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Chemistry of renieramycins. Part 13: Isolation and structure of stabilized renieramycin type derivatives, renieramycins W–Y, from Philippine blue sponge *Xestospongia* sp., pretreated with potassium cyanide

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ABSTRACT

Three new bistetrahydroisoquinoline marine natural products, renieramycins W (**1w**), X (**1x**), and Y (**1y**), along with two known renieramycins M (**1m**) and T (**1t**), were isolated from the pretreated Philippine blue sponge *Xestospongia* sp. with KCN and their structures were elucidated by comparing their spectral data with those of **1m**, **1t**, and *N*-acetylsafracin B (**11**). Renieramycins W (**1w**) and X (**1x**) are the first examples of tiglic acid ester derivatives at the C-1 side chain. Renieramycin Y (**1y**) possesses a characteristic substitution pattern in A-ring and isolation of it from marine organism strongly evidences to link the possible precursor 3-hydroxy-5-methyl-*O*-methyltyrosine with both renieramycin and ecteinascidin marine natural products.

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1. Introduction

Renieramycins and ecteinascidins are tetrahydroisoquinoline marine natural products that are structurally and biologically related to other isoquinoline natural products, including saframycin, naphthyridinomycin, and quinocarcin antibiotics.[1–3] These tetrahydroisoquinoline natural products are relatively unstable and available in only trace amounts because they are easily decomposed during both extraction and isolation procedures. We solved this problem by converting the original natural products having a relatively unstable α-amino alcohol functional group at C-21 position into stable α-aminonitriles by pretreatment with KCN. We have succeeded in identifying a number of biologically active tetrahydroisoquinolines from several Thai marine organisms, such as the tunicate *Ecteinascidia thurstoni*,[4] the blue sponge *Xestospongia* sp.,[5–7] and the nudibranch *Jorunna funebris*.[8]

In 1989, He and Faulkner suggested that renieramycin E (**1e**) underwent oxidative cleavage to furnish renierone (**2**) and mimosamycin (**3**).[9] We have obtained evidence that **1e** decomposed to produce **2** and **3**.[10] After several attempts, the oxidation of **1e** with selenium oxide and *p*-toluenesulfonic acid in 1,4-dioxane at 80 °C for 14 h was found to be the best choice in terms of yield (**2**: 46.4% and **3**: 43.9%) (Fig. 1). We also found that the selenium oxide oxidation of jorumycin (**4**)[11] furnished renierol acetate (**5**)[12] and **3** in 37.2% and 20.5% yields, respectively. These findings indicate that such simple isoquinoline compounds may be oxidative degradation products and/or artifacts of the isolation procedure.

To our knowledge, no renieramycin-related products have been isolated from Philippine marine organisms. Nevertheless, we are very interested in several Philippine marine organisms because of reports of the discovery of **3** along with simple isoquinoline compounds from them. For example, in 1996, Edrada et al.[13] found a new *N*-ethylene methyl ketone derivative of renierone **6**,[14] which was isolated from an unknown blue Philippine marine sponge belonging to the genus *Xestospongia*, along with three known compounds **2**, **3**, and **7**.[15,16] In addition, new isoquinoline marine natural product **8** was isolated by Rashid et al. from an aqueous extract of the Philippine marine sponge *Haliclona* sp. along with **3** (Fig. 2).[17,18]

As part of our search for new metabolites via the isolation and characterization of biologically active compounds, we have succeeded in finding three new compounds from the Philippine blue sponge *Xestospongia* sp., growing in the vicinity of Puerto Galera,

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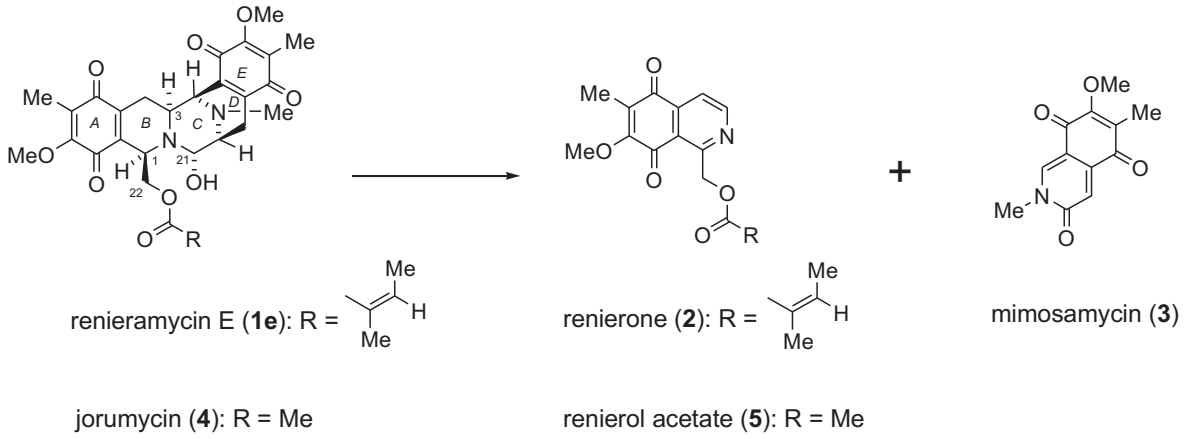

Fig. 1. Oxidative degradation of renieramycin E and jorumycin.

