HETEROCYCLES, Vol. 55, No. 1, 2001, pp. 21 - 28, Received, 10th October, 2000

^{13}C NMR SPECTRAL ASSIGNMENT OF 5-HYDROXY-1,5-IMINO-3-BENZAZOCIN-4,7,10-TRIONE DERIVATIVES: THE REVISED STRUCTURE OF RENIERAMYCIN H

Naoki Saito, [a]* Haruyuki Sakai,[a] Kanit Suwanborirux,[b]
Sunibhond Pummangura,[b] and Akinori Kubo[a]*

[a]Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

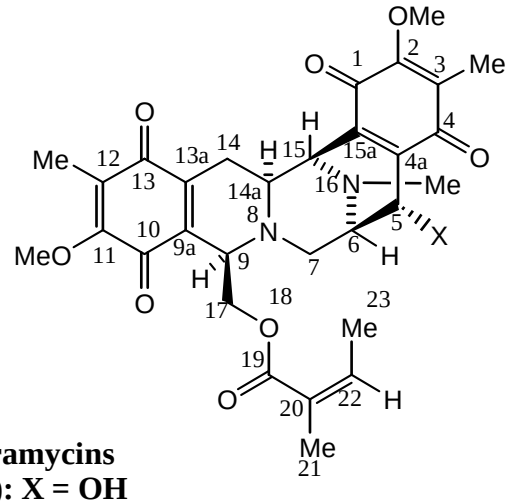
[b]Faculty of Pharmaceutical Sciences, Chularongkorn University, Phyathai Road, Bangkok 10330, Thailand

Tel & Fax: (81)-424-95-8792; E-mail: kubo@my-pharm.ac.jp

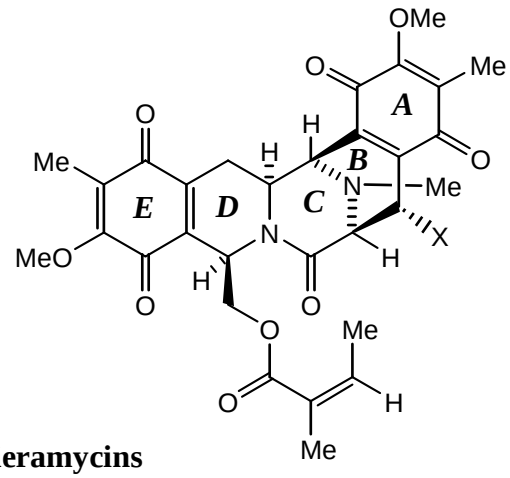
Abstract-^{13}C NMR assignments of the ABC ring model compounds of saframycin H with the proposed structure were performed using a two-dimensional ^{1}H detected heteronuclear multiple-bond correlation (HMBC) experiment. The structure of renieramycin H was reassigned with the revised structure.

During the past two decades, renieramycins have emerged as a class of natural marine products with significant biologic activity (Figure 1)[1] and a structure striking similar to the *Streptomyces* bacterial metabolites, the saframycins.[2] The detailed ^{1}H NMR spectral analysis of this family is a useful tool in structure investigation.[3] The ^{13}C NMR spectral analysis is also an excellent method for structure elucidation, but there are many difficulties, especially in the field of the natural marine products because of the very small amounts of substances available from the marine animals and of the inherent

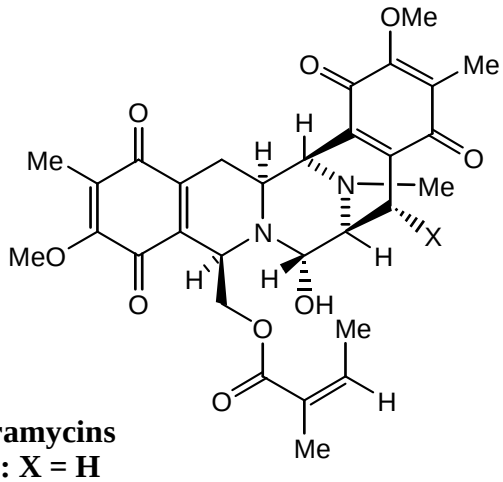
instability of the molecules.[4] Recently, Parameswarene *et al*. reported a new type of alkaloid, renieramycin H (**2**), which was isolated from the methanol extract of the bright blue sponge *Haliclona cribricutis* along with renieramycin I (**1i**) (Figure 2).[5] Renieramycin H (**2**) possesses a unique chemical structure and is the first reported dimeric isoquinolinequinone natural product with a hydroxyl group at the bridge head position 6. During the course of our studies on the chemistry of saframycins, we have recently reported the preparation of the ABC ring model compounds (**3a-c**) of **2** with a hydroxyl group at that position.[6] Comparison of ^{1}H NMR and ^{13}C NMR spectral data of **2** with those of the ABC ring model compound (**3a**), led us to propose the revised structure of **2** to be **1h**. ¶



