieramycin derivative (**1**) from both Philippine[8] and Thai[9,10] *Xestospongia* sp. We report the structure elucidation of compound **1** by means of spectroscopic analysis together with its biological data. We also report an unusual reaction of renieramycin O (**2o**); when exposed to sunlight, **2o** was transformed into compound **1** and renieramycin U (**2u**).

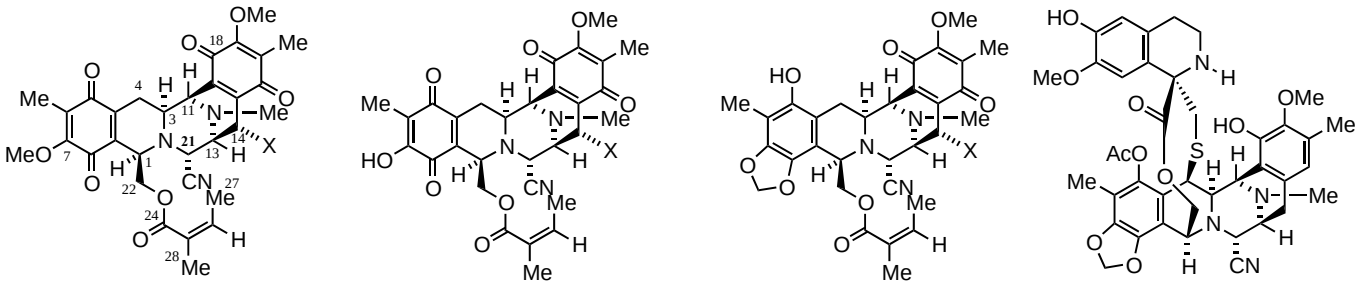

Figure 1. Structures of renieramycin marine natural products

New compound **1**[11] was obtained as a dark yellow amorphous powder. **1** was confirmed to have the molecular formula $C_{30}H_{31}N_3O_9$ by HRFABMS [*m/z* 578.2148 (MH^+)], and to have 16 mass units more than renieramycin S (**2s**). All protons and carbons of **1** were assigned after extensive NMR measurements using COSY, NOESY, HMQC, and HMBC techniques (Table 1). Selected HMBC correlation data confirmed that **1** was 7-demethylrenieramycin O (Fig. 2). The major difference in the ^{13}C NMR spectral data of **1** and **2s** was the downfield shift of the C-14 signal from δ 21.0 (CH_2) for compound **1** to δ 62.0 (CH) ppm for compound **2s**. The ^1H NMR spectrum of **1** was in good agreement with that of **2s** except for the characteristic resonance at δ 4.39 (s, 1H) ppm that was noted in **1** and the two signals of 14-H_2 [δ 2.74 (dd, 1H) and δ 2.31 (d, 1H) ppm] that were observed in **2s**.

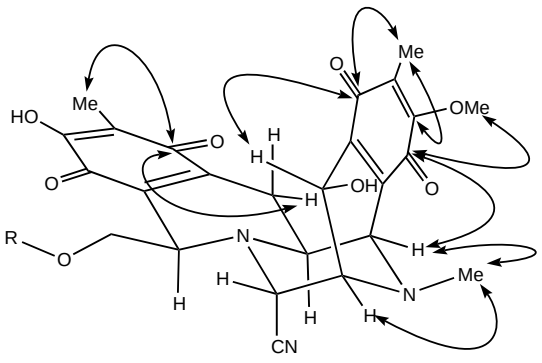

Figure 2. Selected HMBC correlation data of compound **1**

During the course of our research, we observed an unusual phenomenon involving the photochemical transformation of renieramycin O (**2o**) into renieramycin U (**2u**) and compound **1**. Exposing a dilute dichloromethane solution of **2o** to sunlight produced **2u** and **1** in 20.0% and 3.7% yields, respectively.[12] Their spectral data were in complete agreement with those of respective authentic standards. The in vitro IC_{50} cytotoxicity values of compound **1**[13] were much lower than those of renieramycin M and ecteinascidin 770[14-18] (Table 2).

