Chemistry of Renieramycins. Part 3.[1] Isolation and Structure of Stabilized Renieramycin Type Derivatives Possessing Antitumor Activity from Thai Sponge *Xestospongia* Species, Pretreated with Potassium Cyanide

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Renieramycins M (**1m**) and N (**1n**) were isolated from the Thai sponge *Xestospongia* sp., pretreated with potassium cyanide in methanolic buffer solution, and their structures and relative stereochemistries were elucidated on the basis of spectroscopic data. This strategy is the first example of the gram-scale preparation of this series of compounds and presents a potential solution for increasing the gram-scale supply of novel natural products from marine sources.

Introduction

During the past two decades, renieramycins A−I (**1a**−**i**) and cribrostatin 4 (**2**) have been isolated from marine sponges belonging to genera *Reniera*,[2a] *Xestospongia*,[2b] *Haliclona*,[2c] and *Cribrochalina*.[2d] Another compound, jorumycin (**3**), was reported from the nudibranch *Jorunna funebris*.[3] The ring systems of these natural products including their relative stereochemistries were identical with those of the saframycins, which exhibit strong cytotoxicity toward cultured cells and antitumor activity against several experimental tumors.[4] One of the most intriguing problems, however, is that marine natural products are available only in very minute quantities. Therefore, it is difficult to provide a consistent supply of renieramycins for drug development by isolation from marine sources or by total synthesis.[5] Thus, it has been difficult to determine whether the renieramycins have antitumor activities similar to those of saframycins and ecteinascidins.[6] As part of our search for new metabolites via the isolation and characterization of biologically active compounds from Thai marine animals, we found a blue sponge, *Xestospongia* sp., growing around Sichang Island in the Gulf of Thailand. In this paper, we report the isolation and structure elucidation of renieramycins M (**1m**) and N (**1n**) from the Thai sponge *Xestospongia* sp., which was subjected to pretreatment with potassium cyanide.

Results and Discussion

The blue sponge *Xestospongia* sp. was collected by scuba divers in the vicinity of Sichang Island, the Gulf of Thailand, at a depth of 3−5 m in July 1992. Extraction was performed on 15 kg of wet animal using the standard procedure,[2a] followed by LH-20 column chromatography and preparative reversed-phase (C_{18}) chromatography, resulting in the isolation of 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**4**), renierone (**5**), and *N*-formyl-1,2-dihydrorenierone (**6**), each of which was identified by comparison of their spectroscopic data with those of the authentic standard.[2a,7] The more polar fraction was purified by silica gel, Sephadex LH-20, and preparative reversed-phase (C_{18}) chromatography, resulting in the isolation of renieramycins J (**1j**), K (**1k**), and L (**1l**) along with two known compounds, mimosamycin (**7**)[2a] and the *N*-ethylenemethyl ketone derivative of dihydrorenierone (**8**).[8]

Three new renieramycins (**1j**−**l**) were isolated, and their NMR data were very characteristic of renieramycin type marine natural products. Renieramycin J (**1j**) was obtained as a yellow amorphous powder. From HREIMS data, the molecular formula was found to be $C_{33}H_{38}N_2O_9$. This formula indicated that **1j** had three additional carbon atoms and five additional hydrogen atoms as compared to renieramycin E (**1e**). All protons and carbons were assigned after extensive NMR measurements using COSY, NOESY, HMQC, and HMBC techniques. Comparison of the ^{1}H NMR data of **1j** with those of renieramycin E (**1e**) revealed that the two molecules were almost identical except for ^{1}H NMR signals at δ 2.19 (s, 3H), 2.35 (d, 1H, J = 16.5), and 3.44 (m, 1H) ppm. These resonances have been attributed to the presence of a 2-propanone unit and the absence of a hydroxyl signal in the spectra of renieramycin J. Furthermore, the 21-H signal at δ 3.41 of **1j** was shifted upfield relative to the corresponding signal (δ 4.43 ppm) in **1e**. The ^{13}C NMR spectral data of **1j** revealed a signal at δ 58.9 ppm for the C-21 carbon, which was shifted upfield as compared to that of **1e** at δ 82.4 ppm, as well as additional signals for the 2-propanone unit [δ 38.6 (CH_2), 208.1 (CO), 30.9 (CH_3) ppm]. From these data, the structure of renieramycin J was deduced to be **1j**. An observable NOE between 14-Hβ (δ 2.26 ppm) and 21-H revealed the relative stereochemistry at C-21.

Renieramycin K (**1k**: $C_{34}H_{40}N_2O_{10}$) was obtained as a yellow amorphous powder, and its structure was elucidated mainly by interpretation of NMR and MS data as well as through comparison of its spectral data with that of **1j**. The ^{1}H and ^{13}C NMR spectra of **1k** were in good agreement with those of **1j** except for signals indicating the presence of the OCH_3 group (δ_H 3.51 and δ_C 60.4 ppm) at C-14. It was therefore concluded that the structure of renieramycin K (**1k**) is 14-methoxyrenieramycin J. The stereochemistry

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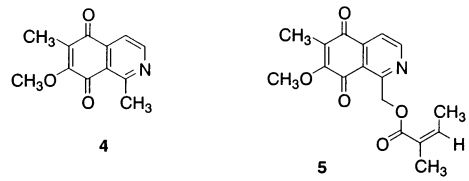
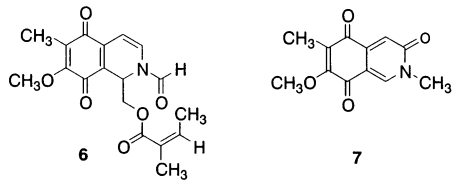
of C-14 revealed an H-14 proton [δ 3.82 ppm (1H, s)] orthogonal to the H-13 proton [δ 2.98 ppm (1H, br s)].

