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RENIERAMYCIN G, A NEW ALKALOID FROM THE SPONGE *XESTOSPONGIA CAYCEDOI*

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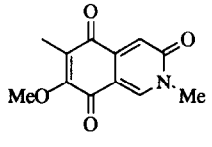
Abstract: A new cytotoxic alkaloid, renieramycin G (**8**), was isolated, along with previously reported metabolites mimosamycin (**1**), renierol (**2**), and *N*-formyl-1,2-dihydrorenierone (**3**), from the Fijian sponge *Xestospongia caycedoi*. The structure of renieramycin G was deduced from spectral data.

Isoquinoline quinones such as mimosamycin (**1**), renierol (**2**), and *N*-formyl-1,2-dihydrorenierone (**3**) belong to a group of compounds which have been isolated both from marine sponges and from terrestrial bacteria.[1] Typically, they have been isolated together with their dimeric analogues. Sponges have been the source of eight dimeric alkaloids, including renieramycins A-F (ie. renieramycins A (**4**), C (**5**), and D (**6**))[2,3] and xestamycin,[4] which show striking similarity to the *Streptomyces* bacterial metabolites the saframycins (ie. saframycin A (**7**))[5] and the safrins.[1] The occurrence of such closely related metabolites in both sponges and bacteria has led to speculation that the renieramycins are produced by an epiphytic or symbiotic bacterium. Further interest in the dimeric compounds has been fueled by their potent antineoplastic activity.



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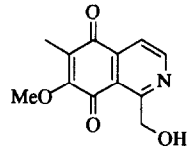

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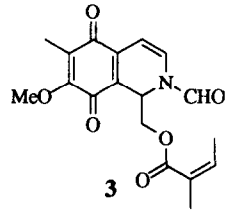

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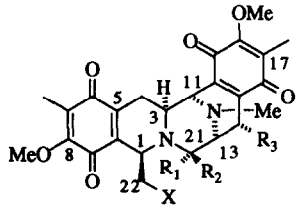
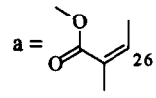
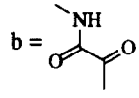
Recently, mimosamycin (**1**) and renierol (**2**) were isolated from the hard blue Fijian sponge *Xestospongia caycedoi* [6] in the absence of corresponding dimers.[7] A more in-depth chemical analysis of this sponge was undertaken, resulting in the isolation of renieramycin G, a new dimeric renieramycin-type alkaloid, depicted below as structure **8**. The re-isolation of mimosamycin (**1**) and renierol (**2**), as well as the isolation of *N*-formyl-1,2-dihydrorenierone (**3**), was also accomplished.

The reinvestigation of *X. caycedoi* was initiated after a preliminary extract (CH_2Cl_2/isopropanol, 1:1) exhibited potent cytotoxicity towards KB and LoVo cell lines with MIC's of 0.08 and 0.0008 μg/mL, respectively. Extraction of the freeze-dried sponge tissue afforded a crude extract (11.94 g) which was subject to a solvent partition scheme to give a biologically active chloroform fraction (0.33 g). Silica gel flash

chromatography (solvent gradient from chloroform to methyl alcohol) provided two primary fractions. The more lipophilic was subjected to reverse-phase (C18) flash chromatography (CH_3CN/H_2O gradient) resulting in the isolation of mimosamycin (**1**, 0.9 mg), renierol (**2**, 16.5 mg), and *N*-formyl-1,2-dihydrorenierone (**3**, 6.1 mg), each of which were identified through a comparison of their ^1H NMR data with that reported in the literature. Purification of the major components of the more polar fraction was hampered by considerable sample decomposition; however, after extensive chromatography, including Sephadex LH-20 ($CH_2Cl_2/MeOH$, 1:1), reverse-phase C18 flash ($MeOH/H_2O$), and reverse-phase HPLC (Rainin Dynamax-60A C18, 10x250 mm, CH_3CN/H_2O, 7:3), an unstable minor metabolite, renieramycin G (**8**), was isolated.[8]







4 R_1=R_2=H R_3=OH X=a
5 R_1,R_2=O R_3=OH X=a
6 R_1,R_2=O R_3=OEt X=a
7 R_1=CN R_2=R_3=H X=b
8 R_1,R_2=O R_3=H X=a

The NMR data was very characteristic of a renieramycin-type alkaloid. The ^{13}C NMR spectrum (Table 1) contained signals for four quinone carbonyls (186.64, 185.58, 182.90, and 186.64 ppm) and an ester carbonyl (167.30 ppm), as well as 10 signals assignable to olefinic or aromatic carbons and 12 aliphatic carbons. The ^1H NMR spectrum (Table 1) showed signals for an N-methyl group, two arylmethoxy groups, two arylmethyl groups, and an angelate ester. The presence of an amide carbonyl signal in the ^{13}C spectrum, together with the broad singlet at δ 5.40 (H1) in the ^1H spectrum was consistent with a structure similar to renieramycin C (**5**) or D (**6**).