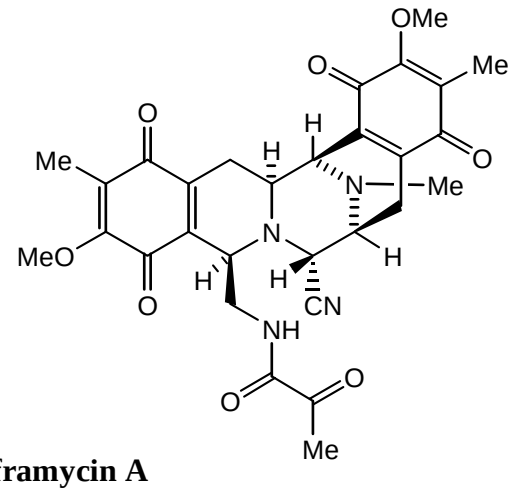
renieramycins
A (1a): X = OH
B (1b): X = OEt



renieramycins
C (1c): X = OH
D (1d): X = OEt
G (1g): X = H

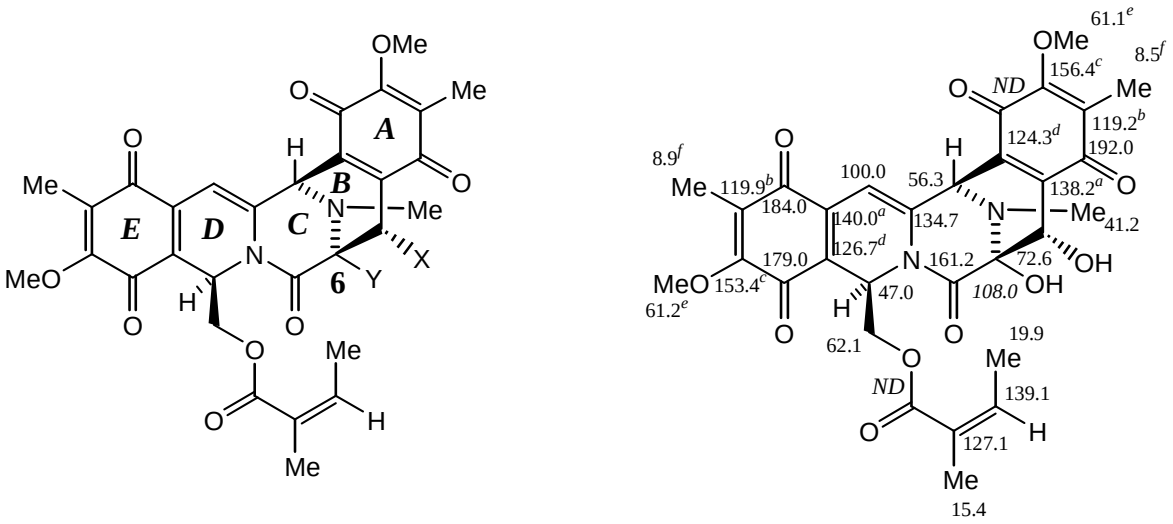


renieramycins
E (1e): X = H
F (1f): X = OMe



saframycin A

Figure 1



renieramycins
H (2): X = Y = OH
I (1i): X = OMe, Y = H

ND: not detected
a-f: assignments may be interchangeable.

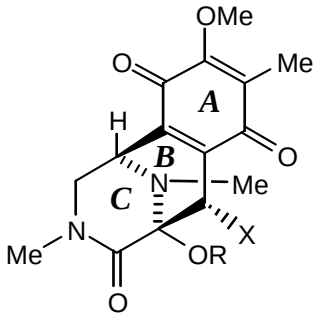
Figure 2. The original structure of renieramycins H and I together with ^{13}C NMR assignments of **2**.