Renieramycin L (**1l**: $C_{33}H_{38}N_2O_{10}$) was obtained as a pale yellow amorphous powder. The molecular formula indicated that **1l** has one more oxygen atom than **1j**. The molar extinction coefficient at maximum UV absorption was reduced to one-half in **1l** as compared to that in the bisquinones (such as **1j**), and only two carbonyl signals (δ 185.9 and 180.9 ppm) of the quinone rings were observed in the ^{13}C NMR spectrum. These data revealed that in renieramycin L one of the quinone rings might have been reduced to form an aromatic ring as that of cribrostatin 4. The ^{13}C NMR spectral data of **1l** revealed the absence of a C-14 signal (δ 23.5 ppm in **1j**) and the presence of a carbonyl signal (δ 203.4 ppm). The ^{1}H NMR spectrum contained two arylmethyl resonances (δ 1.87 and 2.13 ppm), one *N*-methyl signal (δ 2.31 ppm), two arylmethoxyl resonances (δ 3.97 and 3.82 ppm), and resonances for one angelic acid ester moiety [δ 1.31 (3H), 1.71 (3H), and 5.82 (1H) ppm], one 2-propanone moiety [δ 2.21 (s, 3H), 2.51 (1H, dd), and 3.44 (1H, dd) ppm], and the additional two characteristic OH protons [δ 5.42 (18-OH) and 11.81 (15-OH) ppm]. From the data obtained from the diagnostic homoallylic coupling (1.8 Hz) between 1-H and 4-Hβ through five bonds (H−C^4−C^{10}−C^9−C^1−H), the quinone ring of **1l** was concluded to be ring A. These compounds are already known as saframycins D,[9a] F,[9b] and cribrostatin 4 (= revised structure of renieramycin H).[2d]



4 **5**



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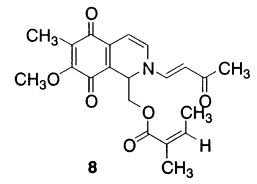

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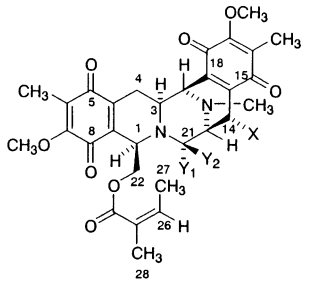
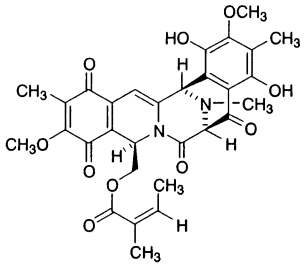
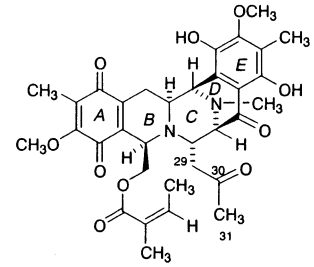
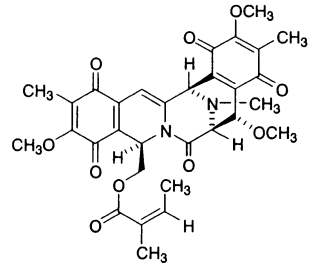




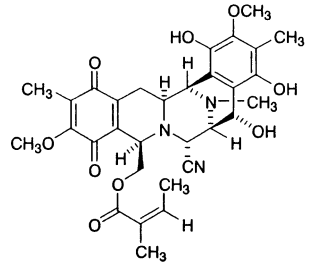
renieramycins
A (**1a**): X = OH, Y_1, Y_2 = H_2
B (**1b**): X = OC_2H_5, Y_1, Y_2 = H_2
C (**1c**): X = OH, Y_1, Y_2 = O
D (**1d**): X = OC_2H_5, Y_1, Y_2 = O
E (**1e**): X = Y_2 = H, Y_1 = OH
F (**1f**): X = OCH_3, Y_1 = OH, Y_2 = H
G (**1g**): X = H, Y_1, Y_2 = O
J (**1j**): X = Y_2 = H, Y_1 = CH_2COCH_3
K (**1k**): X = OCH_3, Y_1 = CH_2COCH_3, Y_2 = H
M (**1m**): X = Y_2 = H, Y_1 = CN
O (**1o**): X = OH, Y_1 = CN, Y_2 = H

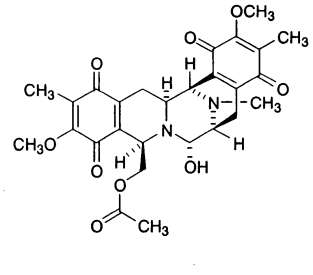
renieramycin H (**1h**)
(= cribrostatin 4: **2**)





renieramycin L (**1l**) renieramycin I (**1i**)





renieramycin N (**1n**) jorumycin (**3**)

One interesting feature in the structures of renieramycins J−L is that they are the first examples of alkylated analogues of renieramycins at the C-21 position, and strong evidence to support the proposed structures was obtained by comparing the 2-propanoyl carbon signals with the CDE ring model compound (**12**).[10]

Thus, we have succeeded in discovering a new marine source of renieramycins, *Xestospongia* sp., in Thailand, although the problem of isolating unprecedented groups of marine natural products, which are prone to be missed by conventional extraction procedures, still remains. Furthermore, it is difficult to rule out the possibility that renieramycins J−L are artifacts resulting from solvent exchange during separation with acetone. The probable mechanistic pathway for the formation of these compounds from **9** (or **10**) is shown in Figure 1.