In summary, minor renieramycin derivative **1** was isolated from both Philippine and Thai blue sponge *Xestospongia* sp., and its structure was elucidated by spectroscopic analysis.[19-21] We discovered that along with renieramycin U (**2u**), compound **1** was produced as a minor product by the unusual photochemical transformation of renieramycin O (**2o**). Mechanistic studies to rationalize this transformation are ongoing in our laboratory.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** and renieramycins O and S measured in $CDCl_3$

	compound **1**			renieramycin O (**2o**)[3]			renieramycin S (**2s**)[4]		
Atom No.	δ_C		δ_H	δ_C		δ_H	δ_C		δ_H
1	52.6	CH	3.99 (m)	56.4	CH	3.98 (m)	56.0	CH	4.03 (ddd, 3.1, 2.9)
3	53.3	CH	3.10 (dt, 10.2, 2.5)	53.4	CH	3.05 (ddd, 11.6, 3.3, 2.3)	54.2	CH	3.12 (ddd, 11.3, 3.1, 2.2)
4	25.8	CH_2	2.93 (dd, 17.6, 2,2)	25.3	CH_2	2.87 (dd, 17.2, 2.3)	26.0	CH_2	2.92 (dd, 17.7, 2.2)
			1.28 (m)			1.27 (ddd, 17.2, 11.6, 2.6)			1.40 (ddd, 17.7, 11.3, 2.8)
5	184.7	C		185.4	C		184.8	C	
6	117.2	C		128.6	C		117.2	C	
7	151.1	C		155.6	C		151.1	C	
8	180.8	C		180.8	C		180.9	C	
9	135.7	C		135.7	C		133.5	C	
10	141.1	C		141.1	C		144.1	C	
11	55.0	CH	4.12 (dd, 3.1, 1.1)	55.0	CH	4.09 (dd, 3.3, 1.3)	54.2	CH	3.99 (dd, 3.1, 1.0)
13	62.3	CH	3.46 (br s)	62.4	CH	3.42 (br s)	54.5	CH	3.38 (ddd, 7.7, 2.5, 1.0)
14	62.0	CH	4.39 (s)	62.0	CH	4.37 (s)	21.0	CH_2	2.74 (dd, 21.1, 7.7)
									2.31 (d, 21.1)
15	184.3	C		187.8	C		185.8	C	
16	128.6	C		128.4	C		128.6	C	
17	155.6	C		155.8	C		155.2	C	
18	182.7	C		182.8	C		182.5	C	
19	135.3	C		135.3	C		135.0	C	
20	141.0	C		141.0	C		142.1	C	
21	56.2	CH	4.23 (d, 2.8)	56.4	CH	4.23 (d, 2.6)	58.3	CH	4.09 (d, 2.5)
22	61.5	CH_2	4.65 (dd, 11.7, 2.8)	62.1	CH_2	4.53 (dd, 11.6, 3.0)	61.4	CH_2	4.65 (dd, 11.9, 3.1)
			4.03 (dd, 11.7, 2.8)			4.09 (dd, 11.6, 3.4)			4.04 (dd, 11.9, 2.9)
24	166.4	C		166.5	C		166.5	C	
25	126.0	C		126.2	C		126.2	C	
26	141.0	CH	5.99 (qq, 7.1, 1.4)	140.6	CH	5.98 (qq, 7.3, 1.2)	140.8	CH	5.96 (qq, 7.4, 1.5)
27	15.8	Me	1.83 (dq, 7.1, 1.6)	15.7	Me	1.82 (dq, 7.3, 1.3)	15.8	Me	1.82 (dq, 7.4, 1.4)
28	20.3	Me	1.57 (dq, 1.6, 1.4)	20.3	Me	1.57 (dq, 1.7, 1.3)	20.4	Me	1.57 (dq, 1.5, 1.4)
6-Me	8.1	Me	1.95 (s)	8.7	Me	1.94 (s)	8.1	Me	1.94 (s)
16-Me	8.4	Me	1.92 (s)	8.4	Me	1.92 (s)	8.5	Me	1.90 (s)
7-OMe	---		---	61.1	Me	4.03 (s)	---		---
17-OMe	61.1	Me	4.03 (s)	61.1	Me	4.02 (s)	60.9	Me	3.98 (s)
N-Me	42.4	Me	2.48 (s)	42.4	Me	2.46 (s)	41.5	Me	2.27 (s)
CN	116.2	C		116.3	C		116.8	C	