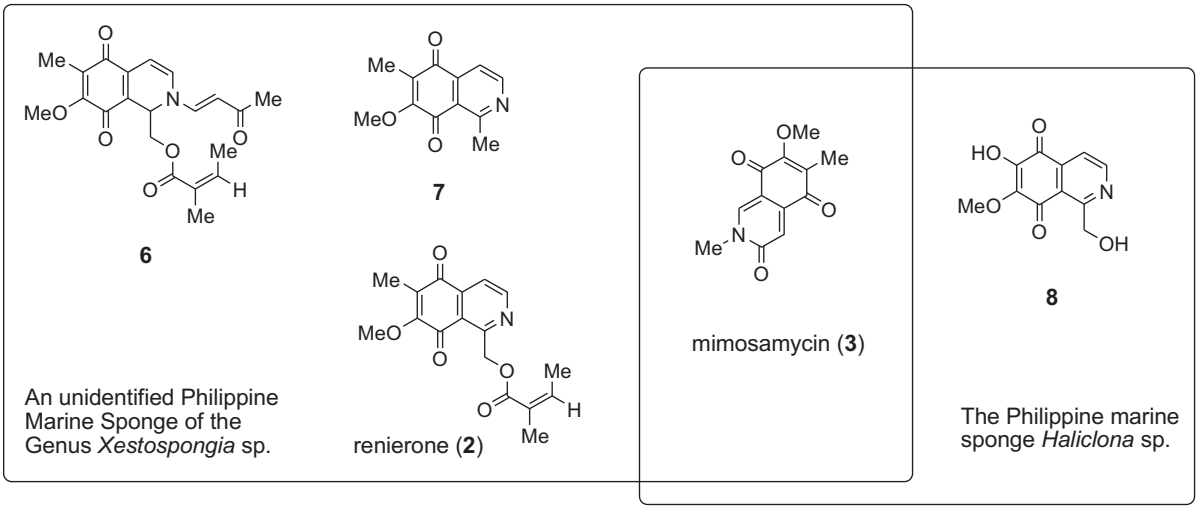

Fig. 2. Simple isoquinoline marine natural products from Philippine marine organisms.

Oriental Mindoro, Mindoro Island. We present here the isolation and structure elucidation of three new stable renieramycins W–Y (**1w**–**y**) along with two known renieramycins M (**1m**) and T (**1t**)[7] from the KCN-pretreated *Xestospongia* sp.

2. Results and discussion

The blue sponge *Xestospongia* sp. (0.2 kg, wet weight) was collected by scuba divers in the vicinity of Puerto Galera, Oriental Mindoro, Mindoro Island (13.50818° N & 120.95877° E) at depths of 3–5 m on 23 September 2011. The collected sponge was homogenized with phosphate buffer solution to adjust the pH to 7. After adding aqueous KCN solution (1.5 mL), the reaction mixture was macerated with methanol and the extract was collected by filtration. The concentrated extract (9.52 g) was partitioned between ethyl acetate and water and the organic layer was concentrated in vacuo to give a residue (560 mg), which was subjected to flash column chromatography on silica gel to give renieramycin M (**1m**) mainly in 33.1% yield based on the weight of the ethyl acetate extract. We also isolated known renieramycin T (**1t**: 0.20% yield based on the weight of the ethyl acetate extract), the identification of which was accomplished by comparing all spectroscopic data (^{1}H NMR, ^{13}C NMR, MS, IR). In addition, we found three new compounds and named them renieramycins W–Y (**1w**–**y**) in 0.25%, 0.20%, and 0.16% yields, respectively.

New compound (**1w**), named renieramycin W, was confirmed to have the molecular formula $C_{31}H_{33}N_3O_8$ on the basis of high-resolution MS, and its molecular formula was the same as that of **1m** (Fig. 3). The ^{1}H and ^{13}C NMR spectral data of **1w** were very similar to those of **1m** except for the signals assigned to the unsaturated tiglic acid ester in **1w** as opposed to those assigned to the angelic acid ester in **1m** (Table 1). The characteristic NMR signals of the tiglate appeared at δ_C 11.9 ppm (2′-CH_3) and δ_H 6.48 ppm (3′-H).[19]

Treatment of jorunnamycin A (**9**) with tigloyl chloride in dichloromethane and DMF at 25 °C for 66 h gave **1w** in 31% yield,[20] which had identical data with those of a natural sample (Chart 1).

Renieramycin X (**1x**) was isolated as a pale yellow amorphous solid. The molecular formula of **1x** was determined to be $C_{31}H_{33}N_3O_8$ by HRFABMS, and was also the same as that of **1m**. Due to the limited availability of the sample, many quaternary carbon signals could not be detected. Comparison of the ^{1}H NMR data of **1x** with those of renieramycin T (**1t**) revealed that the two molecules were identical in that they both had a characteristic pair of doublets at δ 5.86 and 5.92 ppm (J=2 Hz) in addition to the D_2O exchangeable proton signal at δ 4.30 ppm. The presence of only one methoxy proton signal at δ 4.00 ppm indicated that one of the quinone rings should be reduced to form an aromatic ring in **1x**. It was also evidence that the diagnostic homoallylic coupling (approx. 3 Hz) between 1-H and 4-Hβ through five bonds was negligible in **1x** (Table 1).[21] The major differences in the ^{1}H NMR data between **1x** and **1t** were similar to those detected between the unsaturated tiglic acid ester of **1w** and the angelic acid ester of **1m** (Table 1). The characteristic NMR signals of the tiglate in **1x** were detected at δ_C 12.0 ppm (2′-CH_3) and δ_H 6.58 ppm (3′-H).