These results indicate that the precursor of **1j** should be designated as renieramycin E (**1e**), which has a carbinolamine group that may be relatively unstable during the isolation procedure. To obtain sufficient quantities of more stable derivatives with retention of bioactivity, we attempted to stabilize **1e** by converting it into renieramycin M (**1m**) by the addition of potassium cyanide to the homogenized sponge during workup. Extraction of the sponge (8.4 kg wet weight) pretreated with potassium cyanide afforded the crude extract, which was subjected to a solvent partition to give a crude fraction (16.2 g). This fraction was subjected to trituration with methanol to give the precipitate of renieramycin M (**1m**). Silica gel chromatography of the filtrate gave **1m** and renieramycin N (**1n**). This strategy has been realized for the gram-scale production of renieramycin M (**1m**).

Renieramycin M (**1m**) was isolated as dark yellow prisms (mp 194.5−197 °C). The molecular formula of **1m** was determined to be $C_{31}H_{33}N_3O_8$ by EIMS and elemental analysis. The ^{1}H chemical shifts and the proton coupling

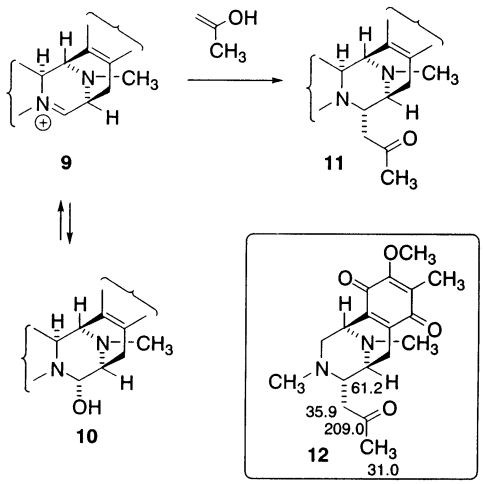


Figure 1.

patterns of **1m** were similar to those of renieramycin E (**1e**). The ^{13}C NMR spectral data of **1m** and **1e** revealed only two major differences. The signal of the α-aminonitrile-containing carbon (C-21) of **1m** was shifted upfield to δ 58.5 ppm compared to that of **1e** at δ 82.4 ppm, and an additional carbon resonance was present at δ 116.9 (21-CN). An observable NOE between 14-Hβ and 21-H revealed the relative stereochemistry at C-21. From these data, the structure of this compound was deduced to be **1m**. Renieramycin M (**1m**) was easily transformed into **1e** in 69% yield by treatment with silver nitrate and was identical with data of an authentic standard in all respects.[2a]

Renieramycin N (**1n**) was isolated as pale yellow prisms (mp 162.5−164 °C). The molecular formula of **1n** was determined to be $C_{31}H_{35}N_3O_9$ by EIMS and elemental analysis, and it was 18 mass units more than **1m**. The extinction coefficient at maximum UV absorption was reduced to one-half in **1n** compared to that in the bisquinones (such as **1m**), and only two carbonyl resonances (δ 183.6 and 179.1 ppm) of the quinone rings were observed in the ^{13}C NMR spectrum. These data revealed that in renieramycin N one of the quinone rings might also have been reduced to form an aromatic ring. Renieramycin N (**1n**) was sensitive to light and oxygen, and to pH above 7. It was rapidly oxidized to afford the corresponding bisquinone (renieramycin O: **1o**) in 69% yield. The major differences in the ^{13}C NMR spectral data of **1o**, in comparison to that for **1m**, were the downfield shift of the C-14 signal from δ 21.3 (CH_2) to δ 62.0 (CH) ppm. The ^1H NMR spectrum of **1o** was in good agreement with that of **1m** except for the two signals at 14-H_2 [δ 2.76 (dd, 1H) and 2.31 (d, 1H) ppm] that were absent and a unique resonance at δ 4.37 ppm that was observable. The structure of renieramycin O (**1o**) was therefore concluded to be 14-hydroxyrenieramycin M.[11,12]

The isolation of renieramycins using the above procedure is advantageous because it provides for a practical way to isolate and purify these types of labile compounds. It is significant to note that we did not obtain any monomeric isoquinolines (such as **4**−**8**) from the producing sponge that was pretreated with potassium cyanide. We believe that these results provide support for the hypothesis that all of the monomeric isoquinolines are degradation products and/or artifacts of isolation procedures.

The in vitro IC_{50} values of these compounds were almost equal to those of saframycin A and ecteinascidin 770 (Table 1).

Table 1. Cytotoxicity of Renieramycins M−O (**1m**−**o**) against Several Cancer Cell Lines[a]

	cell line			
	HCT116	QG56	NCI-H460	DLD1
renieramycin M	7.9	19	5.9	9.6
renieramycin N	5.6	11	6.7	5.7
renieramycin O	28	40	NT	NT
saframycin A	0.4	5.5	2.1	0.6
ecteinascidin 770	1.2	3.9	0.64	2.4

[a] HCT116 = human colon carcinoma; QG56 = human lung carcinoma; NCI-H460 = human lung carcinoma; DLD1 = human colon carcinoma; values reported are IC_{50} in nM. NT: not tested.

In conclusion, we have succeeded in isolating renieramycin M (**1m**) along with renieramycin N (**1n**) from the *Xestospongia* sp. that was pretreated with potassium cyanide. The addition of potassium cyanide for consistency to the homogenized sponge resulted in an approximately 100-fold increase in the yields of the renieramycins and provided for a more consistent and reliable protocol. Details of the biological activities of renieramycins for evaluation of antitumor activity and the isolation and structure elucidation of other minor compounds are under way.[13]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Horiba-SEPA. CD was obtained on a JABCO J-720WI. All melting points were determined using a Yanagimoto micromelting point apparatus and were uncorrected. IR spectra were obtained on a Hitachi 260-10 spectrophotometer. UV spectra were determined with a Hitachi 340. The ^1H and ^{13}C NMR spectra were recorded at 500 and 125.65 MHz, respectively, on a JEOL-JNM-LA 500 FT-NMR spectrometer and at 200 and 50 MHz, respectively, on a Bruker Advance DPX-200 ET-NMR spectrometer (ppm, *J* in Hz with TMS as internal standard). Mass spectra were recorded on JMS-DX 302 and JMS-700 instruments with a direct inlet system operating at 70 eV. Elemental analyses were conducted on a Perkin-Elmer model 240B and a Yanaco MT-6 CHN CORDER elemental analyzer.