Table 2. Cytotoxicities of renieramycins to two carcinoma cell lines (IC_{50} nM)

Carcinoma cell line	sample		
	compound **1**	renieramycin M	ecteinascidin 770[14-18]
HCT116 (human colon carcinoma)	1100 ± 45	16.0 ± 1.7	3.3 ± 0.6
DU145 (human prostate carcinoma)	1040 ± 98	5.3 ± 0.5	5.2 ± 1.8

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REFERENCES AND NOTES

1. A. Kubo and N. Saito, 'Studies in Natural Products Chemistry,' Vol. 10, ed. by Atta-ur-Rahman, Elsevier, Inc., Amsterdam, 1992, pp. 77-145.
2. J. D. Scott and R. M. Williams, *Chem. Rev.*, 2002, **102**, 1669.
3. K. Suwanborirux, S. Amnuoypol, A. Plubrukarn, S. Pummangura, A. Kubo, C. Tanaka, and N. Saito, *J. Nat. Prod.*, 2003, **66**, 1441.
4. S. Amnuoypol, K. Suwanborirux, S. Pummangura, A. Kubo, C. Tanaka, and N. Saito, *J. Nat. Prod.*, 2004, **67**, 1023.
5. N. Daikuhara, Y. Tada, S. Yamaki, K. Charupant, S. Amnuoypol, K. Suwanborirux, and N. Saito, *Tetrahedron Lett.*, 2009, **50**, 4276.
6. N. Saito, M. Yoshino, K. Charupant, and K. Suwanborirux, *Heterocycles*, 2012, **84**, 309.
7. M. Tatsukawa, L. L. C. Punzalan, H. D. S. Magpantay, I. M. Villaseñor, G. P. Concepcion, K. Suwanborirux, M. Yokoya, and N. Saito, *Tetrahedron*, 2012, **68**, 7422.
8. The Philippine blue sponge *Xestospongia* sp. (840 g, wet weight) was collected by scuba diving in the vicinity of Puerto Galera, Oriental Mindorou, Mindoro Island at depths of 3-5 m on 29 October 2011. In accordance with our published extraction procedure,[7] we obtained the EtOAc extract (334.4 mg) of the sponge after pretreatment with KCN in pH 7 buffer. This extract was subjected to silica gel column chromatography to give new renieramycin-type compound **1** (2.8 mg) along with five known renieramycins M (**2m**: 8.8 mg), O (**2o**: 4.5 mg), S (**2s**: 7.0 mg), T (**2t**: 0.9 mg), and U (**2u**: 2.5 mg).
9. For details of the 2013 recollection (9 kg, wet weight) of the Thai sponge *Xestospongia* sp., see ref. 10. Renieramycin M (**2m**: 1.2 g, 0.01% yield based on sponge wet weight) was isolated from the EtOAc extract (18.8 g). After removing crystals of **2m** from the fractions, the combined mother liquor was concentrated in vacuo to give a residue (1.87 g), which was subjected to flash silica gel column chromatography to yield compound **1** (0.8 mg) along with seven known renieramycins M (**2m**: 20.6 mg), O (**2o**, 34.2 mg), R (**2r**: 13.1 mg), Q (**2q**: 1.8 mg), S (**2s**, 3.5 mg), T (**2t**: 5.1 mg), and U (**2u**: 2.7 mg).
10. N. Sirimangkalakitti, S. Chamni, K. Charupant, P. Chanvorachote, N. Mori, N. Saito, and K. Suwanborirux, *J. Nat. Prod.*, 2016, **79**, 2089.