Renieramycin Y (**1y**) was isolated as a pale yellow solid. The molecular formula of **1y** was determined to be $C_{31}H_{35}N_3O_7$ by HRFABMS,

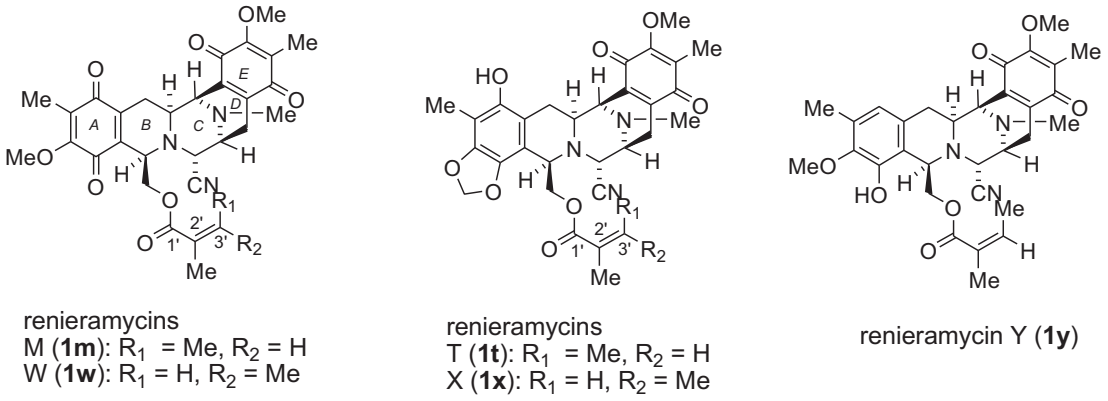

Fig. 3. Structures of new renieramycins W, X, and Y along with known renieramycins M and T.

Table 1
^{1}H and ^{13}C NMR data of renieramycins W (**1w**) and X (**1x**) compared with known renieramycins M (**1m**) and T (**1t**) in $CDCl_3$

Atom	Chemical shift (δ), multiplicity[a] (J in Hz)							
	1m		**1t**		**1w**		**1x**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C[d]	δ_H
1	56.3 d	3.99 (m)[b]	56.4 d	4.16 (dd, 5, 4)	56.4 d	4.00 (m)[b]	56.6 d	4.16 (dd, 5, 3)
3	54.1 d	3.11 (dt, 11, 3)	56.2 d	3.24 (ddd, 12, 3, 2)	54.3 d	3.09 (dt, 11, 2)	56.4 d	3.23 (dt, 12, 3)
4	25.4 t	2.89 (dd, 17, 8)	26.8 t	2.87 (dd, 15, 2)	25.4 t	2.91 (dd, 17, 2)	26.8 t	2.87 (dd, 15, 3)
		1.36 (ddd, 17, 11, 3)		1.67 (dd, 15, 12)		1.31 (ddd, 17, 11, 3)		1.63 (dd, 15, 12)
5	185.4 s		144.7 s		185.5 s		nd	
6	128.6 s		106.2 s		128.4 s		nd	
7	155.8 s		144.9 s		155.7 s		nd	
8	180.9 s		136.8 s		181.0 s		nd	
9	135.7 s		112.1 s		135.6 s		nd	
10	141.3 s		113.1 s		141.6 s		nd	
11	54.2 d	4.01 (dd, 3, 2)	54.9 d	4.00 (dd, 3, 1)	54.2 d	4.01 (dd, 2, 1)	54.9 d	3.99 (dd, 2, 1)
13	54.6 d	3.40 (ddd, 8, 3, 2)	54.8 d	3.37 (ddd, 7, 2, 1)	54.5 d	3.38 (ddd, 8, 3, 1)	54.8 d	3.35 (ddd, 8, 3, 1)
14	21.3 d	2.76 (dd, 21, 8)	21.2 d	2.75 (dd, 21, 7)	21.1 d	2.74 (dd, 21, 8)	21.2 t	2.73 (dd, 21, 8)
		2.30 (d, 21)		2.30 (d, 21)		2.29 (d, 21)		2.27 (d, 21)
15	185.9 s		186.1 s		186.0 s		nd	
16	128.6 s		129.0 s		128.5 s		nd	
17	155.2 s		155.4 s		155.1 s		nd	
18	182.5 s		182.8 s		182.5 s		nd	
19	135.0 s		135.7 s		134.8 s		nd	
20	142.0 s		141.8 s		142.1 s		nd	
21	58.5 d	4.07 (d, 3)	59.8 d	4.11 (d, 2)	58.6 d	4.05 (d, 3)	59.8 d	4.06 (d, 3)
22	62.0 t	4.10 (dd, 12, 3)	64.6 t	3.99 (dd, 11, 5)	62.5 t	4.01 (dd, 12, 3)	65.1 t	3.95 (dd, 11, 5)
		4.53 (dd, 12, 3)		4.41 (dd, 11, 4)		4.59 (dd, 12, 4)		4.41 (dd, 11, 3)
1′	166.5 s		167.1 s		166.9 s		167.1 s	
2′	126.3 s		126.8 s		128.5 s		nd	
3′	140.5 d	5.96 (qq, 7, 2)	139.7 d	6.00 (qq, 7, 2)	137.9 d	6.48 (qq, 8, 1)	139.8 d	6.58 (qq, 7, 1)
4′	15.7 q	1.82 (dq, 7, 2)	15.7 q	1.85 (dq, 7, 2)	14.3 q	1.66 (qq, 8, 1)	14.4 q	1.72 (qq, 7, 1)
2′-CH_3	20.4 q	1.58 (dq, 2, 2)	20.5 q	1.69 (dq, 2, 2)	11.9 q	1.58 (qq, 1, 1)	12.0 q	1.67 (qq, 1, 1)
6-CH_3	8.7 q	1.94 (s)	8.8 q	2.11 (s)	8.8 q[c]	1.93 (s)	8.8 q	2.12 (s)
16-CH_3	8.6 q	1.90 (s)	8.7 q	1.94 (s)	8.7 q[c]	1.96 (s)	8.8 q	1.96 (s)
7-OCH_3	60.9 q[c]	4.02 (s)			61.0 q[c]	4.02 (s)		
OCH_2O			101.1 t	5.85 (d, 2)			101.1 t	5.86 (d, 2)
				5.92 (d, 2)				5.92 (d, 2)
17-OCH_3	61.0 q[c]	3.99 (s)	60.9 q	3.98 (s)	61.1 q[c]	4.01 (s)	61.0 q	4.00 (s)
N-CH_3	41.5 q	2.28 (s)	41.4 q	2.29 (s)	41.5 q	2.27 (s)	41.5 q	2.29 (s)
CN	116.9 s		117.4 s		116.9 s		nd	
5-OH				4.55 (br s)				4.30 (br s)

