# Antimicrobial Metabolites of the Sponge *Reniera* sp.

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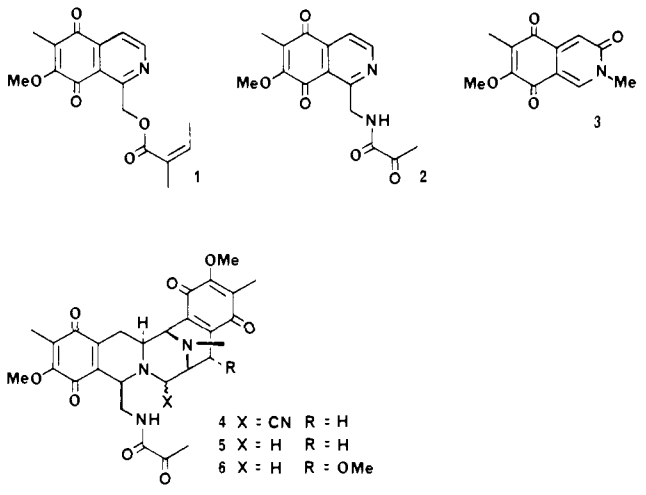
**Abstract:** The sponge *Reniera* sp. contains renierone (**1**), mimosamycin (**3**), *N*-formyl-1,2-dihydrorenierone (**7**), *O*-demethylrenierone (**8**), 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**), 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**), and renieramycins A–D (**11–14**). Mimosamycin (**3**) was first isolated from *Streptomyces lavendulae* no. 314, which also contained mimosin (**2**) and the saframycins (**4–6**), compounds similar in structure to renierone (**1**) and the renieramycins (**11–14**). The structure of 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**), the first naturally-occurring isoindole to be described, was confirmed by an unambiguous synthesis. The structures of the renieramycins were determined by analysis of spectral data, particularly the $^1$H NMR data.

We have recently described the isolation and structural elucidation of renierone (**1**), the major antimicrobial metabolite of *Reniera* sp., a bright-blue sponge found near Isla Grande, Mexico.[1] The similarity between renierone (**1**) and mimocin (**2**), a metabolite of *Streptomyces lavendulae* No. 314,[2] is striking: mimocin (**2**) contains a pyruvamide side chain in place of the angelate ester side chain of renierone (**1**). *Streptomyces lavendulae* No. 314 also contained the antibiotics mimosamycin (**3**),[3] saframycin A (**4**),[4] saframycin B (**5**), and saframycin C (**6**).[5] Further studies of the metabolites of *Reniera* sp. have resulted in the isolation of mimosamycin (**3**), *N*-formyl-1,2-dihydrorenierone (**7**), *O*-demethylrenierone (**8**), 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**), 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**), and four "dimeric"metabolites that we have called renieramycins A–D (**11–14**).

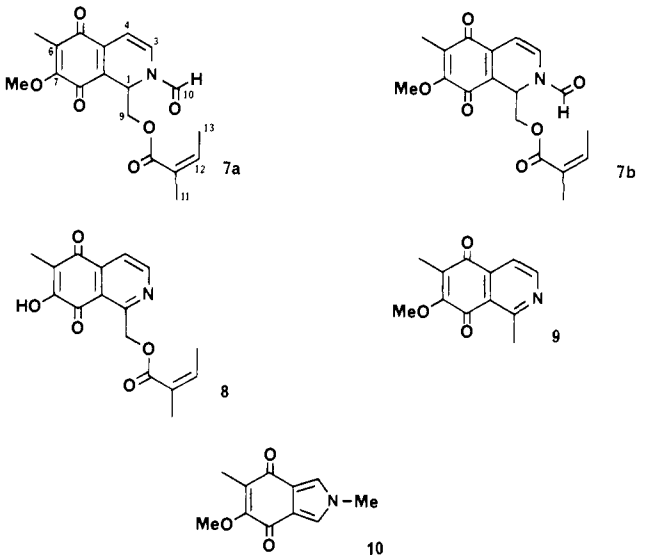



The chloroform-soluble material from an ethanol extract of the lyophilized sponge was chromatographed on silica gel to obtain five orange-red bands. Each of the colored bands showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*. Each fraction was subjected to further chromatographic separations in order to obtain pure compounds. The structure of the major antimicrobial metabolite renierone (**1**) (0.03% dry weight) and determined by single-crystal X-ray diffraction analysis and has been reported previously.[1] We also isolated the known metabolite mimosamycin (**3**) (0.01% dry weight), identical in all respects with an authentic sample.[6]

Table I. Assignment of $^1$H NMR Chemical Shift Data for Renierone (1) and the Two Isomers of *N*-Formyl-1,2-dihydrorenierone (7a and 7b)[a]

| H at C no. | 1 | 7a | 7b |
|---|---|---|---|
| 1 | | 5.99 (dd, 4, 3) | 5.37 (dd, 9, 4) |
| 3 | 8.91 (d, 5) | 6.92 (d, 8) | 7.45 (d, 8) |
| 4 | 7.87 (d, 5) | 6.03 (d, 8) | 6.25 (d, 8) |
| 6-Me | 2.09 (s) | 1.95 (s) | 1.98 (s) |
| 7-OMe | 4.15 (s) | 4.05 (s) | 4.07 (s) |
| 9 | 5.78 (s) | 4.37 (dd, 12, 4) | 4.24 (dd, 12, 9) |
| 9 | | 4.21 (dd, 12, 3) | 3.91 (dd, 12, 4) |
| 10 | | 8.43 (s) | 8.22 (s) |
| 11 | 1.99 (bs) | 1.77 (br s) | 1.87 (br s) |
| 12 | 6.10 (q, 7) | 6.06 (q, 7) | 6.15 (q, 7) |
| 13 | 2.04 (d, 7) | 1.91 (d, 7) | 2.00 (d, 7) |

[a] Multiplicities and coupling constants in parentheses.