In the case of renieramycin H, a ^{13}C NMR spectral signal at *108.0* ppm was assigned to the bridged head C-6 carbon of structure (**2**). The unusual chemical shift at the C-6 carbon could not be readily explained, but we suspect it was an aminal carbon linked to amide carbonyl. We had already prepared the ABC ring model compound (**3a**).[6, 7] We were able to assign ^{13}C NMR spectral resonance for all carbons of **3a** through a series of HMBC experiments. The quaternary carbon at C-6 appears at *85.6* ppm (Table 1). Particularly convincing evidence is found in the comparison of the two hydroxyl proton chemical shifts (5.70 and 11.34 ppm) with the hydroquinones (**4a**) (5.58 and 11.52 ppm), (**4b**) (5.45 and 11.85 ppm), and (**4c**) (5.61 and 11.82 ppm), which were the ABC model compounds of saframycin D.[8] These results indicated that renieramycin H is *not* a dimeric isoquinolinequinone. This assignment was supported by comparison of ^{13}C NMR chemical shifts of the six carbons at the A ring, including C-4a in **4a** (*109.8* ppm) with the corresponding resonance of saframycin D and renieramycin H (Table 2). Thus, the structure of renieramycin H must be **1h**. The relative stereochemistry at C-9 could not be determined from the data. While this paper was under review, Pettit and co-workers has discovered cribrostatin 4 from the blue sponge *Cribrochalina* sp. collected in reef passages in the Republic of Maldives, the structure of which was determined by an X-Ray crystallography.[9] The NMR spectra of renieramycin H (structure **1h**) and cribrostatin 4 were identical. Accordingly, renieramycin H is now

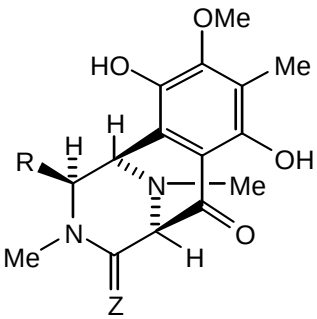
assigned structure (**1h**) (Table 3 and 4).[10]

Table 1: NMR assignments of the ABC model compounds (**3a-c**) in $CDCl_3$.

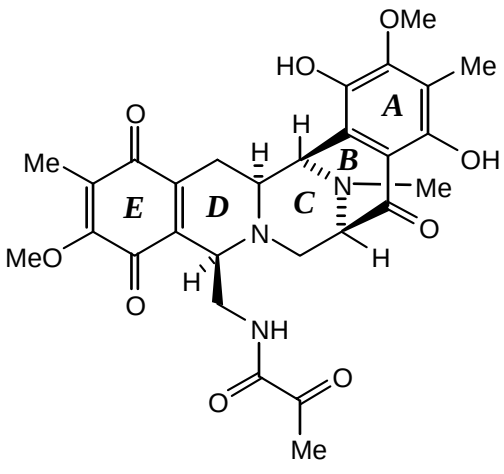
	3a				**3b**		**3c**	
atom no.	^{13}C NMR	mult.	^{1}H NMR (mult. Integral, *J* (Hz))	correlations from C				
1	182.5	s		15-H	182.4	s	182.4	s
2	155.4	s		2-OMe, 3-Me	155.4	s	155.4	s
3	130.3	s		3-Me	129.6	s	129.7	s
4	186.1	s		3-Me, 5-H	186.0	s	185.8	s
4a	139.3	s		5-H, 15-H	141.0	s	140.5	s
15a	137.3	s		5-H, 14a-Hβ, 15-H	135.6	s	135.7	s
5	73.1	d	4.03 (s, 1H)	5-OMe	28.7	t	25.6	s
6	85.6	s		5-H, 15-H, 16-Me	83.0	s	87.8	s
7	169.3	s		5-H, 8-Me, 14a-Hα	170.2	s	168.4	s
14a	49.9	t	2.94 (dd, 1H, 12.5, 1.0)	8-Me, 15-H	52.1	t	52.1	t
			3.04 (dd, 1H, 12.5, 5.3)					
15	52.9	d	4.23 (dd, 1H, 5.3, 1.0)	14a-H_2, 16-Me	53.7	d	54.5	d
2-OMe	61.0	q	4.00 (s, 3H)		61.0	q	61.0	q
3-Me	8.9	q	2.00 (s, 3H)		8.8	q	8.9	q
8-Me	34.8	q	2.88 (s, 3H)		34.8	q	35.0	q
16-Me	36.1	q	2.59 (s, 3H)		34.6	q	34.4	q
5-OMe	62.4	q	3.74 (s, 3H)	5-H	———		———	
6-OAc	———				———		21.1	q
							166.4	s



3a: X = OMe, R = H
3b: X = R = H
3c: X = H, R = Ac



4a: R = H, Z = O
4b: R = H, Z = H_2
4c: R = Me, Z = O



saframycin D

Table 2. ^{13}C NMR spectra of the 12 carbons at the A and E rings.

atom no.	**4a**	**4b**	**4c**	saframycin D	renieramycin H revised structure (**1h**)
1	137.8	137.8	139.0	139.3	138.2
2	153.4	152.3	156.2	153.3	153.4
3	118.5	117.2	117.9	118.6	119.9
4	155.8	154.5	153.3	154.8	156.4
4a	109.8	112.8	109.1	112.2	108.0
15a	120.7	122.3	118.9	118.3	119.2
9a				136.6	134.7
10				181.2	179.0
11				156.3	not detected
12				127.5	127.1
13				186.1	184.0
13a				141.7	140.0

Table 3. ^{1}H NMR assignments of renieramycin H, cribrostatin 4, and saframycin D.