nd=not detected.
[a] Proton assignments were based on COSY and homonuclear decoupling experiments. Carbon multiplicities were determined on the basis of either DEPT or HMQC data.
[b] The signal overlapped with the methyl singlet.
[c] Assignments are interchangeable.
[d] Carbon resonances were observed through proton resonances by HMQC experiment due to the limited amount of sample available.

and was 14 mass units less than those of **1m** and **1t**. All proton and carbon signals of **1y** were assigned after extensive NMR measurements using COSY, HMQC, and HMBC techniques (Table 2). The molecular formula indicated 16 degrees of unsaturation and the detected ^{13}C carbon resonance attributable to 12 olefinic carbons, three carbonyl groups, and one nitrile carbon in **1y** accounted for 13 degrees of unsaturation. This compound was presumed to have five rings. Observed were the characteristic D_2O exchangeable proton signal at δ 5.80 ppm, two carbonyl resonances (δ 186.2 and 182.8 ppm) indicative of a quinone ring, and an aromatic CH carbonyl resonance (δ 120.9 ppm). These data revealed that **1y** might have one quinone ring and a penta-substituted phenol ring. As the diagnostic homoallylic coupling (approx. 3 Hz) between 1-H and 4-Hβ through five bonds was negligible in **1y**, **1y** might have a *p*-quinone at the E ring. The

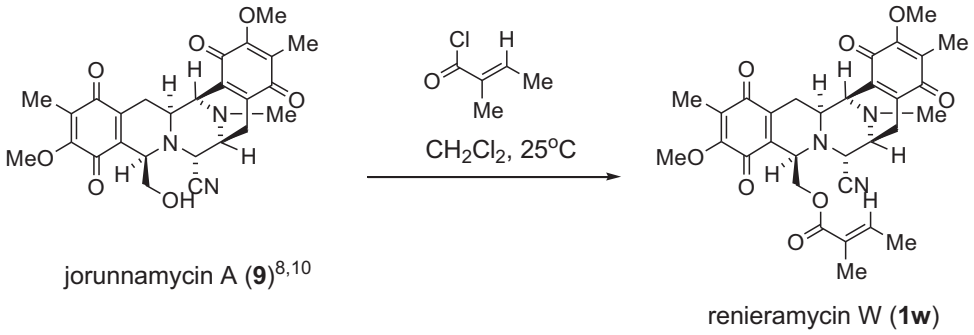

Chart 1. Preparation of renieramycin W (**1w**) from jorunnamycin A (**9**).

Table 2
^{1}H and ^{13}C data of renieramycin Y (**1y**) and related *N*-acetylsafracin B (**11**) in $CDCl_3$