*N*-Formyl-1,2-dihydrorenierone (**7**) was obtained as a noncrystalline red solid (0.027% dry weight) having the molecular formula $C_{18}H_{19}NO_6$. An infrared band at 1715 cm$^{-1}$ was assigned to the unsaturated ester and the band at 1650 cm$^{-1}$ was attributed to both the formamide and quinone functionalities. The ultraviolet spectrum [515 nm ($\epsilon$ 3500), 340 nm ($\epsilon$ 6000), 265 nm ($\epsilon$ 16 500), and 216 nm ($\epsilon$ 31 000)] was not readily assigned. The mass

(1) McIntyre, D. E.; Faulkner, D. J.; Van Engen, D.; Clardy, J. *Tetrahedron Lett.* **1979**, 4163.
(2) Kubo, A.; Nakahara, S.; Iwata, R.; Takahashi, K.; Arai, T. *Tetrahedron Lett.* **1980**, *21*, 3207.
(3) Fukumi, H.; Kurihara, H.; Hata, T.; Tamura, C.; Mishima, H.; Kubo, A.; Arai, T. *Tetrahedron Lett.* **1977**, 3825. Fukumi, H.; Kurihara, H.; Mishima, H. *Chem. Pharm. Bull.* **1978**, *26*, 2175.
(4) Arai, T.; Takahashi, K.; Nakahara, H.; Kubo, A. *Experientia* **1980**, *36*, 1025.
(5) Arai, T.; Takahashi, K.; Kubo, A.; Nakahara, S.; Sato, S.; Aiba, K.; Tamura, C. *Tetrahedron Lett.* **1979**, 2355.
(6) An authentic sample of mimosamysin (**3**) was obtained from Dr. H. Fukumi, Sankyo Co., Ltd., Japan.

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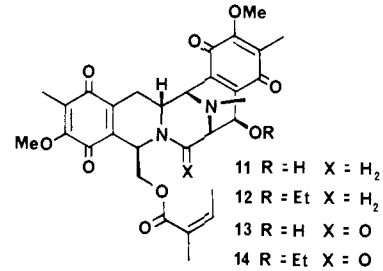
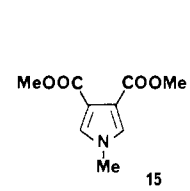
spectrum of *N*-formyl-1,2-dihydrorenierone (**7**) was almost identical to that of renierone (**1**) with the exception of the presence of a small molecular ion peak at $m/z$ 345. The facile loss of 30 amu could be explained by loss of the formyl group and a neighboring hydrogen from *N*-formyl-1,2-dihydrorenierone (**7**). Both the $^1H$ and $^{13}C$ NMR spectra indicated that the formamide **7** was a 2:1 mixture of two physically inseparable isomers. These spectra can be readily interpreted by assuming that the partial double bond character of the *N*-formyl bond permits the observation of two stereoisomers on the NMR time scale. The signals of the $^1H$ NMR spectrum were visually differentiated and assigned to the major or minor stereoisomers (Table I). The signals for the angelate ester and the methyl and methoxyl groups were at similar chemical shifts to the corresponding signals for renierone (**1**). The $^1H$ NMR spectrum of the major stereoisomer **7a** contained signals at $\delta$ 4.21 (dd, 1 H, $J$ = 12, 3 Hz), 4.37 (dd, 1 H, $J$ = 12, 4 Hz), and 5.99 (dd, 1 H, $J$ = 4, 3 Hz) which were assigned to a methylene group bearing the angelate ester adjacent to the C-1 methine group of an N-substituted 1,2-dihydroisoquinoline. The remaining signals at $\delta$ 6.03 (d, 1H, $J$ = 8 Hz), 6.92 (d, 1 H, $J$ = 8 Hz), and 8.43 (s, 1 H) were assigned to the C-4, C-3, and *N*-formyl protons respectively. Similar assignments were made for the minor stereoisomer **7b**. The geometry of the stereoisomers was determined by comparison of the chemical shifts of the C-1 and C-3 proton signals.[7] In order to confirm that the methyl and methoxyl groups on the quinone ring were at C-6 and C-7, respectively, a dichloromethane solution of *N*-formyl-1,2-dihydrorenierone (**7**) was treated with 0.01 N aqueous methanolic sodium hydroxide solution in a two-phase reaction to obtain renierone (**1**) as the major product.

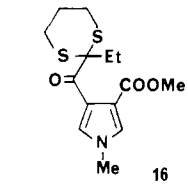
*O*-Dimethylrenierone (**8**) (0.002% dry weight) was obtained as beige crystals, mp 135–136 °C. The molecular formula $C_{16}H_{15}NO_5$, the presence of a hydroxyl band (3450 $cm^{-1}$) in the infrared spectrum, and the lack of a methoxyl signal in the $^1H$ NMR spectrum suggested that the 7-methoxy group in renierone (**1**) was replaced by a hydroxyl group in *O*-demethylrenierone (**8**). Since the methoxyl group in renierone (**1**) may be considered part of a vinylogous ester, we were able to convert renierone (**1**) into *O*-demethylrenierone (**8**) by treatment with methanolic sodium hydroxide solution. Treatment of *O*-demethylrenierone (**8**) with excess ethereal diazomethane solution gave renierone (**1**), identical in all respects with authentic material.

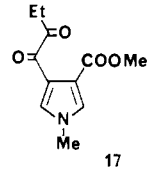
The remaining isoquinoline quinone, 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**) (0.007% dry weight) had the molecular formula $C_{12}H_{11}NO_3$. Comparison of the $^1H$ NMR spectrum of quinone **9** with that of renierone (**1**) suggested that the angelate ester side chain and the methylene group [$\delta$ 5.78 (s, 2 H)] at C-1 had been replaced by a methyl group [$\delta$ 2.94 (s, 3 H)]. The similarity between the remaining signals in both spectra indicated that the substitution pattern about the isoquinoline ring in 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**) was the same as that in renierone (**1**). Comparison of the $^{13}C$ NMR spectrum of 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**) with that of renierone (**1**) supported the structural assignment.