Although the FAB mass spectrum exhibited only a very weak $[M+H]^+$ ion at *m/z* 565, a strong $[M+3H]^+$ ion at *m/z* 567, which is characteristic of quinone moieties and is also consistent with results reported for related compounds,[9] was apparent.[10] These results, together with the NMR data, supported a molecular formula of $C_{30}H_{32}N_2O$, 16 mass units less than that reported for renieramycin C; therefore, structure **8**, bearing an amide carbonyl at C21 and lacking the C14 hydroxyl of renieramycin C, can be assigned to renieramycin G.

The COSY spectrum of **8** supported the proposed structure, clearly defining four isolated spin systems (H1, H22A, H22B; H3, H4A, H4B, H11; H13, H14A, H14B; and angelate ester H26, Me15, Me26). The first three of these may be interconnected through the homoallylic coupling of H1 and H4B (J=1.9 Hz) and the W-coupling of H11 and H13. The HMBC experiment (Table 1) allowed the unambiguous assignment of almost all of the quaternary carbons, confirming the proposed structure

Based on comparisons of the ^1H NMR data with results reported in the literature, the relative stereochemistry of compound **8** is proposed to be identical to that determined for the saframycins[11] and that proposed for all of the previously reported renieramycins.[2] The high-field chemical shift of H4B at δ 1.49 and the strong homoallylic coupling between H4B and H1 requires both protons to be directed perpendicular to the quinone ring. Attempts to measure dipolar coupling between H1 and H3 met with unsatisfactory results.

Although the crude extract exhibited potent cytotoxicity, compound **8** was only moderately active, exhibiting MIC values of 0.5 and 1.0 μg/mL against the KB and LoVo cell lines, respectively. Apparently, oxidation of C21 to the amide may provide added stability relative to the carbinolamine found in related compounds, such as saframycins B and S,[1] renieramycins E and F,[3] and the ecteinascidins,[12,13] allowing easier isolation but decreased biological activity.

Table 1. NMR Assignments of Renieramycin G (**8**)[a]

Atom no.	^{13}C NMR		^{1}H NMR (mult., integral, *J* (Hz))	LR ^{13}C to ^{1}H corr.
1	50.52		5.40 (brs, 1H)	H22
3	56.63		3.85 (dt, 1H, 12.2, 3.0)	H11
4	26.09	A	3.01 (dd, 1H, 16.5, 3.0)	
		B	1.49 (ddd, 1H, 16.5, 12.2, 1.6)	
5	142.15			H4A
6	185.58			H4A, ArMe
7	129.63†			
8	155.99			ArMe, OMe (3.98)
9	180.84			
10	136.59			H1, H4, H22A, H22B
11	53.2[d]		4.12 (brd, 1H, 4.0)	H13, NMe
13	59.60		3.67 (d, 1H, 7.1)	H11, H14A, H14B, NMe
14	24.00	A	2.87 (dd. 1H, 20.6, 6.1)	H13
		B	2.64 (d, 1H, 20.6)	
15	142.56			H11, H13, H14A, H14B
16	186.64			H14B, ArMe
17	128.79†			ArMe
18	156.56			ArMe, OMe (4.01)
19	182.90			H11
20	135.45			H11, H14A, H14B
21	170.71			H13, H14A, H14B
22	63.34	A	4.67 (dd, 1H, 11.7, 2.8)	
		B	4.32 (dd, 1H, 11.7, 2.6)	
24	167.30			H22A, H22B, Me (25)
25	127.26			Me (25), Me (26)
26	139.50		5.90 (m, 1H)	Me (25), Me (26)
Me (25)	15.60		1.52 (t, 3H, 1.6)	
Me (26)	20.51		1.68 (dq, 3H, 7.3, 1.6)	H26
Ar Me	8.76		1.93 (s, 6H)	
OMe	61.28§		4.01 (s, 3H)	
OMe	61.24§		3.98 (s, 3H)	
NMe	40.06		2.36 (s, 3H)	H11, H13

[a]All data were recorded in CD_2Cl_2 and to the solvent signal (5.32 ppm for ^{1}H, 53.8 ppm for ^{13}C); [b]Measured at 500 MHz; [c]Measured at 125 MHz; [d]Approximate chemical shift--signal was under solvent peak; §,†Signals are interchangeable.

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8. UV (MeOH) λ_{max} 269 nm (ε 20,000); IR (film) 2923, 1713, 1658, 1652, 1308, 1230, 1150 cm^{-1}; NMR data given in Table 1.

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10. Also apparent in the FAB mass spectrum were ions at *m/z* 599, assigned to $[M+3H+MeOH]^+$, and *m/z* 581, which can be assigned to $[M+3H+MeOH-H_2O]^+$.

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