Atom no.	**1y**			*N*-Acetylsafracin B (**11**)[23,a]	
	δ_C	δ_H	HMBC correlation (from C)	δ_C	δ_H
1	56.4 d	4.30 (dd, 6, 3)	3-H, 21-H, 22-Ha, 22-Hb	58.2 d	4.20 (m)
3	56.5 d	3.27 (dt, 12, 3)	1-H, 4-Hα, 4-Hβ, 21-H	50.4 d	
4	33.1 t	2.56 (dd, 15, 3)	5-H, 11-H	25.4 t	2.90 (dd, 17, 4)
		1.95 (dd, 15, 12)			1.50 (ddd, 17, 11, 2.8)
5	120.9 d	6.43 (s)	4-Hα, 4-Hβ, 6-CH3	187.5 s	
6	129.3 s		6-CH_3	128.9 s	
7	143.8 s		5-H, 6-CH_3, 7-OCH_3, 8-OH	156.3 s	
8	145.1 s		1-H, 8-OH	182.3 s	
9	116.7 s		1-H, 4-Hα, 4-Hβ, 5-H, 8-OH	134.1 s	
10	131.7 s		1-H, 4-Hα, 4-Hβ	142.2 s	
11	54.9 d	3.97 (dd, 3, 2)	4-Hα, 4-Hβ, 13-H	53.8 d	4.00 (d, 1.6)
13	54.9 d	3.35 (ddd, 8, 3, 2)	11-H, 14-Hα, 14-Hβ, 21-H	50.1 d	3.15 (dd, 7.7, 2)
14	21.3 t	2.75 (dd, 21,8)	13-H, 21-H	24.8 t	2.85 (dd, 19, 7.7)
		2.28 (d, 21)			2.28 (d, 19)
15	186.2 s		14-Hα, 14-Hβ, 16-CH_3	121.9 d	6.73 (s)
16	129.1 s		16-CH_3	130.9 s	
17	155.3 s		16-CH_3, 17-OCH_3	137.2 s	
18	182.8 s		11-H	144.7 s	
19	135.7 s		11-H, 14-Hα, 14-Hβ	112.1 s	
20	141.9 s		11-H, 13-H, 14-Hα, 14-Hβ	118.0 s	
21	60.1 d	4.09 (d, 3)	1-H, 3-H, 13-H, 14-Hα, 14-Hβ	82.0 d	4.40 (d, 2)
22	65.8 t	4.00 (dd, 11, 6)	1-H	42.8 t	2.90 (d, 16)
	4.37 (dd, 11, 3)				3.70 (dd, 16, 4)
1′	167.2 s		22-Ha, 22-Hb, 2′-CH_3, 3′-H	173.1 s	
2′	126.9 s		2′-CH_3, 4′-H_3, 3′-H	50.3 d	3.38 (q, 7)
3′	139.6 d	6.02 (dq,7, 2)	2′-CH_3, 4′-H_3	17.8 q	0.80 (d, 7)
4′	15.8 q	1.87 (dq, 7, 1)	3′-H		
2′-CH_3	20.6 q	1.73 (dq, 2, 1)	3′-H		
6-CH_3	15.7 q	2.24 (s)	5-H	9.6 q	1.86 (s)
7-OCH_3	60.9 q	3.77 (s)		61.4 q	4.03 (s)
16-CH_3	8.7 q	1.94 (s)		16.4 q	2.23 (s)
17-OCH_3	61.0 q	3.99 (s)		60.5 q	3.76 (s)
N-CH_3	41.5 q	2.28 (s)	11-H, 13-H	40.8 q	2.31 (s)
CN	117.7 s		11-H, 21-H		
8-OH	5.80 (s)				

[a] *N*-Acetyl chemical shifts (δ_C) are 22.8 and 175.2 ppm ($COCH_3$).

substituents on phenol ring A were assigned as follows: the chemical shifts of C-7 (δ 143.8 ppm) and C-8 (δ 145.1 ppm) indicated oxygen substitution, and the former was observed to have long-range $^{1}H—^{13}C$ correlations to 6-CH_3 protons (δ 2.24 ppm) and 7-OCH_3 protons (δ 3.77 ppm). The long-range $^{1}H—^{13}C$ correlations of the characteristic 5-H singlet proton (δ 6.43 ppm) to C-4 (δ 33.1 ppm), C-6 (δ 129.3 ppm), C-7, and C-9 (δ 116.7 ppm) revealed the positions of these carbons.

While renieramycin Y (**1y**) is the first example of a penta-substituted phenol at the A-ring, it is well known antitumor antibiotic, safracins A (**10a**)[22] and B (**10b**) have a same phenol at the E-ring[22,23] (Fig. 4). The structure of **1y** was elucidated by comparing its spectral data to those of safracins A, B and cyanosafracin A (**10c**). Safracins possess a penta-substituted phenol at the E-ring, which is the same as that of ecteinascidin 743.[4,24] In Table 2, the ^{1}H and ^{13}C NMR data of **1y** were listed together with those of *N*-acetylsafracin B (**11**),[23] which was prepared from **10b** and acetic anhydride in methanol. As the key EI mass fragment peak of **1y**, *m*/*z* 204^{+} (fragment A), was negligible, both 448^{+} and 218^{+} ion peaks were assigned to fragments B and C, respectively. These data confirm the locations of the *p*-quinone ring and the penta-substituted phenol ring with respect to each other and eliminate the possibility of reversal (Chart 2).

3. Conclusion

Three new bistetrahydroisoquinoline marine natural products, renieramycins W (**1w**), X (**1x**), and Y (**1y**), along with two known renieramycins M (**1m**) and T (**1t**), were isolated from the Philippine blue sponge *Xestospongia* sp. It is much better source to take renieramycin M (**1m**) to an approximately 3-fold increasing in the