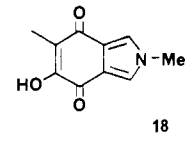
The remaining quinone **10**, mp 153–154 °C, had the molecular weight $C_{11}H_{11}NO_3$. The $^1H$ NMR spectrum contained an aromatic methyl signal at $\delta$ 2.00 (s, 3 H), an *N*-methyl signal at 3.73 (s, 3 H), an *O*-methyl signal at 4.01, and two aromatic proton signals at 7.15 (d, 1 H, $J$ = 1.5 Hz) and 7.20 (d, 1 H, $J$ = 1.5 Hz). Assuming that the methyl and methoxyl groups are attached to an intact quinone ring (IR 1650 $cm^{-1}$) the only reasonable structures are those based on either the *N*-methylindole or *N*-methylisoindole ring systems. A nuclear Overhauser enhancement difference spectrum (NOEDS) showed an equal enhancement of both aromatic proton signals when the *N*-methyl signal was irradiated. This result, together with comparison of the NMR data with those of model compounds,[8] indicated that the quinone was 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**).



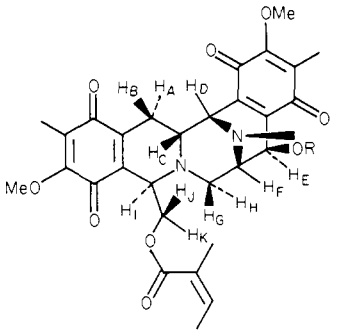




The structure of the quinone **10** was confirmed by an unambiguous synthesis. Condensation of 1 equiv of 2-lithio-2-ethyl-1,3-dithiane with 3,4-dicarbomethoxy-1-methylpyrrole (**15**) gave the keto ester **16** in 27% yield (51% based on starting material consumed). Use of more than 1 equiv of dithiane reagent gave unacceptable yields of the bisaddition product. Hydrolysis of the dithiane group was difficult since reagents commonly used for this reaction, such as *N*-bromosuccinimide or bromine, reacted preferentially at the pyrrole ring. However, treatment of the keto-ester **16** with 4 equiv of *N*-chlorosuccinimide and 5 equiv of silver nitrate in aqueous acetonitrile at 0 °C for 15 min gave the diketone **17** in high yield. The diketone **17** was cyclized using sodium hydride in dimethylformamide to obtain a hydroxyquinone **18** in 30% yield. Methylation of the hydroxyquinone **18** could be carried out using dimethyl sulfate or methyl iodide and potassium carbonate but the product hydrolyzed back to starting material during aqueous workup. Treatment of the hydroxyquinone **18** with excess ethereal diazomethane solution gave products in which the excess diazomethane had added to the pyrrole ring. We therefore prepared a sample of the quinone **10** by treatment of the hydroxyquinone **18** with ~0.5 equiv of diazomethane and separated the product from starting material by LC on Partisil. Although quinonoid isoindoles have been reported as synthetic products,[8] this is the first report of a number of this class of compounds as a natural product.

The "dimeric" quinones **11–14** were all isolated as minor metabolites of the sponge. The major dimer, renieramycin A (**11**), $[\alpha]_D$ −36° (0.008% dry weight), had the molecular formula $C_{30}H_{34}N_2O_9$. The infrared spectrum contained bands at 3160 $cm^{-1}$ (H-bonded hydroxyl), 1720 $cm^{-1}$ ($\alpha,\beta$-unsaturated ester) and 1660 $cm^{-1}$ (quinone). The ultraviolet spectrum [265 nm ($\epsilon$ 15 800), 365 nm ($\epsilon$ 1370)] was characteristic of a quinone. The $^1H$ NMR spectrum contained signals at $\delta$ 1.91 (s, 3 H), 1.92 (s, 3 H), 4.00 (s, 3 H), and 4.01 (s, 3 H) due to the methyl and methoxyl groups on the two quinone rings, at 2.43 (s, 3 H) due to the *N*-methyl group and at 1.55 (br s, 3 H), 1.78 (d, 3 H, $J$ = 7 Hz), and 5.92 (br q, 1 H, $J$ = 7 Hz) due to the angelate ester. The remaining signals were assigned as shown in Table II. Spin-decoupling experiments indicated the presence of four groups of adjacent protons. The signals at $\delta$ 4.49 (dd, 1 H, $J$ = 11.5, 3 Hz, $H_K$) and 4.19 (dd, 1 H, $J$ = 11.5, 2 Hz, $H_J$) were coupled to a signal at 3.60 (m, 1 H, $w_{1/2}$ = 9 Hz, $H_I$) and were assigned to the methylene bearing the angelate ester moiety coupled to a proton adjacent to both the quinone ring and a nitrogen atom. The signals at $\delta$

(7) Lewin, A. H.; Frucht, M. *Org. Magn. Reson.* **1975**, *7*, 206.
(8) Cragg, G. M. L.; Giles, R. G. F.; Roos, G. H. P. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1339.