Animal Material. The sponge sample (QMG306998) was identified as *Xestospongia* sp. #2133 (family Petrosiidae) by Dr. John N. A. Hooper and deposited at Queensland Museum, Australia. This sponge exhibits thick, encrusted, lobate growth. Its texture is hard, brittle, and easily crumbled. It is light bluish-gray when alive and pinkish in ethanol. Oscules are numerous and of moderate size and are found, on apexes of surface lobes, with slightly raised lips. The surface has prominent bulbous surface lobes with some that are nearly digitate in size. The surface is translucent, membranous, optically smooth, macroscopically bulbous, and microscopically even, with choanosomal drainage canals that are slightly visible below the surface. Ectosomal skeleton membranes have no specialized speculation or structure. The choanosomal skeleton with isotropic reticulation of paucispicular tracts of oxeas forms tight oval meshes. Many free oxeas are scattered between tracts. Small to moderately sized subdermal cavities are observed throughout skeleton. There are no visible fibers and only small amounts of collagen in the mesophyll. The oxeas are robust, straight or slightly curved at the center, sharply pointed, and hastate (190−210 × 12−18 μm). This sponge is probably a new species.

Collection and Extraction of *Xestospongia* Sponge. The sponge *Xestospongia* sp. was collected by scuba divers in the vicinity of Sichang Island at a depth of 3−5 m in July 1992 and frozen until used. The collected sponge (15 kg, wet weight) was homogenized and extracted with methanol and then partitioned with CH_2Cl_2 followed by hexane and methanol to give the methanolic extract (57 g). Silica gel chromatography (solvent gradient from hexane to ethyl acetate, and finally methanol) provided two primary fractions. The more lipophilic

fraction (2.0 g) was consequently chromatographed by a silica gel column (15% ethyl acetate/hexane), Sephadex LH-20 column (hexane/ethyl acetate/methanol = 85:10:5), resulting in the isolation of 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**4**, 10 mg), renierone (**5**, 30 mg), and *N*-formyl-1,2-dihydrorenierone (**6**, 35 mg), each of which was identified by comparing their spectroscopic data with those of the authentic standard.[2a,7] The more polar fraction (6.0 g) was purified by a silica gel column (25% acetone/CH_2Cl_2), a Sephadex LH-20 column (25% ethyl acetate/hexane), a silica gel column (25% acetone/hexane), and preparative reversed-phase (C_{18}) chromatography (75% methanol/water), resulting in the isolation of renieramycins J (**1j**, 2 mg), K (**1k**, 7 mg), and L (**1l**, 30 mg) along with two known compounds, mimosamycin (**7**, 35 mg)[2a] and the *N*-ethylenemethyl ketone derivative of dihydrorenierone (**8**, 5 mg).[8]

Renieramycin J (1j): yellow amorphous powder; IR (film) 1720, 1660 cm^{-1}; UV λ_{max} (log ϵ) 273 (4.32), 370 (3.00) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.93 (1H, qq, J = 7.3, 1.6 Hz, 26-H), 4.16 (1H, dd, J = 11.0, 2.7 Hz, 1-CH), 4.09 (1H, dd, J = 11.0, 3.4 Hz, 1-CH), 4.00, 3.96 (each 3H, s, OCH_3), 3.86 (1H, br s, 11-H), 3.81 (1H, br s, 1-H), 3.44 (1H, m, 21-CH), 3.41 (1H, m, 21-H), 2.90−2.93 (2H, dd, m, 13-H and 3-H), 2.82 (1H, br d, J = 16.8 Hz, 4-Hα), 2.74 (1H, dd, J = 20.8, 7.3 Hz, 14-Hα), 2.35 (1H, d, J = 16.5 Hz, 21-CH), 2.26 (1H, d, J = 20.8 Hz, 14-Hβ), 2.19 (3H, s, $COCH_3$), 2.16 (3H, s, NCH_3), 1.90, 1.90 (each 3H, s, 6-CH_3 and 16-CH_3), 1.79 (3H, dq, J = 7.3, 1.6 Hz, 26-CH_3), 1.59 (3H, dq, J = 1.6 Hz, 25-CH_3), 1.25 (1H, ddd, J = 16.8, 11.2, 2.0 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 208.1 (COCH_3), 186.7 (C-15), 185.6 (C-5), 182.9 (C-18), 181.2 (C-8), 167.0 (C-24), 156.0 (C-7), 155.1 (C-17), 143.4 (C-20), 141.6 (C-10), 139.4 (C-26), 137.2 (C-9), 135.0 (C-19), 128.7 (C-6), 128.0 (C-16), 126.8 (C-25), 64.6 (C-22), 60.8 (OCH_3), 60.8 (OCH_3), 58.9 (C-21), 55.1 (C-13), 55.1 (C-11), 53.5 (C-1), 52.3 (C-3), 41.7 (NCH_3), 38.6 (C-29), 30.9 (C-31), 25.5 (C-4), 23.1 (C-14), 20.3 (C-28), 15.5 (C-27), 8.6 (6-CH_3), 8.6 (16-CH_3); EIMS m/z (%) 606 (M^+, 42), 493 (75), 388 (20), 290 (30), 272 (51), 220 (100); HREIMS m/z 606.2582 [M^+] (calcd for $C_{33}H_{38}N_2O_9$, 606.2577).

