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# CHEMISTRY OF RENIERAMYCINS. PART 10: STRUCTURE OF RENIERAMYCIN V, A NOVEL RENIERAMYCIN MARINE NATURAL PRODUCT HAVING A STEROL ETHER AT C-14 POSITION

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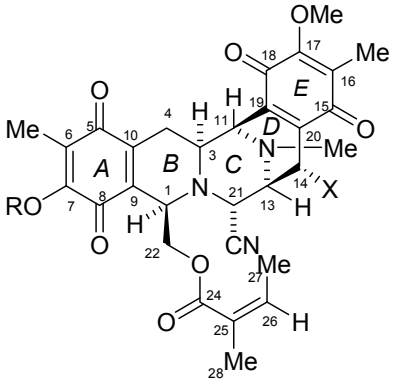
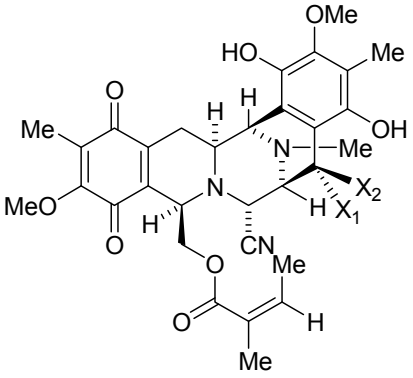
This paper is dedicated to Dr. Albert Padwa on the occasion of his 75th birthday.

**Abstract** — A new renieramycin-type marine natural product, renieramycin V, was isolated from the low polar fraction obtained from the Thai blue sponge *Xestospongia* sp., and its structure was elucidated by comparing its spectral data with those of renieramycin O and cholesterol. This is the first example of renieramycin that has a sterol moiety attached to it. It is interesting that renieramycin V does not show any cytotoxicity to several human cancer cell lines.

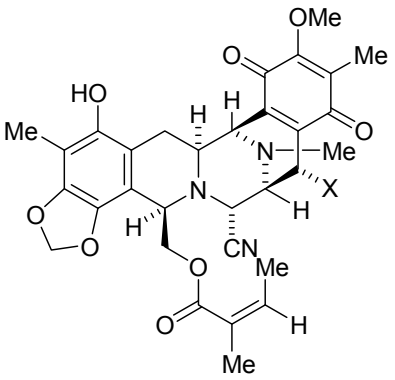
Renieramycins are isoquinoline marine natural products that are structurally and biologically related to other isoquinoline natural products, such as saframycins and ecteinascidins.[1] In our ongoing search for new anticancer metabolites in Thai marine animals, we were able to isolate and elucidate the structures of renieramycins M-O and Q-U from the Thai blue sponge, *Xestospongia* sp., after stabilization of the sponge homogenized in phosphate buffer solution by the addition of potassium cyanide.[2-4] In the course of our chemical studies on isoquinoline marine natural products, we found renieramycin V (**2**), which is the first example of renieramycin (specifically renieramycin O (**1o**)) with a sterol moiety attached to it. We present here the structure of **2**, which was elucidated by means of spectroscopic analyses, along with its biological data (Figure 1).



renieramycins
M (**1m**)[2]: R = $CH_3$, X = H
O (**1o**)[2]: R = $CH_3$, X = OH
R (**1r**)[3]: R = $CH_3$, X = $OCH_3$
S (**1s**)[3]: R = X = H



renieramycins
N (**1n**)[2]: $X_1$ = OH, $X_2$ = H
Q (**1q**)[3]: $X_1$, $X_2$ = O



renieramycins
T (**1t**)[4]: X = H
U (**1u**)[4]: X = OH

Figure 1. Structures of renieramycin marine natural products from Thai blue sponge