Table II. $^1$H NMR Chemical Shift Data for Renieramycins A-D (11-14)[a]




11-14

| | 11 | 12 | 13 | 14 |
|---|---|---|---|---|
| $H_A$ | 1.26 (17, 11, 3) | 1.23 (18, 11, 3) | 1.41 (17, 12, 2) | 1.41 (17, 12, 1) |
| $H_B$ | 2.75 (17, 2.5, 1) | 2.74 (18, 3) | 3.02 (17, 2.5) | 3.02 (17, 2.5) |
| $H_C$ | 2.64 (11, 2.5, 2.5) | 2.62 (11, 3, 2.5) | 3.86 (12, 3.5, 2.5) | ~3.87 |
| $H_D$ | 4.04 (2.5, 1) | 4.04 (3, 1) | 4.19 (3.5, 1) | 4.22 (2) |
| $H_E$ | 4.44 (2) | 4.05 (s) | 4.78 (br s) | 4.36 (br s) |
| $H_F$ | 3.18 ($w_{1/2}$ = 5) | 3.19 (br s) | 3.73 (s) | 3.67 (br s) |
| $H_G$ | 2.71 (11, 3.5) | 2.76 (11, 3.5) | | |
| $H_H$ | 3.18 (11) | 3.10 (11, 2) | | |
| $H_I$ | 3.60 ($w_{1/2}$ = 9) | 3.61 (br s) | 5.48 (br s) | 5.49 (br s) |
| $H_J$ | 4.19 (11.5, 2) | 4.27 (11.5, 2.5) | 4.72 (11.5, 2.5) | 4.63 (12, 2.5) |
| $H_K$ | 4.49 (11.5, 3) | 4.32 (11.5, 3) | 4.36 (11.5, 2.5) | 4.42 (12, 2.5) |
| ring A | | | | |
| $OCH_3$ | 4.00*[b] | 4.00*[b] | 4.01*[b] | 3.97*[b] |
| $CH_3$ | 1.91**[b] | 1.90**[b] | 1.93**[b] | 1.92**[b] |
| ring E | | | | |
| $OCH_3$ | 4.01*[b] | 3.95*[b] | 4.05*[b] | 4.04*[b] |
| $CH_3$ | 1.92**[b] | 1.93**[b] | 1.95**[b] | 1.95**[b] |
| N-$CH_3$ | 2.43 | 2.48 | 2.61 | 2.62 |
| angelate | | | | |
| $CH_3$ | 1.55 (br s) | 1.56 (br s) | 1.50 (br s) | 1.48 (br s) |
| $CH_3$ | 1.78 (d, 7) | 1.79 (d, 7) | 1.69 (d, 7) | 1.68 (d, 7) |
| H | 5.92 (br q, 7) | 5.91 (br q, 7) | 5.90 (br q, 7) | 5.89 (br q, 7) |
| OEt | | 1.19 (t, 7) | | 1.23 (t, 7) |
| | | 3.76 (q, 7) | | 3.87 (q, 7) |

[a] Coupling constants (Hz) in parentheses. [b] Asterisk and double asterisk numbers may be exchanged.

1.26 (m, 1 H, $J$ = 17, 11, 3 Hz, $H_A$) and 2.75 (m, 1 H, $J$ = 17, 2.5, 1 Hz, $H_B$) were coupled to a signal at 2.64 (dt, 1 H, $J$ = 11, 2.5, 2.5 Hz, $H_C$) that was in turn coupled to a signal at 4.04 (dd, 1 H, $J$ = 2.5, 1 Hz, $H_D$). A third group of signals at $\delta$ 3.18 (bd, 1 H, $J$ = 11 Hz, $H_H$), 2.71 (dd, 1 H, $J$ = 11, 3.5 Hz, $H_G$), and 3.18 (bs, 1 H, $w_{1/2}$ = 5 Hz, $H_F$) were assigned to the N-$CH_2$-CH-N moiety while the remaining methine signal at $\delta$ 4.64 (bs, 1 H, $H_E$) was coupled only to the hydroxyl proton ($D_2O$ exchangeable) at 3.40 (bs, 1 H). The relatively large homoallylic coupling (3 Hz) between the signals at $\delta$ 1.26 and 3.60 indicated that both protons were orthogonal to the plane of the quinone ring, suggesting a pseudoboat conformation for the nitrogen-containing ring B. The long-range coupling (1 Hz) between signals at $\delta$ 4.04 and 3.18 was expected for the bridgehead protons in a bicyclo-[3.3.1] ring system. The absence of coupling between the signals at $\delta$ 3.18 and 4.44 required a dihedral angle of 80–90° between $H_F$ and $H_E$. A series of nuclear Overhauser enhancement difference spectra (NOEDS)[9] allowed us to define the geometry of renieramycin A (**11**). On irradiation of the *N*-methyl signal, nuclear Overhauser enhancement of the signals due to $H_D$, $H_F$, and the hydroxy proton was observed. Irradiation of $H_E$ caused enhancement of the signals due to $H_H$ and $H_I$ while irradiation of $H_I$ caused a strong enhancement of the $H_E$ signal. Irradiation of $H_A$ caused enhancement of the $H_D$ signal. Only the configuration shown for renieramycin A (**11**) satisfied the requirement that the $H_E$–$H_I$ distance be less than 3 Å. The large upfield shift of the $H_A$ signal at $\delta$ 1.26 relative to the $H_B$ signal at 2.75 served to confirm the renieramycin A geometry that required $H_A$ to lie in the ring current of the quinone ring (ring E). The positions of the methyl and methoxyl groups on rings A and E were assigned on the assumption that renieramycin A (**11**) was derived from one molecule of *N*-formyl-1,2-dihydrorenierone (**7**) and one molecule of mimosamycin (**3**). The ring system of renieramycin A (**11**) was identical with that of the saframycins (**4**–**6**) but the shapes of the two series of molecules were quite different due to inversion at the BC ring junction.

Renieramycin B (**12**) had the molecular formula $C_{32}H_{38}N_2O_9$. Comparison of the spectral data, particularly the $^1$H NMR data (Table II), with those of renieramycin A (**11**) revealed that the molecules were almost identical. The $^1$H NMR spectrum contained signals at $\delta$ 1.19 (t, 3 H, $J$ = 7 Hz) and 3.76 (q, 2 H, $J$ = 7 Hz) due to an ethoxy group and lacked the hydroxyl signal. The signal for $H_E$ ($\delta$ 4.05) in renieramycin B (**12**) was shifted upfield from the corresponding signal ($\delta$ 4.44) in renieramycin A (**11**) while all other signals were virtually unchanged in both chemical shifts and coupling constants. Irradiation of $H_I$ ($\delta$ 3.61) again caused an enhancement (NOEDS) of the $H_E$ ($\delta$ 4.44) signal. Renieramycin B (**12**) was probably an artifact resulting from solvent exchange during storage of the sponge in ethanol.