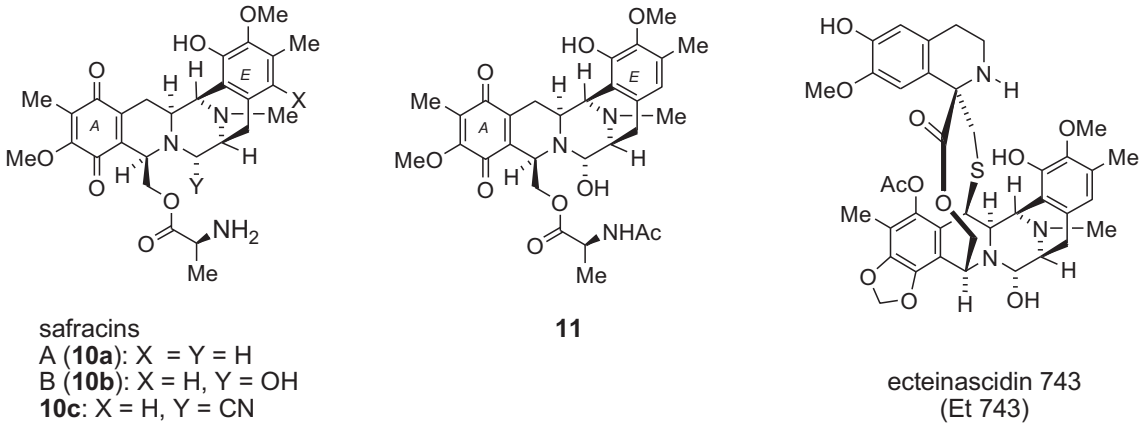

Fig. 4. Structures of safracins and ecteinascidins.

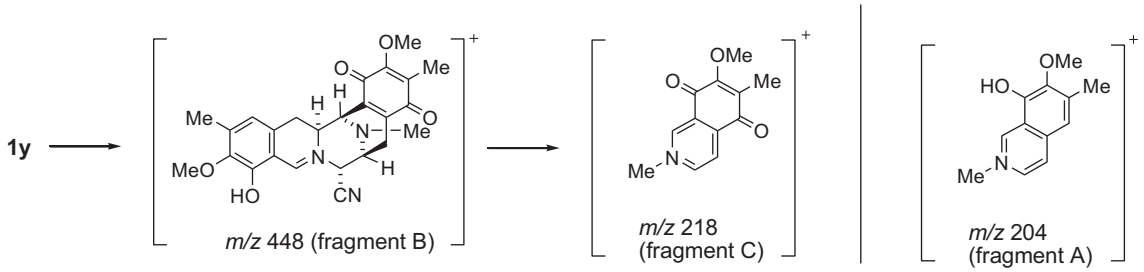

Chart 2. EI mass spectral fragmentation of **1y**.

yields of **1m** from the Thai blue sponge. Both **1w** and **1x** are the first examples of tiglic acid ester derivatives and are the geometrical isomers at the C-1 unsaturated ester of known angelate derivatives **1m** and **1t**, respectively. We also found renieramycin Y (**1y**), which is the first example having a penta-substituted phenol in the A-ring in this series.

One study of the biosynthesis of antitumor isoquinoline natural products has demonstrated that 3-hydroxy-5-methyl-*O*-methyltyrosine, a nonproteinogenic amino acid, may be the precursor of the tetrahydroisoquinoline core.[25] Although several groups have proposed biosynthetic schemes for these natural products,[26–28] no solid experimental evidence has been offered regarding the biosynthetic intermediates or the enzymatic conversion in vitro. In-depth studies of the biological activities of new renieramycins for the evaluation of antitumor activity, and the isolation of an additional amount of **1y**, which is necessary to understand its biosynthetic pathway, are underway (Fig. 5).[29–31]

4. Experimental section

4.1. General experimental procedure

Optical rotations were measured on a Horiba-SEPA polarimeter. Circular dichroism (CD) measurements were carried out on a Jasco 820 spectropolarimeter. IR spectra were obtained with a Shimadzu Prestige 21/IRA Affinity-1 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-JNM-ECA500 FT NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, on a JEOL-JNM-AL400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, and on a JEOL-JNM-AL300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C (ppm, *J* in Hz with TMS as internal standard). All proton and carbon signals were assigned by extensive NMR measurements using COSY, HMBC, and HMQC techniques. Mass spectra were recorded on a JEOL JMS 700 instrument with a direct inlet system operating at 70 eV.

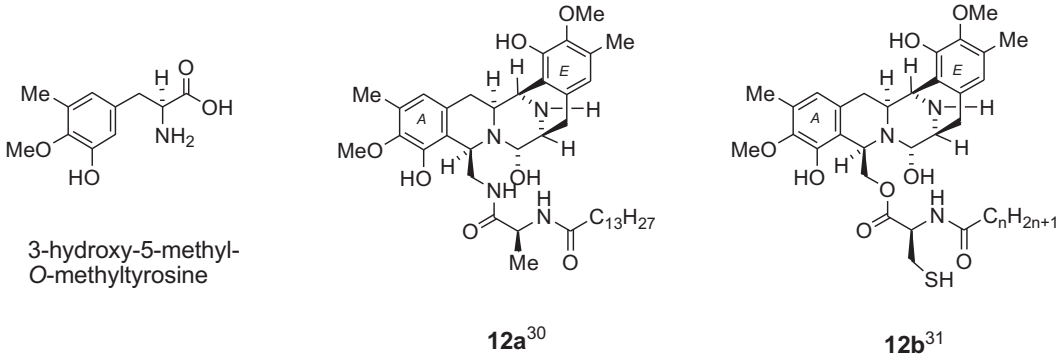

Fig. 5. Structure of the fatty-acid-bound pentacyclic framework that acts as the scaffold of the natural products.