New compound **2**,[5] named renieramycin V,[6] was confirmed to have the molecular formula $C_{58}H_{77}N_3O_9$ [*m/z* 959.5659 ($M^+$)] by HRFABMS. All proton and carbon signals of **2** were assigned after extensive NMR measurements using COSY, HMQC, and HMBC techniques. The molecular formula of **2** indicated 22 degrees of unsaturation and the detected resonance attributable to 12 olefinic carbons, five carbonyl groups, and one nitrile carbon in **2** accounted for 13 degrees of unsaturation. This compound was presumed to have nine rings. Comparison of the $^{13}C$ NMR data of **2** with those of renieramycins O (**1o**) and R (**1r**) revealed that the 31 carbon signals of **2**, including the characteristic signals of both 10 $sp^2$ and five carbonyl carbons, were almost identical with those of the renieramycin framework (Table 1-1). $^1H$ and $^{13}C$ NMR spectra led us to speculate that **2** might be 14-*O*-alkylated renieramycin M with a characteristic 14-H proton signal at δ 4.09.[7] The remaining proton and carbon signals could be assigned to a sterol moiety whose formula was presumed to be $C_{27}H_{45}$. To discuss the NMR spectra of the sterol moiety, the data for 27 carbons, including some characteristic protons, are listed in Table 1-2. The $^{13}C$ NMR data of the sterol moiety of renieramycin V are quite similar to those of cholesterol[8] and the slight differences in the chemical shifts of C-2, C-3, and C-4 carbon signals are thought to be due to the presence of bulky renieramycin at C-3 position. The $^1H$ NMR spectrum of this moiety contained five methyl proton signals [δ 0.69 (3H, s), 0.87 (3H, d, *J* = 6.7 Hz), 0.87 (3H, d, *J* = 6.7 Hz), 0.92 (3H, d, *J* = 6.7 Hz), 1.00 (3H, s)] (Table 1-2). The $^1H$ NMR spectrum also had a characteristic oxygenated methine proton signal at δ 3.52 ppm and the $^{13}C$ NMR spectrum revealed that the carbon signal corresponding to this appeared at δ 80.9 ppm. Furthermore, a one-proton signal appeared at δ 5.43, which indicated the presence of an olefinic proton at C-6' owing to the double bond between C-5' (δ 140.5 ppm) and C-6' (δ

122.1 ppm) of the sterol moiety.

Evidence of the attachment of the sterol moiety to the renieramycin framework was found in a 3-bond correlation from H-14 at 4.09 to C-3' at 80.9 in the HMBC spectrum. Thus, the structure of **2** was confirmed to be cholesterol-3-*O*-renieramycin O.

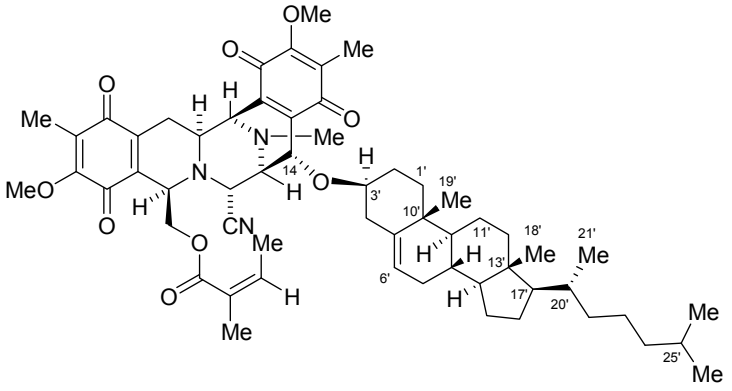



Figure 2. Structure of renieramycin V

The cytotoxicity of renieramycin V (**2**) to three cancer cell lines is shown in Table 2. It was surprising that renieramycin V (**2**) did not show any cytotoxicity. Nevertheless, we expect that the sterol moiety in renieramycin V, which was introduced by oxidation process, will play a role in protecting the life of the marine organism.

In conclusion, a minor marine natural product having a novel renieramycin structure, renieramycin V, was isolated from the Thai blue sponge, *Xestospongia* sp., and its structure was elucidated by spectroscopic analysis. To our knowledge, this is the first example of a nontoxic renieramycin derivative, the structure of which consists of cholesterol at C-14 position of renieramycin M.