Renieramycin C (**13**) had the molecular formula $C_{30}H_{32}N_2O_{10}$. The infrared spectrum contained a broad band at 3200 cm$^{-1}$ due to a hydrogen-bonded hydroxyl group, an ester band at 1720 cm$^{-1}$, the quinone band at 1660 cm$^{-1}$, and an amide band at 1650 cm$^{-1}$. The presence of the amide band suggested that renieramycin C (**13**) was a tertiary amide related to renieramycin A (**11**) by substitution of a carbonyl for the only methylene group adjacent to nitrogen. The $^1$H NMR spectrum (Table II) supported this assignment. The downfield shift of the $H_I$ signal from $\delta$ 3.60 in renieramycin A to 5.48 (bs, 1 H, $w_{1/2}$ = 6.5 Hz) in renieramycin C reflected both the change from an adjacent tertiary amine to

(9) Hall, L. D.; Saunders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.

a tertiary amide and the change of geometry that caused $H_I$ to be situated further away from the quinone ring E. The signal at $\delta$ 1.41 (ddd, 1 H, $J$ = 17, 12.5, 2 Hz) was assigned to $H_A$ that was again in the ring current of the quinone ring E, implying that the stereochemistry of the BC and CD ring junctions in renieramycin C (**13**) was the same as in renieramycin A (**11**). The signals at $\delta$ 4.78 (bs, 1 H) and 3.73 (bs, 1 H) due to $H_E$ and $H_F$ respectively again required a dihedral angle of ~90° for these protons: the configuration of the hydroxyl group in renieramycin C (**13**) was therefore the same as that in renieramycin A (**11**).

Renieramycin D (**14**) had the molecular formula $C_{32}H_{36}N_2O_{10}$. Inspection of the spectral data, particularly the $^1H$ NMR spectrum (Table II), led to the conclusion that renieramycin D (**14**) was the ethyl ether of renieramycin C (**13**).

We have screened the new natural products from *Reniera* sp. against a variety of terrestrial and marine microorganisms and have found antimicrobial activity in all compounds. Since we obtained insufficient quantities of the renieramycins (**11–14**), we have been unable to determine whether they have antitumor properties similar to those reported for the saframycins (**4–6**). Renierone (**1**) and *N*-formyl-1,2-dihydrorenierone (**7**) both inhibit cell division in the fertilized sea urchin egg assay.[10] Since the sponge contained relatively small quantities of metabolites resembling those of *S. lavendulae*, it seems possible that the metabolites were produced by a symbiotic microorganism. By a quite remarkable coincidence, we have recently found specimens of the same bright blue *Reniera* sp. in marine lake[11] in Palau, Western Caroline Islands. A preliminary examination of the Palau sample suggests that the same metabolites are present.

## Experimental Section

Infrared spectrum were recorded on a Perkin-Elmer Model 137 spectrophotometer. Ultraviolet spectra were recorded on a Varian Cary 219 double-beam spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 10-cm microcell. $^1H$ NMR spectra were recorded on a custom-built instrument based on a 360-MHz Oxford narrow-bore magnet and a Nicolet FT data system. $^{13}C$ NMR spectra were recorded on a Varian CFT-20 spectrometer. Low-resolution mass spectra were recorded on a Hewlett-Packard 5930A mass spectrometer. High-resolution mass measurements were obtained from the Department of Chemistry, UCLA and the Department of Chemistry and Space Sciences Laboratory, UC Berkeley. Melting points were measured on a Fisher-Johns apparatus and reported uncorrected. All solvents used were either spectral grade or distilled from glass prior to use.

**Collection and Extraction.** *Reniera* sp. was collected by hand using SCUBA (−10 to −20 m) in the vicinity of Isla Grande, Mexico (near Zihuatenejo), during the TEPE 1978 expedition on R/V Alpha Helix. The sponge (350 g dry weight) was homogenized in ethanol and filtered and the ethanol extract evaporated under reduced pressure. The resulting aqueous suspension was extracted sequentially with chloroform (2 L), ethyl acetate (2 L), and *n*-butyl alcohol (2 L). Each of the extracts was concentrated to obtain a black tar-like oil.

**Chromatography of the Chloroform Extract.** The chloroform extract (16.5 g) was chromotographed on a column (40 cm × 6 cm diameter) of silica gel using eluants of increasing polarity from ether through acetone to methanol. The earlier fractions eluted with ether were combined and rechromatographed on a Merck LOBAR 'C' LiChroprep Si 60 medium pressure column using solvents of increasing polarity from ether to 5% methanol in acetone. Fractions were collected on the basis of the clearly defined colored bands. The first yellow band contained renierone (**1**, 180 mg, 0.03% dry weight), obtained as yellow needles, mp 91–92 °C, from ether–hexane. A red band contained *N*-formyl-1,2-dihydrorenierone (**7**, 93 mg, 0.027% dry weight) that was separated from sterols by rechromatography on silica gel using 10% ether in dichloromethane as eluant. A second yellow band contained 1,6-dimethyl-7-methoxy-5,8-dihdyroisoquinoline-5,8-dione (**9**, 25 mg, 0.007% dry weight), obtained as yellow needles, mp 188–190 °C dec, from ether–hexane. The remaining material eluted from the medium-pressure chromatography was combined with the later fractions eluted with ether from the initial silica gel separation and the material was chromatographed on the medium-pressure LOBAR column. Material eluted with 5% acetone in ether was rechromatographed by LC on Partisil using 20% acetone in ether as solvent to obtain, in order of retention, renieramycin D (**14**, 4 mg, 0.001% dry weight), renieramycin B (**12**, 7 mg, 0.002% dry weight), and 2,5-dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**, 4 mg, 0.001% dry weight). Material eluted with 50% acetone in ether gave crystals of mimosamycin (**3**, 35 mg, 0.01% dry weight) from ether. Material eluted from the initial silica gel column with acetone was rechromatographed by LC on Partisil using 20% acetone in ether as solvent to obtain renieramycin A (**11**, 28 mg, 0.008% dry weight) and renieramycin C (**13**, 8 mg, 0.002% dry weight). Material eluted from the silica gel column with 1:1 acetone–methanol was crystallized from dichloromethane/ether to obtain *O*-demethylrenierone (**8**, 7 mg, 0.002% dry weight).