4.2. Animal material

The sponge sample (OMID-312-B) was identified as *Xestospongia* sp. (Family Petrosidae) by Ms. Fleurdeliz Panga and deposited at the University of the Philippines Marine Science Institute Museum (UPMSI). This sponge takes a digitate to fused lobate habit. It grows on live coral or rock at depths of 2–4 m. It has a fine micropunctipore surface. At the tips of the tubes are oscules with diameters of approximately 6 mm. General consistency is hard, although the sponge is brittle and easily crumbles. Fresh specimens are bright blue in color, but turn brown to orange in alcohol. Megascleres are lightly bent oxeas (range: 212.80 μm×7.6 μm–296.40 μm×7.6 μm, average: 234.84 μm×7.6 μm). No microscleres are noted. The skeleton is neither specialized nor distinct apart from a mesh of free oxeas or endings of choasomal tracts. The choasomal skeleton is a reticulation of pauci- to multi-spicular (6–10 spicules) forming polygonal meshes.

4.3. Collection and extraction of *Xestospongia* sponge

The sponge *Xestospongia* sp. was collected by scuba divers of MPMSI in the vicinity of Puerto Galera, Oriental Mindoro, Mindoro Island (13.50818° N & 120.95877° E) at depths of 3–5 m on 23 September 2011 and frozen until used. The collected sponge (0.2 kg, wet weight) was homogenized and phosphate buffer solution was added to the resulting homogenized solution (200 mL) to adjust pH to 7. After 10% potassium cyanide solution (1.5 mL) was added dropwise to the suspension, the reaction mixture was stirred for 5 h. The reaction mixture was macerated with methanol (3×200 mL) and the combined extracts were filtered. The combined filtrates were concentrated in vacuo to give a residue (9.52 g). This residue was diluted with water (150 mL) and extracted with ethyl acetate (300, 200, 100 mL). The combined extracts were washed with brine (200 mL), dried, and concentrated in vacuo to give a residue (560 mg). This residue was subjected to step-gradient silica gel (50 g) column chromatography with a solvent system consisting of 0–100% hexane/ethyl acetate to yield five fractions (fractions A–E). Fraction B (138.1 mg) yielded dark brown crystals and recrystallization from ethyl acetate/ether gave renieramycin M (**1m**: 77.7 mg). The mother liquor was concentrated in vacuo to afford fraction B1 (58.7 mg). Fraction B1 was combined with fraction C (84.9 mg) and the whole was chromatographed on a silica gel column (CH_2Cl_2/MeOH=250:1) to give **1m** (fraction F1: 106.8 mg, total 184.5 mg, 33.1% yield based on the weight of the ethyl acetate extract) and fraction F2 (6.7 mg). Fraction F2 was subjected to silica gel column chromatography (hexane/ethyl acetate=5:1) to give renieramycin W (**1w**, 1.4 mg, 0.25% yield based on the weight of the ethyl acetate extract) and renieramycin X (**1x**, 1.1 mg, 0.20% yield based on the weight of the ethyl acetate extract). Fraction D (5.0 mg) was subjected to silica gel column chromatography (hexane/ethyl acetate=5:1) to give renieramycin Y (**1y**, 0.9 mg, 0.16% yield based on the weight of the ethyl acetate extract) and renieramycin T (**1t**, 1.1 mg, 0.20% yield based on the weight of the ethyl acetate extract).

4.4. Renieramycin M (1m)

Mp 196.5–197 °C (lit.,[5] mp 194.5–197 °C); $[\alpha]_D^{25}$ −49.5 (*c* 0.2, $CHCl_3$); CD Δ ε (*c*: 60 μM, methanol, 22 °C) −4.5 (355), −1.6 (304), −10.3 (281), +8.9 (257), −3.7 (228), +6.2 (211); IR (KBr) 3441, 2922, 2851, 2230w, 1713, 1699, 1655, 1616, 1449, 1412, 1375, 1348, 1312, 1234, 1190, 1152, 1103, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 575 (M^+, 9), 260 (12), 243 (8), 221 (21), 220 (100), 219 (14), 218 (23); EIHRMS *m*/*z* 575.2269 (M^+, calcd for $C_{31}H_{33}N_3O_8$, 575.2268).

4.5. Renieramycin T (1t)

$[\alpha]_D^{21}$ −19.5 (*c* 0.04, $CHCl_3$); CD Δ ε (*c*: 52 μM, methanol, 22 °C) −4.8 (345), +7.1 (268), −0.2 (235), +9.6 (212); IR (KBr) 3435, 2926, 2855, 1713, 1653, 1616, 1460, 1437, 1375, 1308, 1234, 1190, 1152, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; FABMS *m*/*z* 576 $[M+H]^+$; HRFABMS *m*/*z* 576.2350 (M^++1, calcd for $C_{31}H_{34}N_3O_8$, 576.2346).

4.6. Renieramycin W (1w)

$[\alpha]_D^{25}$ −55.1 (*c* 0.03, $CHCl_3$); CD Δ ε (*c*: 63 μM, methanol, 22 °C) −2.2 (353), −1.1 (306), −5.1 (281), +3.0 (257), −1.4 (228), +2.3 (206); IR (KBr) 3429, 2941, 2852, 1710, 1645, 1616, 1449, 1375, 1312, 1263, 1234, 1190, 1151, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; FABMS *m*/*z* 576 $[M+H]^+$; HRFABMS *m*/*z* 576.2341 (M^++1, calcd for $C_{31}H_{34}N_3O_8$, 576.2346).