**ACKNOWLEDGEMENTS**

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Table 1-1. $^{1}H$ (500 MHz) and $^{13}C$ NMR (125 MHz) data of renieramycin V (**2**) in $CDCl_3$

| Atom no. | $\delta_C$ | $\delta_H$ | HMBC correlation | *Known* | | | |
|---|---|---|---|---|---|---|---|
| | | | | renieramycin O[*1] | | renieramycin R[*2] | |
| | | | | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 56.0 | 4.01 (dt, 3.7, 2.7) | 3-H, 21-H, 22-$H_2$ | 56.4 | 3.98 (ddd, 3.4, 3.0, 2.8) | 56.3 | 4.01 (dt, 3.4, 2.8) |
| 3 | 53.5 | 3.01 (dt, 11.4, 2.7) | 4-H, 11-H | 53.4 | 3.05 (ddd, 11.6, 3.3, 2.3) | 53.4 | 3.02 (ddd, 11.6, 3.1, 2.8) |
| 4 α | 25.1 | 2.85 (dd, 17.2, 2.3) | | 25.3 | 2.87 (dd, 17.2, 2.3) | 25.1 | 2.86 (dd, 17.1, 2.5) |
| β | | 1.26 (ddd, 17.2, 11.4, 2.7) | | | 1.27 (ddd, 17.2, 11.6, 2.8) | | 1.26 (ddd, 17.1, 11.6, 2.5) |
| 5 | 185.0 | | 4-Hα 6-$CH_3$ | 185.4 | | 185.3 | |
| 6 | 129.9 | | 6-$CH_3$ | 128.6 | | 129.8 | |
| 7 | 155.8 | | 6-$CH_3$, 7-$OCH_3$ | 155.6 | | 155.8 | |
| 8 | 180.8 | | | 180.8 | | 180.8 | |
| 9 | 135.6 | | 1-H, 4-Hα, 4-Hβ, 22-H | 135.7 | | 135.6 | |
| 10 | 141.2 | | 1-H, 4-Hα, 4-Hβ | 141.1 | | 141.2 | |
| 11 | 54.3 | 4.09 (s) | 4-Hα, 4-Hβ, 13-H, *N*-$CH_3$ | 55.0 | 4.09 (dd, 3.3, 1.3) | 54.5 | 4.08 (dd, 3.1, 1.0) |
| 13 | 61.8 | 3.38 (s) | 11-H, 14-H, 21-H, *N*-$CH_3$ | 62.4 | 3.42 (br s) | 60.0 | 3.44 (br d) |
| 14 | 66.2 | 4.09 (s) | 13-H, 21-H | 62.0 | 4.37 (s) | 70.0 | 3.84 (s) |
| 15 | 185.3 | | 14-H, 16-$CH_3$ | 187.8 | | 185.1 | |
| 16 | 128.3 | | 16-$CH_3$ | 128.4 | | 128.3 | |
| 17 | 154.9 | | 16-$CH_3$, 17-$OCH_3$ | 155.8 | | 154.9 | |
| 18 | 183.0 | | 11-H | 182.8 | | 182.9 | |
| 19 | 135.4 | | 3-H, 11-H, 14-H | 135.3 | | 135.2 | |
| 20 | 141.3 | | 11-H, 13-H, 14-H | 141.0 | | 140.8 | |
| 21 | 56.3 | 4.18 (d, 2.4) | 1-H, 3-H, 13-H, 14-H | 56.4 | 4.23 (d, 2.6) | 56.6 | 4.17 (d, 2.4) |
| 22 | 63.0 | 4.31 (dd, 11.4, 2.7) | | 62.1 | 4.53 (dd, 11.6, 3.0) | 62.8 | 4.34 (dd, 11.6, 3.4) |
| | | 4.14 (dd, 11.4, 3.7) | | | 4.09 (dd, 11.6, 3.4) | | 4.13 (dd, 11.6, 3.4) |
| 24 | 166.7 | | 22-H, 26-H, 25-$CH_3$ | 166.5 | | 166.7 | |
| 25 | 126.3 | | 26-$CH_3$, 25-$CH_3$ | 126.2 | | 126.3 | |
| 26 | 140.2 | 5.98 (qq, 7.3, 1.5) | 26-$CH_3$, 25-$CH_3$ | 140.6 | 5.98 (qq, 7.3, 1.7) | 140.3 | 5.97 (qq, 7.3, 1.5) |
| 26-$CH_3$ | 15.7 | 1.84 (dq, 7.3, 1.5) | 26-H | 15.7 | 1.82 (dq, 7.3, 1.3) | 15.7 | 1.83 (dq, 7.3, 1.6) |
| 25-$CH_3$ | 20.3 | 1.60 (dq, 1.5, 1.5) | 26-H | 20.3 | 1.57 (dq, 1.7, 1.3) | 20.3 | 1.59 (dq, 1.6, 1.5) |
| 6-$CH_3$ | 8.8 | 1.92 (s) | | 8.7 | 1.94 (s) | 8.7 | 1.92 (s) |
| 16-$CH_3$ | 8.7 | 1.92 (s) | | 8.4 | 1.92 (s) | 8.7 | 1.92 (s) |
| 7$OCH_3$ | 61.0 | 4.01 (s) | | 61.1 | 4.03 (s) | 61.0 | 4.01 (s) |
| 17-$OCH_3$ | 60.8 | 3.97 (s) | | 61.1 | 4.02 (s) | 60.9 | 3.97 (s) |
| *N*-$CH_3$ | 42.9 | 2.52 (s) | 11-H, 13-H | 42.4 | 2.46 (s) | 42.7 | 2.48 (s) |
| CN | 116.6 | | 13-H, 21-H | 116.3 | | 116.5 | |
| 14-OH | | | | | 3.52 (br s) | | |
| 14-$OCH_3$ | | | | | | 59.5 | 3.54 (s) |