***N*-Formyl-1,2-dihydrorenierone (7)**: $[\alpha]^{20}_D$ −227° (*c* 0.023 MeOH); UV (MeOH) 216 nm ($\epsilon$ 31 000), 265 ($\epsilon$ 16 500), 340 ($\epsilon$ 6000), 515 ($\epsilon$ 3500); IR ($CH_2Cl_2$) 1715, 1650 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) see Table I; $^{13}C$ NMR ($CDCl_3$), major isomer (**7a**), $\delta$ 184.6 (s), 180.1 (s), 167.2 (s), 161.9 (d), 156.1 (s), 153.6 (s), 140.4 (d), 133.1 (d), 126.9 (s), 126.5 (s), 123.8 (s), 102.7 (d), 62.9 (t), 60.9 (q), 47.3 (d), 20.3 (q), 15.5 (q), 8.4 (q), minor isomer (**7b**) $\delta$ 184.0 (s), 180.0 (s), 166.5 (s), 161.1 (d), 155.8 (s), 154.0 (s), 139.5 (d), 129.2 (d), 126.8 (s), 126.4 (s), 123.0 (s), 100.7 (d), 62.9 (t), 60.9 (q), 49.6 (d), 20.3 (q), 15.7 (q), 8.5 (q); mass spectrum $m/z$ 345, 315, 232, 204, 117, 83; high-resolution mass spectrum, obsd $m/z$ 345.1180, $C_{18}H_{19}NO_6$ requires 345.1212.

***O*-Demethylrenierone (8)**: mp 135–136 °C; UV (MeOH) 208 nm ($\epsilon$ 12 500), 248 ($\epsilon$ 7640), 290 ($\epsilon$ 3260), 316 ($\epsilon$ 1610); IR ($CH_2Cl_2$) 3450, 1517, 1660 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.99 (br s, 3 H), 2.01 (d, 3 H, $J$ = 7 Hz), 2.11 (s, 3 H), 5.80 (s, 2 H), 6.12 (q, 1 H), $J$ = 7 Hz), 7.94 (d, 1 H, $J$ = 5 Hz), 8.96 (d, 1 H, $J$ = 5 Hz); mass spectrum, $m/z$ 301, 202, 162, 83; high-resolution mass spectrum, obsd $m/z$ 301.0949, $C_{16}H_{15}NO_5$ requires 301.0950.

**1,6-Dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (9)**: mp 188–190 °C dec; UV (MeOH) 223, 276, 283, 292 nm; IR ($CH_2Cl_2$) 1675 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.04 (s, 3 H), 2.94 (s, 3 H), 4.15 (s, 3 H), 7.94 (d, 1 H, $J$ = 5 Hz), 8.76 (d, 1 H, $J$ = 5 Hz); $^{13}C$ NMR ($CDCl_3$) $\delta$ 183.0, 180.0, 158.0 (2 c), 153.7, 139.0, 130.2, 122.0, 117.4, 61.3, 29.7, 9.1; mass spectrum, $m/z$ 217, 202, 187, 174, 146, 130, 118; high-resolution mass measurement, obsd $m/z$ 217.0724, $C_{12}H_{11}NO_3$ requires 217.0739.

**2,5-Dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (10)**: mp 153–154 °C; UV (MeOH) 224 nm ($\epsilon$ 13 640), 233 ($\epsilon$ 12 000), 275 ($\epsilon$ 11 000), 362 nm ($\epsilon$ 3030); IR ($CH_2Cl_2$) 1650 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.00 (s, 3 H), 3.73 (s, 3 H), 4.01 (s, 3 H), 7.15 (d, 1 H, $J$ = 1.5 Hz), 7.17 (d, 1 H, $J$ = 1.5 Hz); mass spectrum, $m/z$ 205, 190, 176, 162, 134, 118; high-resolution mass spectrum, obsd $m/z$ 205.0737, $C_{11}H_{11}NO_3$ requires 205.0739.

**Renieramycin A (11)**: $[\alpha]_D^{20}$ −36.3° (*c* 0.16 MeOH); UV (MeOH) 268 nm ($\epsilon$ 15 800), 365 ($\epsilon$ 1370); IR ($CHCl_3$) 3160, 1720, 1660, 1650, 1645, 1625 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) see Table II; $^{13}C$ NMR ($C_6D_6$) $\delta$ 166.0, 156.8, 156.4, 143.1, 141.9, 139.8, 137.7, 137.1, 130.8, 130.0, 126.3, 64.2, 62.8, 61.5, 61.0, 59.5, 57.1, 56.7, 42.8, 26.9, 20.9, 16.2, 9.0, 8.8 (six signals not observed); mass spectrum, $m/z$ 566, 466, 443, 437, 332, 315, 282, 236, 234, 232, 220, 218, 208, 192; high-resolution mass spectrum, obsd $m/z$ 566.2262, $C_{30}H_{34}N_2O_9$ requires 566.2264.