Renieramycin K (1k): yellow amorphous powder; IR (film) 1720, 1660 cm^{-1}; UV λ_{max} (log ϵ) 271 (4.40), 372 (3.11) nm; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.92 (1H, qq, J = 7.3, 1.4 Hz, 26-H), 4.12 (1H, dd, J = 11.1, 3.4 Hz, 1-CH), 3.98 (3H, s, OCH_3), 3.94 (1H, m, 11-H), 3.92 (3H, s, OCH_3), 3.90 (1H, dd, J = 11.1, 3.4 Hz, 1-CH), 3.82 (1H, s, 14-H), 3.79 (1H, ddd, J = 3.4, 3.4, 1.9 Hz, 1-H), 3.51 (3H, s, 14-OCH_3), 3.47 (1H, m, 21-H), 3.43 (1H, m, 21-CH), 2.98 (1H, br s, 13-H), 2.82 (1H, m, 3-H), 2.79 (1H, m, 4-Hα), 2.35 (1H, br d, J = 15.6 Hz, 21-CH), 2.34 (3H, s, NCH_3), 2.19 (3H, s, $COCH_3$), 1.91, 1.87 (each 3H, s, 6-CH_3 and 16-CH_3), 1.78 (3H, dq, J = 7.3, 1.4 Hz, 26-CH_3), 1.58 (3H, dq, J = 1.4 Hz, 25-CH_3), 1.12 (1H, ddd, J = 17.0, 11.9, 1.9 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 208.1 (COCH_3), 185.9 (C-15), 185.5 (C-5), 183.4 (C-18), 181.1 (C-8), 167.2 (C-24), 156.0 (C-7), 154.9 (C-17), 141.8 (C-20), 141.5 (C-10), 139.5 (C-26), 137.1 (C-9), 135.0 (C-19), 130.0 (C-6), 128.1 (C-16), 126.7 (C-25), 72.3 (C-14), 65.1 (C-22), 60.9 (OCH_3), 60.8 (OCH_3), 60.4 (OCH_3), 59.0 (C-21), 56.8 (C-1), 55.4 (C-13), 53.4 (C-11), 51.6 (C-3), 42.9 (NCH_3), 38.1 (C-29), 30.9 (C-31), 25.1 (C-4), 20.3 (C-28), 15.5 (C-27), 8.7 (6-CH_3), 8.7 (16-CH_3); EIMS m/z (%) 636 (M^+, 11), 533 (9), 372 (100), 279 (67); HREIMS m/z 636.2688 [M^+] (calcd for $C_{34}H_{40}N_2O_{10}$, 636.2672).

Renieramycin L (1l): pale yellow amorphous powder; IR (film) 3290 (br), 1712, 1655 cm^{-1}; UV λ_{max} (log ϵ) 244 (4.02), 276 (4.07), 375 (3.75) nm; ^1H NMR ($CDCl_3$, 200 MHz) δ 11.81 (1H, s, 15-OH), 5.82 (1H, qq, J = 7.2, 1.4 Hz, 26-H), 5.42 (1H, s, 18-OH), 4.20 (1H, dd, J = 2.7, 0.5 Hz, 11-H), 4.13 (1H, dd, J = 13.0, 3.6 Hz, 1-CH), 3.97 (3H, s, 7-OCH_3), 3.83 (1H, ddd, J = 4.3, 3.6, 1.8 Hz, 1-H), 3.82 (3H, s, 17-OCH_3), 3.82 (1H, dd, J = 13.0, 3.6 Hz, 1-CH), 3.81 (1H, ddd, J = 9.3, 2.1, 1.1 Hz, 21-H), 3.44 (1H, dd, J = 17.2, 9.3 Hz, 21-CH), 3.14 (1H, ddd, J = 10.9, 2.8, 2.7 Hz, 3-H), 3.09 (1H, dd, J = 1.0, 0.5 Hz, 13-H), 2.99 (1H, dd, J = 17.0, 2.8 Hz, 4-Hα), 2.51 (1H, dd, J = 17.2, 2.1 Hz, 21-CH), 2.31 (3H, s, NCH_3), 2.21 (3H, s, $COCH_3$), 2.13 (3H, s, 16-CH_3), 1.87 (3H, s, 6-CH_3), 1.71 (3H, dq, J = 7.2, 1.4 Hz, 26-CH_3), 1.46 (1H, ddd, J = 17.0, 10.9, 1.8 Hz, 4-Hβ), 1.31 (3H, dq, J = 1.4 Hz, 25-CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 207.2 (COCH_3), 203.4 (C-14), 185.9 (C-5), 180.9 (C-8), 167.0 (C-24), 156.1 (C-7), 154.2 (C-15), 152.1 (C-17), 141.5 (C-10), 139.1 (C-26), 138.9 (C-18), 137.0 (C-9), 127.7 (C-6), 126.8 (C-25), 117.9 (C-16), 117.8 (C-17), 112.6 (C-20), 67.6 (C-13), 64.3 (C-22), 60.9 (OCH_3), 60.7 (OCH_3), 57.2 (C-11), 55.9 (C-21), 53.4 (C-1), 51.5 (C-3), 42.6 (NCH_3), 37.5 (C-29), 30.7 (C-31), 24.1 (C-4), 19.8 (C-28), 15.3 (C-27), 8.7 (6-CH_3), 8.5 (16-CH_3); EIMS m/z (%) 622 (M^+, 1), 388 (14), 274 (64), 236 (100); HREIMS m/z 622.2533 [M^+] (calcd for $C_{33}H_{38}N_2O_{10}$, 622.2516).