*1: see ref 2; *2: see ref 3

Table 1-2. $^{1}H$ (500 MHz) and $^{13}C$ NMR (125 MHz) data of renieramycin V (**2**) in $CDCl_3$

| Atom no. | $\delta_C$ | $\delta_H$[a] | HMBC correlation | Original $\delta_C$ data of cholesterol[b] |
|---|---|---|---|---|
| 1’ | 37.3 ($CH_2$) | | 2’-$H_2$, 19’-$H_3$ | 37.2 |
| 2’ | 29.8 ($CH_2$) | | 4’-H | 31.6 |
| 3’ | 80.9 (CH) | 3.52 (m) | 14-H, 2’-$H_2$, 4’-$H_2$ | 71.5 |
| 4’ | 38.7 ($CH_2$) | | 6’-H | 42.2 |
| 5’ | 140.5 (C) | ------ | 3’-H, 4’-$H_2$, 19’-$H_3$ | 140.8 |
| 6’ | 122.1 (CH) | 5.43 (br d) | 4’-$H_2$, 7’-$H_2$, 8’-H, | 121.7 |
| 7’ | 31.8 ($CH_2$) | | 6’-H, 8’-H, 9’-H, 14’-H | 31.8 |
| 8’ | 31.9 (CH) | | 11’-$H_2$, 15’-$H_2$, 6’-H | 31.8 |
| 9’ | 50.2 (CH) | 1.00 (m) | 11'-$H_2$, 12'-$H_2$, 8'-H, 7'-$H_2$, 10'-$CH_3$ | 50.0 |
| 10’ | 36.7 (C) | ------ | 1’-$H_2$, 2’-$H_2$, 4’-H, 6’-H, 6’-H, 8’-H | 37.0 |
| 11’ | 21.1 ($CH_2$) | | 8’-H, 9’-$H_2$ | 21.1 |
| 12’ | 39.8 ($CH_2$) | | 9’-H, 11’-$H_2$, 14’-H, 17’-H, 18’-$H_3$ | 39.7 |
| 13’ | 42.3 (C) | ------ | 8’-H, 14’-$H_2$, 15’-$H_2$, 17’-H, 18’-$H_3$, 20’-H | 42.2 |
| 14’ | 56.8 (CH) | | 7’-$H_2$, 8’-H, 12’-$H_2$, 15’-$H_2$, 16’-$H_2$, 19’-$H_3$ | 56.0 |
| 15’ | 24.3 ($CH_2$) | | 8’-H, 14’-H, 16’-$H_2$, 17’-H | 24.2 |
| 16’ | 28.3 ($CH_2$) | | 14’-H, 15’-$H_2$, 17’-H | 28.2 |
| 17’ | 56.2 (CH) | | 14’-H, 15’-$H_2$, 16’-$H_2$, 18’-$H_3$, 20’-H, 21’-$H_3$ | 56.1 |
| 18’ (13’-$CH_3$) | 11.9 ($CH_3$) | 0.69 (s) | 12’-$H_2$, 14’-H, 17’-H | 12.0 |
| 19’(10’-$CH_3$) | 19.4 ($CH_3$) | 1.00 (s) | 1’-$H_2$, 9’-H | 11.8 |
| 20’ | 35.8 (CH) | | 16’-$H_2$, 21’-$H_3$, 22’-$H_2$, 23’-$H_2$ | 35.8 |
| 21’ (20’-$CH_3$) | 18.7 ($CH_3$) | 0.92 (d, $J$ = 6.7 Hz) | 17’-H, 20’-H, 22’-$H_2$ | 18.7 |
| 22’ | 36.2 ($CH_2$) | | 20’-H, 21’-$H_2$, 23’-$H_2$, 24’-$H_2$ | 36.0 |
| 23’ | 23.8 ($CH_2$) | | 20’-H, 22’-$H_2$, 24’-$H_2$, 25’-H | 23.8 |
| 24’ | 39.5 ($CH_2$) | | 22’-$H_2$, 25’-H, 26’-$H_3$, 27’-$H_3$ | 39.5 |
| 25’ | 28.0 (CH) | | 23’-$H_2$, 24’-$H_2$, 26’-$H_3$, 27’-$H_3$ | 28.2 |
| 26’ (25’-$CH_3$) | 22.6 ($CH_3$) | 0.87 (d, $J$ = 6.7 Hz) | 24’-$H_2$, 25’-H, 27’-$H_3$ | 22.4 |
| 27’ (25’-$CH_3$) | 22.8 ($CH_3$) | 0.87 (d, $J$ = 6.7 Hz) | 24’-$H_2$, 25’-H, 26’-$H_3$ | 22.7 |