**Renieramycin B (12)**: $[\alpha]^{20}_D$ −32.2° (*c* 0.15 MeOH); UV (MeOH) 268 nm ($\epsilon$ 17 400), 365 ($\epsilon$ 1460); IR ($CHCl_3$) 1715, 1660, 1645, 1620 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) see Table II; mass spectrum, $m/z$ 594, 550, 494, 483, 481, 437, 435, 332, 265, 218; high-resolution mass spectrum, obsd $m/z$ 594.2553, $C_{32}H_{38}N_2O_9$ requires 594.2577.

**Renieramycin C (13)**: $[\alpha]^{20}_D$ −89.2° (*c* 0.065 MeOH); UV (MeOH) 266 nm ($\epsilon$ 14 900), 360 ($\epsilon$ 21 600); IR ($CHCl_3$) 3100, 1720, 1680, 1660, 1650, 1620 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) see Table II; mass spectrum, $m/z$ 580, 564, 451, 439, 437, 421, 235, 220, 218; high-resolution mass spectrum, obsd $m/z$ 580.2045, $C_{30}H_{32}N_2O_{10}$ requires 580.2056.

**Renieramycin D (14)**: $[\alpha]^{20}_D$ −100.7° (*c* 0.092 MeOH); UV (MeOH) 264 nm ($\epsilon$ 16 100), 370 ($\epsilon$ 1450); IR ($CHCl_3$) 1720, 1680, 1665, 1645, 1620 cm$^{-1}$; $^1H$ NMR ($CDCl_3$) see Table II; mass spectrum, $m/z$ 608, 564, 495, 467, 451, 421, 264, 262, 218; high-resolution mass spectrum, obsd $m/z$ 608.2340, $C_{32}H_{36}N_2O_{10}$ requires 608.2369.

**Conversion of *N*-Formyl-1,2-dihydrorenierone (7) to Renierone (1).** A solution of the formamide **7** (2 mg, 0.006 mmol) in dichloromethane (0.5 mL) was added to aqueous methanolic sodium hydroxide solution [0.2 mL of 5% NaOH in 1:1 methanol–water (2 mL)]. The two-phase reaction mixture was shaken vigorously at room temperature for 1 min and allowed to separate. A blue dichloromethane layer was separated and dried over anhydrous sodium sulfate and the solvent evaporated to obtain a blue oil. Chromatography of the blue oil by LC on $\mu$-porasil using 1:3 hexane–ether as eluant gave renierone (**1**, ~1 mg), identical in all respects with authentic material.

(10) Jacobs, R. S.; White, S.; Wilson, L. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1981**, *40*, 26.

(11) Several of the marine lakes in Palau contained blue-purple sponges but only one lake contained the *Reniera* sp. The two collecting sites are approximately 8500 miles apart, an unusual situation.

**Hydrolysis of Renierone (1).** A solution of sodium hydroxide (0.1 mL of 0.1 N) in methanol (2 mL) was added to a stirred solution of renierone (**1**, 5 mg, 0.016 mmol) in methanol (5 mL). The reaction mixture turned red and was stirred at room temperature for 15 min. The reaction mixture was cooled by addition of ice (~10 mL) and acidified with 0.1 N hydrochloric acid and the resulting yellow solution extracted with dichloromethane (3 × 25 mL). The combined extracts were washed with water (10 mL) and dried over anhydrous sodium sulfate and the solvent evaporated to obtain a brown solid. The product was chromatographed on sephadex LH-20 using 1:1 methanol–dichloromethane as eluant to obtain *O*-demethylrenierone (**8**, 4 mg, 84% theoretical) identical in all respects with the natural material.

**Methylation of *O*-Demethylrenierone (8).** Ethereal diazomethane solution was added dropwise to a cooled (0 °C) solution of *O*-demethylrenierone (**8**, 2 mg, 0.007 mmol) in 1:1 ether–dichloromethane (5 mL) and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was quenched with water and the organic material extracted with dichloromethane. The dichloromethane extract was dried over sodium sulfate and the solvent evaporated to obtain renierone (**1**, 2 mg, quantitative) identical in all respects with an authentic sample.

**Synthesis of 2,5-Dimethyl-6-methoxy-4,7-dihydrolsoindole-4,7-dione (10).** A solution of *n*-butyllithium (4.05 mL of 2.1 M, 8.5 mmol) in hexane was added to a solution of freshly distilled 2-ethyl-1,3-dithiane (1.68 g, 11.4 mmol) in tetrahydrofuran (46 mL) at −43 °C under a nitrogen atmosphere for 5 min. The solution was allowed to stand at 0 °C for 40 min then cooled to −78 °C. This solution was added dropwise to a stirred solution of 1-methyl-3,4-dicarbomethoxypyrrole (1.83 g, 9.3 mmol) in dry tetrahydrofuran (130 mL) at −43 °C under a nitrogen atmosphere. The reaction mixture was stirred at −43 °C for 10 min, warmed to 0 °C, and stirred for an additional 30 min. The reaction mixture was poured onto ice (200 g), extracted with ether (2 × 200 mL), acidified with 3 N hydrochloric acid, and extracted with dichloromethane (3 × 200 mL). The combined organic extracts were dried over sodium sulfate and the solvent evaporated to obtain an oil. The product was chromatographed on a medium-pressure LiChroprep Si 60 column using 60% ether–dichloromethane as eluant to obtain the bisaddition product (340 mg, 9% theoretical), 1-methyl-3,4-dicarbomethoxypyrrole (884 mg, 48% recovery), and the required keto ester **16** (772 mg, 27% theoretical, 51% based on starting material consumed): mp 95–96 °C; UV (MeOH) 263 nm ($\epsilon$ 4840); IR ($CHCl_3$) 1725, 1690 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.05 (t, 3 H, $J$ = 7 Hz), 1.86 (m, 1 H), 2.08 (q, 2 H, $J$ = 7 Hz), 2.11 (m, 1 H), 2.66 (m, 2 H), 3.26 (m, 2 H), 3.66 (s, 3 H), 3.78 (s, 3 H), 7.16 (d, 1 H, $J$ = 2 Hz), 7.35 (d, 1 H, $J$ = 2 Hz); mass spectrum, $m/z$ 313, 282, 207, 192, 166, 147; high-resolution mass spectrum, obsd $m/z$ 313.0804, $C_{14}H_{19}NO_3S_2$ requires 313.0806.