Simple Isoquinoline Marine Natural Products (4−8). The spectroscopic data of these natural products were identical with those reported in the literature.[2a,7,8]

Collection and Extraction of *Xestospongia* sp. Pretreated with KCN. The sponge *Xestospongia* sp. was collected by scuba divers in the vicinity of Sichang Island at a depth of 3−5 m in December 2001 and frozen until used. The sponge described above (8.4 kg wet weight) was homogenized, and phosphate buffer solution was added to the resulting homogenized solution (9 L) to pH 7. Then, 10% potassium cyanide solution (60 mL) was added dropwise to the suspension, and the mixture was stirred for 5 h. Thereafter, the mixture was macerated with methanol (5 L × 3), the extract was filtered, and the filtrate was concentrated under reduced pressure. The aqueous methanolic solution (800 mL) was partitioned with ethyl acetate (500 mL × 4), and the solvent was removed to give a residue (16.2 g).

Isolation of Renieramycins M and N. The ethyl acetate extract was subjected to trituration with methanol to give the orange precipitate renieramycin M (**1m**, 681 mg). The mother liquor was concentrated in vacuo to give the residue (13.9 g), which was further chromatographed on a silica gel flash column with hexane/ethyl acetate (7:3 to 1:1) as mobile phase to afford **1m** (1.141 g; 1.822 in total g, 12.04% based on the ethyl acetate extract). Further elution with hexane/ethyl acetate, 1:1, gave renieramycin N (**1n**, 247 mg) as orange rods.

Renieramycin M (1m): dark yellow prisms from ethyl acetate, mp 194.5−197 °C; $[\alpha]^{20}_D$ −49.5 (*c* 1.0, $CHCl_3$); CD $\Delta\epsilon$ nm (*c* 68 μM, methanol, 22 °C) −5.2 (359), −1.9 (305), −8.7 (282), 0 (270), +9.5 (258), 0 (243), −2.2 (232), 0 (223), +10.5 (211); IR (KBr) 3270, 2940, 2320w, 1705, 1690, 1650, 1640, 1605 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.63), 370 (3.11) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.96 (1H, qq, J = 7.3, 1.5 Hz, 26-H), 4.53 (1H, dd, J = 11.6, 3.1 Hz, 1-CH), 4.10 (1H, dd, J = 11.6, 2.5 Hz, 1-CH), 4.07 (1H, d, J = 2.5 Hz, 21-H), 4.02 (3H, s, OCH_3), 4.01 (1H, d, J = 3.1 Hz, 11-H), 3.99 (each 3H, s, OCH_3), 3.99 (1H, m, 1-H)*, 3.40 (1H, ddd, J = 7.6, 2.5, 1.8 Hz, 13-H), 3.11 (1H, ddd, J = 11.3, 3.1, 2.8 Hz, 3-H), 2.89 (1H, dd, J = 17.4, 2.8 Hz, 4-Hα), 2.76 (1H, dd, J = 20.6, 7.6 Hz, 14-Hα), 2.30 (1H, d, J = 20.6 Hz, 14-Hβ), 2.28 (3H, s, NCH_3), 1.94, 1.90 (each 3H, s, 6-CH_3 and 16-CH_3), 1.82 (3H, dq, J = 7.3, 1.5 Hz, 26-CH_3), 1.58 (3H, dq, J = 1.5, 1.2 Hz, 25-CH_3), 1.36 (1H, ddd, J = 17.4, 11.3, 2.7 Hz, 4-Hβ) (*the signal overlapped with the O-methyl signal); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.9 (C-15), 185.4 (C-5), 182.5 (C-18), 180.9 (C-8), 166.5 (C-24), 155.8 (C-7), 155.2 (C-17), 142.0 (C-20), 141.3 (C-10), 140.5 (C-26), 135.7 (C-9), 135.0 (C-19), 128.6 (C-6), 128.6 (C-16), 126.3 (C-25), 116.9 (21-CN), 62.0 (C-22), 61.0 (OCH_3), 60.9 (OCH_3), 58.5 (C-21), 56.3 (C-1), 54.6 (C-13), 54.2 (C-11), 54.1 (C-3), 41.5 (NCH_3), 25.4 (C-4), 21.3 (C-14), 20.4 (C-28), 15.7 (C-27), 8.7 (6-CH_3), 8.6 (16-CH_3); EIMS m/z (%) 575 (M^+, 3), 464 (4), 462 (3), 260 (11), 221 (22), 220 (100), 219 (16), 218 (25), 204 (11), 83 (11), 55 (14); *anal.* C 64.52%, H 5.84%, N 7.11%, calcd for $C_{31}H_{33}N_3O_8$, C 64.68%, H 5.78%, N 7.30%.

Renieramycin N (1n): pale yellow prisms from ethanol, mp 162.5−164 °C; $[\alpha]^{20}_D$ −24.7 (*c* 0.015, methanol); CD $\Delta\epsilon$ nm (*c* 45 μM, methanol, 22 °C) 0 (398), −1.0 (355), 0 (310), +0.5 (300), 0 (287), −1.7 (266), 0 (248), +11.8 (222), +25.2 (206); IR (KBr) 3700−3070, 2230w, 1740, 1720, 1655, 1630 cm^{-1}; UV λ_{max} (log ϵ) 273 (3.99), 370 (2.70) nm; ^1H NMR (pyridine-d_5, 270 MHz) δ 10.68, 9.30, 7.60 (each 1H, br s, OH), 5.70 (1H, qq, J = 7.3, 1.3 Hz, 26-H), 5.18 (1H, s, 14-Hβ), 4.94 (1H, d, J = 2.6 Hz, 21-H), 4.74 (1H, d, J = 2.0 Hz, 11-H), 4.58 (1H, dd, J = 11.6, 2.6 Hz, 1-CH), 4.51 (1H, dd, J = 11.6, 2.0 Hz, 1-CH), 4.37 (1H, ddd, J = 2.6, 2.6, 2.0 Hz, 1-H), 3.96 (1H, dd, J = 2.6, 2.0 Hz, 13-H), 3.94 (3H, s, 7-OCH_3), 3.74 (3H, s,