11. Compound **1**: dark yellow amorphous powder, ^{1}H and ^{13}C NMR data, see Table 1: EIMS *m/z* (%) 577 (M^+, 5), 462 (9), 446 (12), 301 (12), 235 (12), 234 (11), 230 (16), 229 (100), 220 (37), 219 (16), 218 (69), 203 (14), 201 (12); HRFABMS *m/z* 578.2148 [$(M+H)^+$, calcd for $C_{30}H_{32}N_3O_9$, 578.2139]; IR (KBr) 3392 br, 2926, 2855, 2380, 1717, 1655, 1456, 1377, 1341, 1306, 1234, 1153, 1084, 1045 cm^{-1}; $[\alpha]_D^{25}$ +239 (*c*, 0.07, $CHCl_3$); CD (*c* 0.016 mmol/L, MeOH, 24 ºC) –1.29 (363), –2.57 (285), –3.07 (280), +3.58 (261), +3.37 (257), +3.05 (242), +3.42 (228), +3.36 (232), +3.37 (221).
12. A solution of renieramycin O (**2o**: 16.7 mg, 0.028 mmol) in CH_2Cl_2 (70 mL) was exposed to sunlight for 3.5 h. The reaction mixture was concentrated in vacuo to give a residue (18.5 mg), which was subjected to flash silica gel column chromatography (CH_2Cl_2/MeOH, 49:1) to give compound **1** (0.6 mg, 3.7%) and renieramycin U (**2u**: 3.4 mg, 20.0%).
13. Cell growth inhibition assay (IC_{50}): A single-cell suspension of each cell line (2 x 10^3 cells/well) was added to the serially diluted test compounds in a microplate. The cells were then cultured for 4 d. Cell growth was measured with a cell counting kit (DOJINDO, Kumamoto, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with the untreated control.
14. K. Suwanborirux, K. Charupant, S. Amnuoypol, S. Pummangura, A. Kubo, and N. Saito, *J. Nat. Prod.*, 2002, **65**, 935.
15. P. Puthongking, C. Patarapanich, S. Amnuoypol, K. Suwanborirux, A. Kubo, and N. Saito, *Chem. Pharm. Bull.*, 2006, **54**, 1010.
16. P. Saktrakulkla, S. Toriumi, M. Tsujimoto, C. Patarapanich, K. Suwanborirux, and N. Saito, *Bioorg. Med. Chem.*, 2011, **19**, 4421.
17. M. Tsujimoto, W. Lowtangkitcharoen, N. Mori, W. Pangkruang, K. Suwanborirux, and N. Saito, *Chem. Pharm. Bull.*, 2013, **61**, 1052.
18. R. Toyoshima, N. Mori, T. Suzuki, W. Lowtangkitcharoen, K. Suwanborirux, and N. Saito, *Chem. Pharm. Bull.*, 2016, **64**, 961.
19. Recently, several new renieramycin-type marine natural products were isolated from the South China Sea nudibranch *Jorunna funebris* and its possible sponge prey *Xestospongia* sp.[20,21]
20. W.-F. He, Y. Li, M.-T. Feng, M. Gavagnin, E. Mollo, S.-C. Mao, and Y.-W. Guo, *Fitoterapia*, 2014, **96**, 109.
21. R.-Y. Huang, W.-T. Chen, T. Kurtán, A. Mándi, J. Ding, J. Li, X.-W. Li, and Y.-W. Guo, *Future Med. Chem.*, 2016, **8**, 17.