A solution of the keto ester **16** (70 mg, 0.224 mmol) in 4:1 acetonitrile–water (1 mL) was added dropwise to a cooled solution of *N*-chlorosuccinimide (120 mg, 0.9 mmol) and silver nitrate (172 mg, 1.11 mmol) in 4:1 acetonitrile–water (60 mL) at 0 °C. The resulting heterogeneous mixture was stirred for 15 min at 0 °C then quenched with 10% sodium sulfite solution (30 mL). The organic material was extracted with dichloromethane (3 × 100 mL) and the combined extracts were washed with water (3 × 100 mL) then brine (50 mL) and dried over sodium sulfate. The solvent was evaporated to obtain the diketone **17** (47 mg, 94% theoretical): oil; UV (MeOH) 271 nm ($\epsilon$ 3400), 297 ($\epsilon$ 2500); IR ($CHCl_3$) 1720, 1665 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.19 (t, 3 H, $J$ = 7 Hz), 2.94 (q, 2 H, $J$ = 7 Hz), 3.71 (s, 3 H), 3.76 (s, 3 H), 7.20 (d, 1 H, $J$ = 2 Hz), 7.31 (d, 1 H, $J$ = 2 Hz); mass spectrum, $m/z$ 223, 183, 166, 149, 137; high-resolution mass spectrum, obsd $m/z$ 223.0846, $C_{11}H_{13}NO_4$ requires 223.0844.

A solution of the diketone **17** (349 mg, 1.57 mmol) in dry dimethylformamide (1 mL) was added over a 20-min period to a stirred suspension of sodium hydride (prewashed with benzene) (112 mg of 60% dispersion, 2.8 mmol) in dry dimethylformamide (35 mL) at 80 °C. The deep red reaction mixture was stirred for 30 min., cooled, and poured onto a mixture of 3 N hydrochloric acid (25 mL) and ice (100 g). The resulting yellow solution was extracted with dichloromethane (4 × 200 mL), the combined extracts were dried over sodium sulfate, and the solvent was evaporated to obtain a yellow oil (247 mg). The product was chromatographed on Sephadex LH-20 using 1:1 methanol–dichloromethane as eluant to obtain the starting diketone **17** (107 mg) and the isoindole **18** (63 mg, 30% theoretical, based on starting material consumed): mp 149–150 °C; UV (MeOH) 232 nm ($\epsilon$ 2890), 278 ($\epsilon$ 2580), 338 ($\epsilon$ 680); IR ($CHCl_3$) 3200 (br), 1660, 1615 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.99 (s, 3 H), 3.74 (s, 3 H), 7.15 (d, 1 H, $J$ = 2 Hz), 7.23 (d, 1 H, $J$ = 2 Hz), 7.31 (bs, 1 H); mass spectrum, $m/z$ 191, 164, 163, 152, 135; high-resolution mass spectrum, obsd $m/z$ 191.0582, $C_{10}H_9NO_3$ requires 191.0582.

An ethereal soluton of diazomethane was added dropwise to a cooled solution of the isoindole **18** (6 mg, 0.031 mmol) in 1:1 dichloromethane–ether (5 mL) at 0 °C until approximately half of the starting material had reacted, as judged by TLC. The reaction mixture was allowed to warm to room temperature and the solvent was evaporatd to obtain a yellow solid. The product was chromatographed on Partisil using 5% acetone in ether to obtain starting isoindole **18** (3.6 mg, 60% recovery) and isoindole **10** (2.5 mg, 40% theoretical, quantitative conversion), identical in all respects with the natural product.

**Antimicrobial Screening Data.** Paper disks (6.5-mm diameter) bearing 100, 50, and 10 $\mu$g of the compounds to be tested were placed on agar plates preseeded with a test organism. The plates were incubated at 37 °C for *Staphylococcus aureus* (Sa), *Bacillus subtilis*, (Bs), *Escherichia coli* (Ec), *Candida albicans* (Ca), *Pseudomonas aeriginosa* (Pa), and *Enterobacter aerogenes* (Ea) or at 30 °C for *Vibrio anguilarium* (Va) and B-392, a marine pseudomonad. After 24 h, the diameter of a zone of inhibition was recorded. [Compound: organism (quantity ($\mu$g) on disk) diameter (mm) of zone of inhibition.] Renierone (**1**): Sa (10) 8, Bs (10) 10, Ec (10) 8. Mimosamycin (**3**): Sa (50) 14, Bs (50) 11, Ca (50) 9, Va (10) 11, B-392 (10) 10. *N*-Formyl-1,2-dihydrorenierone (**7**): Sa (50) 9, Bs (10) 10, Ec (10) 9. *O*-Demethylrenierone (**8**): Sa (100) 12, Bs (100) 8, Va (100) 9. 1,6-Dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**9**): Sa (50) 18, Bs (10) 11, Ca (50) 8, Va (10) 8, B-392 (50) 10. 2,5-Dimethyl-6-methoxy-4,7-dihydroisoindole-4,7-dione (**10**): Sa (100) 9, Bs (50) 9, Va (50) 9, B-392 (100) 9. Renieramycin A (**11**): Sa (10) 14, Bs (10) 10. Renieramycin B (**12**): Sa (50) 9, Bs (10) 8, Va (100) 9. Renieramycin C (**13**): Bs (10) 8. Renieramycin D (**14**): Sa (100) 8, Bs (10) 8.

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