17-OCH_3), 3.60 (1H, dd, J = 17.8, 2.6 Hz, 4-Hα), 3.44 (1H, ddd, J = 11.2, 2.6, 2.6 Hz, 3-H), 2.70 (3H, s, NCH_3), 2.40 (3H, s, 16-CH_3), 2.20 (1H, ddd, J = 17.8, 11.2, 2.6 Hz, 4-Hβ), 1.92 (3H, s, 6-CH_3), 1.74 (3H, dq, J = 7.3, 1.7 Hz, 26-CH_3), 1.44 (3H, dq, J = 1.7, 1.3 Hz, 25-CH_3); ^{13}C NMR (pyridine-d_5, 67.5 MHz) δ 183.6 (C-5), 179.1 (C-8), 164.8 (C-24), 153.8 (C-7), 145.2 (C-15), 144.2 (C-17), 140.3 (C-10), 139.4 (C-18), 136.5 (C-26), 133.9 (C-9), 125.4 (C-25), 124.9 (C-6), 117.8 (21-CN), 115.9 (C-19), 115.7 (C-16), 114.7 (C-20), 63.1 (C-13), 62.0 (C-14), 60.5 (C-22), 58.3 (7-OCH_3), 57.7 (17-OCH_3), 55.2 (C-21), 54.6 (C-11), 54.4 (C-1), 53.1 (C-3), 40.8 (NCH_3), 22.9 (C-4), 17.7 (C-28), 13.1 (C-27), 7.3 (16-CH_3), 6.3 (6-CH_3); EIMS m/z (%) 593 (M^+, 0.1), 455 (4), 315 (10), 245 (50), 244 (17), 243 (100), 204 (12), 100 (18), 83 (25), 82 (15), 57 (12), 55 (42), 45 (19), 43 (66); *anal.* C 61.00%, H 6.07%, N 6.68%, calcd for $C_{31}H_{35}N_3O_9-H_2O$, C 60.88%, H 6.10%, N 6.87%.

Oxidation of Renieramycin N (1n). A solution of **1n** (23.7 mg, 0.04 mmol) in ethyl acetate (5 mL) and 5% $NaHCO_3$ (5 mL) was stirred in air at 25 °C overnight. After dilution with water (5 mL), the mixture was extracted with ethyl acetate (5 mL × 3). The combined extracts were washed with water (10 mL), dried, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 3:1) to afford the corresponding quinone (**1o**, 16.3 mg, 69%) as a pale yellow amorphous powder; $[\alpha]^{20}_D$ −134.4 (*c* 0.7, $CHCl_3$); CD $\Delta\epsilon$ nm (*c* 88 μM, methanol, 22 °C) 0 (380), −0.5 (352), 0 (310), −1.9 (278), −0.4 (246), 0 (241), +7.1 (213); IR ($CHCl_3$) 3513, 1720, 1670, 1630 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.62), 370 (3.10) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.98 (1H, qq, J = 7.3, 1.7 Hz, 26-H), 4.53 (1H, dd, J = 11.6, 3.0 Hz, 1-CH), 4.37 (1H, s, 14-H), 4.23 (1H, d, J = 2.6 Hz, 21-H), 4.09 (1H, dd, J = 11.1, 3.4 Hz, 1-CH), 4.09 (1H, dd, J = 3.3, 1.3 Hz, 11-H), 4.03, 4.02 (each 3H, s, OCH_3), 3.98 (1H, m, 1-H), 3.52 (1H, br s, OH), 3.42 (1H, br s, 13-H), 3.05 (1H, ddd, J = 11.6, 3.3, 2.3 Hz, 3-H), 2.87 (1H, dd, J = 17.2, 2.3 Hz, 4-Hα), 2.46 (3H, s, NCH_3), 1.94, 1.92 (each 3H, s, 6-CH_3 and 16-CH_3), 1.82 (3H, dq, J = 7.3, 1.3 Hz, 26-CH_3), 1.57 (3H, dq, J = 1.7, 1.3 Hz, 25-CH_3), 1.27 (1H, ddd, J = 17.2, 11.6, 2.6 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.8 (C-15), 185.4 (C-5), 182.8 (C-18), 180.8 (C-8), 166.5 (C-24), 155.8 (C-17), 155.6 (C-7), 141.1 (C-10), 141.0 (C-20), 140.6 (C-26), 135.7 (C-9), 135.3 (C-19), 128.6 (C-6), 128.4 (C-16), 126.2 (C-25), 116.3 (21-CN), 62.4 (C-13), 62.1 (C-22), 62.0 (C-14), 61.1 (OCH_3), 61.1 (OCH_3), 56.4 (C-1), 56.4 (C-21), 55.0 (C-11), 53.4 (C-3), 42.4 (NCH_3), 25.3 (C-4), 20.3 (C-28), 15.7 (C-27), 8.7 (6-CH_3), 8.4 (16-CH_3); EIMS m/z (%) 591 (M^+, 3), 577 (7), 575 (6), 566 (7), 476 (9), 464 (5), 462 (8), 460 (8), 315 (7), 260 (14), 243 (43), 236 (18), 235 (25), 221 (24), 220 (100), 219 (23), 218 (50), 205 (11), 204 (24), 83 (14), 55 (11); HREIMS m/z 591.2222 [M^+] (calcd for $C_{31}H_{33}N_3O_9$, 591.2217).