4.7. Transformation of jorunnamycin A (9) into renieramycin W (1w)

A solution of tigloyl chloride was prepared by stirring tiglic acid (45.3 mg, 0.45 mM) in dry ether (2.3 mL) and mixing it with oxalyl chloride (38.1 mL, 0.45 mM) in dry DMF (6.9 μL, 0.082 mM) at 25 °C for 2 h. A solution of **9** (11.0 mg, 22.3 μM) in dichloromethane (1.5 mL) was added to the above acid chloride solution at 0 °C for 5 min and the mixture was concentrated in vacuo with a stream of argon gas to give a residue. Dichloromethane (0.8 mL) was added to the residue and the resulting mixture was stirred at 25 °C for 66 h. After concentration, the crude product was subjected to column chromatography on silica gel (4 g) with ethyl acetate/hexane (1:2) to give **1w** (4.0 mg, 31%) as a pale yellow amorphous powder. CD Δ ε (*c*: 63 μM, methanol, 22 °C) −4.8 (353), −2.2 (306), −12.4 (281), +7.0 (257), −2.3 (228), +4.4 (204); IR (KBr) 3429, 2941, 2852, 1710, 1645, 1616, 1449, 1375, 1312, 1263, 1234, 1190, 1151, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.31 (1H, ddd, *J*=17, 11, 3 Hz, 4-Hβ), 1.58 (3H, dq, *J*=2, 1 Hz, 2′-CH_3), 1.66 (3H, dq, *J*=7, 1 Hz, 4′-H_3), 1.93 (3H, s, 6-CH_3), 1.96 (3H, s, 16-CH_3), 2.27 (3H, s, NCH_3), 2.29 (1H, d, *J*=21 Hz, 14-Hβ), 2.74 (1H, dd, *J*=21, 8 Hz, 14-Hα), 2.91 (1H, dd, *J*=17, 2 Hz, 4-Hα), 3.09 (1H, dt, *J*=11, 2 Hz, 3-H), 3.38 (1H, ddd, *J*=8, 3, 1 Hz, 13-H), 4.00 (3H, s, 17-OCH_3), 4.00 (1H, overlapped, 1-H), 4.01 (1H, dd, *J*=12, 3 Hz, 22-H), 4.01 (1H, dd, *J*=2, 1 Hz, 11-H), 4.02 (3H, s, 7-OCH_3), 4.05 (1H, d, *J*=3 Hz, 21-H), 4.59 (1H, dd, *J*=12, 4 Hz, 22-H), 6.48 (1H, qq, *J*=8, 1 Hz, 3′-H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 8.7 (ArCH_3), 8.8 (ArCH_3), 11.9 (2′-CH_3), 14.3 (C4′), 21.1 (C14), 25.4 (C4), 41.5 (NCH_3), 54.2 (C11), 54.3 (C3), 54.5 (C13), 56.4 (C1), 58.6 (C21), 61.0 (OCH_3), 61.1 (OCH_3), 62.5 (C22), 116.9 (CN), 128.5 (C2′), 128.4 (C6), 128.5 (C16), 134.8 (C19), 135.6 (C9), 137.9 (C3′), 141.6 (C10), 142.1 (C20), 155.1 (C17), 155.7 (C7), 166.9 (C1′), 181.0 (C8), 182.5 (C18), 185.5 (C5), 186.0 (C15); EIMS *m*/*z* 575 (M^+, 12), 243 (8), 221 (15), 220 (100), 219 (15), 218 (26); EIHRMS *m*/*z* 575.2271 (M^+, calcd for $C_{31}H_{33}N_3O_8$, 575.2268).

4.8. Renieramycin X (1x)

$[\alpha]_D^{21}$ −12.5 (*c* 0.04, $CHCl_3$); CD Δ ε (*c*: 52 μM, methanol, 22 °C) −2.4 (348), +1.9 (262), −0.4 (236), +4.8 (209); IR (KBr) 3442, 2926, 2855, 1707, 1653, 1618, 1456, 1379, 1307, 1263, 1236, 1150, 1091 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; FABMS *m*/*z* 576 $[M+H]^+$; HRFABMS *m*/*z* 576.2340 (M^++1, calcd for $C_{31}H_{34}N_3O_8$, 576.2346).

4.9. Renieramycin Y (1y)

$[\alpha]_D^{21}$ +4.2 (*c* 0.05, $CHCl_3$); CD Δ ε (*c*: 52 μM, methanol, 22 °C) −1.9 (355), +2.6 (262), −1.3 (234), +7.9 (207); IR (KBr) 3435, 2941, 1714, 1653, 1616, 1420, 1387, 1373, 1310, 1234, 1152 cm^{-1}; ^1H NMR

($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m*/*z* 561 (M^+, 12), 448 (28), 229 (11), 221 (18), 220 (100), 219 (22), 218 (21); FABMS *m*/*z* 562 $[M+H]^+$; HRFABMS *m*/*z* 562.2562 (M^++1, calcd for $C_{31}H_{36}N_3O_7$, 562.2553).

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Supplementary data

Color picture of Philippine *Xestospongia* sp. along with a map of collecting place is available. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tet.2012.06.067.

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