a) Because many proton signals overlapped with each other around δ 2.50-0.90, only the assignable proton signals are listed in Table 1-2.
b) Bruker WM-360 NMR spectrometer ($CDCl_3$): see ref 8.

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

| Compound | HCT116 | QG56 | DU145 |
|---|---|---|---|
| renieramycin V (**2**) | > 1 | > 1 | > 1 |
| renieramycin M (**1m**) | 0.011 | 0.023 | 0.0029 |
| renieramycin O (**1o**)[b] | 0.028 | 0.040 | NT |

a) HCT116 = human colon carcinoma; QG56 = human lung carcinoma; DU145 = human prostate carcinoma.
b) See ref. 2

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5 For details of the 2006 recollection (18 kg, wet weight) of the Thai blue sponge *Xestospongia* sp., see ref. 4. Silica gel chromatography (solvent gradient from hexane to ethyl acetate) provided three primary fractions. The most lipophilic fraction (9.47 g) was purified by silica gel (several times), followed by preparative TLC to give renieramycin V (**2**: 10.5 mg) along with renieramycin O (**1o**: 57.0 mg).

6 Renieramycin V (**2**): amorphous powder, $^{1}$H and $^{13}$C NMR data, see Table 1: UV $\lambda_{max}$ (log ε) 269 (4.62), 370 (3.09) nm; EIMS *m/z* (%) no $M^+$, 575 (3), 398 (46), 386 (78), 368 (30), 314 (100), 300 (45), 299 (30), 275 (31), 273 (37), 272 (30), 271 (63), 255 (62), 243 (35), 220 (32), 213 (40), 161 (31), 159 (39), 147 (31), 145 (43), 133 (34), 107 (42), 105 (33), 95 (34), 93 (30), 81 (37), 55 (47); HRFABMS *m/z* 959.5659 ($M^+$, calcd for $C_{58}H_{77}N_3O_9$, 959.5660): IR (KBr) 3435, 2945, 2868, 2320, 1717, 1655, 1616, 1456, 1375, 1307, 1290, 1261, 1231, 1150, 1080, 1045, 1028, 883 cm$^{-1}$: $[\alpha]_D^{17}$ −41.6 (*c*, 0.6, $CHCl_3$); CD (*c* 0.013 mmol/L, MeOH, 24 °C) −2.6 (353), −1.6 (310), −20.3 (275), −2.0 (246), 1.7 (239), 26.5 (212), 25.9 (210).

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