Transformation of Renieramycin M (1m) to Renieramycin E (1e). Renieramycin M (**1m**, 46.0 mg, 0.08 mmol) was dissolved in a mixture of acetonitrile and water [3:2 (v/v), 10 mL], and silver nitrate (350.0 mg, 2.0 mmol, 25 equiv) was added to this solution. The mixture was stirred at 40 °C for 8 h. The reaction mixture was filtered and then washed with chloroform (50 mL), and the combined filtrates were concentrated in vacuo. The residue was was diluted with water (30 mL) and extracted with chloroform (30 mL × 3). The combined extracts were washed with brine (30 mL), dried, and concentrated in vacuo to give the residue (59 mg). The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 1:1) to afford renieramycin E (**1e**, 31.5 mg, 69.6%) as a yellow solid, which gave spectral data (^{1}H NMR, ^{13}C NMR, IR, HRFABMS) that were in complete agreement with those of the authentic standard: $[\alpha]^{22}_D$ −121.9 (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) 3390, 1700, 1655, 1615 cm^{-1}; UV λ_{max} (log ϵ) 269 (4.63), 370 (3.11) nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92 (1H, qq, J = 7.3, 1.5 Hz, 26-H), 4.45 (1H, d, J = 2.7 Hz, 21-H), 4.43 (1H, dd, J = 11.9, 3.1 Hz, 1-CH), 4.43 (1H, m, 1-H), 4.15 (each 1H, dd, J = 11.9, 3.1 Hz, 1-CH), 4.00, 3.98 (each 3H, s, OCH_3), 3.92 (1H, dd, J = 2.4, 1.0 Hz, 11-H), 3.21 (1H, br d, J = 7.3 Hz, 13-H), 3.16 (1H, ddd, J = 11.3, 2.4, 2.4 Hz, 3-H), 2.78 (1H, dd, J = 16.8, 2.4 Hz, 4-Hα), 2.66 (1H, dd, J = 21.1, 7.6 Hz, 14-Hα), 2.25 (3H, s, NCH_3), 2.21 (1H, d, J = 21.1 Hz, 14-Hβ), 1.91, 1.91 (each 3H, s, 6-CH_3 and 16-CH_3), 1.80 (3H, dq, J = 7.3, 1.5 Hz, 26-CH_3), 1.57 (3H, dq, J = 1.5 Hz, 25-CH_3), 1.32 (1H, ddd, J = 16.8, 11.3, 2.4 Hz, 4-Hβ); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.0 (C-15), 185.8 (C-5), 182.6 (C-18), 181.2 (C-8), 166.8 (C-24), 156.0 (C-17), 155.3 (C-7), 141.7 (C-20), 141.3 (C-10), 139.6 (C-26), 137.6 (C-9), 134.7 (C-19), 128.7 (C-6), 127.9 (C-16), 126.7 (C-25), 82.4 (C-21), 63.1 (C-22), 60.9 (OCH_3), 60.9 (OCH_3), 57.5 (C-13), 54.1 (C-1), 53.0 (C-11), 50.9 (C-3), 41.4 (NCH_3), 25.7 (C-4), 20.5 (C-14), 20.3 (C-28), 15.6 (C-27), 8.6 (6-CH_3), 8.5 (16-CH_3); HRFABMS m/z 549.2258 [$M + H - H_2O$] (calcd for $C_{30}H_{33}N_2O_9$, 549.2237).

Cytotoxicity Assay. A single-cell suspension of HCT116 cells (2×10^3 cells/well) was added to the serially diluted test compounds in a microplate. The cells were then cultured for 4 days. The degree of 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 control.

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Supporting Information Available: MS, IR, ^{1}H NMR, ^{13}C NMR, and CD spectra for renieramycin M (**1m**) and renieramycin N (**1n**), as well as ^{1}H NMR spectra of renieramycins J−K (**1j**,**k**), renieramycin O (**1o**), and the known compounds **4**−**8**, **1e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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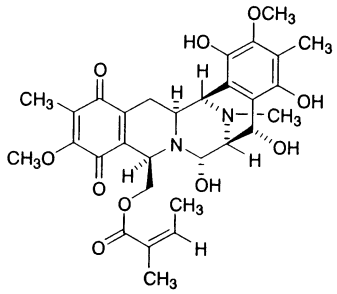
(9) Structure of saframycin D, see: Kubo, A.; Saito, N.; Kitahara, Y.; Takahashi, K.; Yazawa, K.; Arai, T. *Chem. Pharm. Bull.* **1986**, *35*, 440−442. (b) Structures of saframycins F and G, see: Mikami, Y.; Takahashi, K.; Yazawa, K.; Hour-Young, C.; Arat, T.; Saito, N.; Kubo, A. *J. Antibiot.* **1988**, *41*, 734−740.
(10) The synthesis of **12** was reported in a separate paper; see ref 1.
(11) The structure of renieramycins is closely related to that of saframycins. Renieramycin O (**1o**) has an angelic acid ester side chain in place of the pyruvamide side chain of saframycin G.[9b] On the other hand, renieramycin N (**1n**) has the same substitution of the saframycin derivative, which is the unnatural 21-cyanated analogue of saframycin E; however, it can be obtained from saframycin G in two steps (H_2, Pd/C, ethyl acetate followed by air oxidation) in 52% overall yield.
(12) Structure of saframycin E, see: Saito, N.; Harada, S.; Nishida, M.; Inouye, I.; Kubo, A. *Chem. Pharm. Bull.* **1995**, *43*, 777−782.
(13) While this paper was under review, Fusetani and co-workers reported a new renieramycin derivative **13**, named renieramycin J, from a marine sponge *Neopetrosia* sp. collected in southern Japan; see: Oku, N.; Matsunaga, S.; van Soest, R. W. M.; Fusetani, N. *J. Nat. Prod.* **2003**, *66*, 1136−1139. However, we have already named structures **1j**−**l** renieramycins J−L, respectively. Thus structure **13**, named renieramycin J by the Fusetani group, should be renamed renieramycin P.



13 (renieramycin P)

NP030262P