Proton	renieramycin H[1] revised structure (**1h**)	cribrostatin 4[2]	saframycin D[3]
6	4.10 (d, 1.3)	4.10*	3.27 (ddd, 2.7, 2.7, 0.5)
7			2.92 (dd, 10.5, 2.7)
			3.28 (dd, 10.5, 2.7)
9	6.20 (dd, 6.3, 2.7)	6.18	3.67 (ddd, 3.7, 2.7, 1.4)
14	6.26 (s)	6.22	1.57 (ddd, 17.8, 10.5, 2.7)
			2.97 (dd, 17.8, 2.0)
14a			2.93 (ddd, 10.5, 2.7, 2.0)
15	4.80 (d, 1.3)	4.85*	4.35 (dd, 2.7, 0.5)
17	3.82 (dd, 11.8, 3.1)	3.81	3.06 (ddd, 14.1, 3.7, 3.7)
	4.05 (dd, 11.8, 3.1)	4.04	3.70 (ddd, 14.1, 9.7, 1.4)
2-OMe	3.85	3.84	3.94
11-OMe	4.05	4.04	4.02
3-Me	2.15	2.14	2.15
12-Me	1.95	1.93	1.89
16-Me	2.56	2.55	2.42
NH			6.28 (dd, 9.7, 3.7)
21	1.46 (d, 1.5)	1.45	2.26
22	5.91 (qq, 7.3, 1.5)	5.90 (q)	
22-Me	1.74 dq (7.3, 1.5)		
1-OH	5.70		5.53
4-OH	11.34		11.88

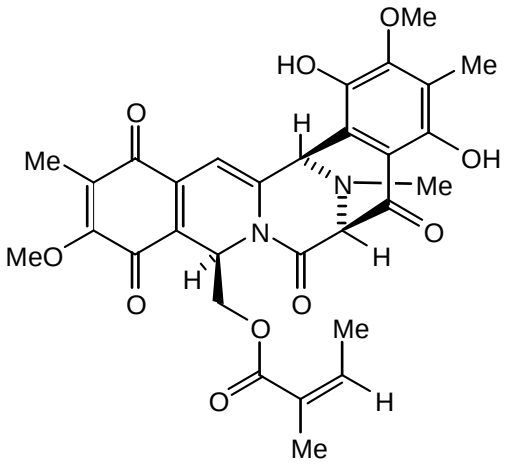
1: at 400 MHz (in $CDCl_3$) (ref. 4).
2: at 500 MHz (in $CDCl_3$). No data for two hydroxy protons presented. 6-H and 15-H assignments were exchanging (ref. 7).
3: at 400 MHz (in $CDCl_3$) (ref. 6).

In our knowledge, renieramycin H (= cribrostatin 4) is the first example of a naturally occurring

Table 4. ^{13}C NMR assignments of renieramycin H, cribrostatin 4, and saframycin D.

atom no.	renieramycin H revised structure (**1h**)	cribrostatin 4	saframycin D
1	138.2 s	138.5 s	139.3 s
2	153.4 s	153.3 s	153.3 s
3	119.9 s[a]	119.8 s	118.6 s
4	156.4 s	156.2 s	154.8 s
4a	108.0 s	108.6 s[b]	112.2 s
15a	119.2 s[a]	119.2 s[b]	118.3 s
5	192.0 s	192.7 s	203.4 s
6	72.6 d	72.5 d	65.5 d
7	161.2 s	161.1 s	54.7 t
9	47.0 d	46.9 d[c]	57.6 d
9a	134.7 s	134.6 s	136.6 s
10	179.0 s	179.9 s	181.2 s
11	not detected	156.3 s	156.3 s
12	127.1 s	127.1 s	127.5 s
13	184.0 s	185.0 s	186.1 s
13a	140.0 s	139.8 s	141.7 s
14	100.0 d	100.0 d	24.5 t
14a	124.3 s	124.2 s	56.9 d
15	56.3 d	56.2 d[c]	57.4 d
2-OMe	61.1 q	61.1 q	60.9 q
10-OMe	61.2 q	61.2 q	61.0 q
3-Me	8.9 q	9.0 q	8.9 q
11-Me	8.5 q	8.6 q	8.6 q
16-Me	41.2 q	41.2 q	42.3 q
17	62.1 t	62.0 t	40.8 t
19	not detected	166.8 s	160.3 s
20	127.1 s	126.6 s	195.8 s
21	19.9 q	19.9 q	24.3 q
22	139.1 d	139.3 d	
23	15.4 q	15.4 q	

a Assignments may be interchanged.
b, *c* Original assignments were revised.



renieramycin H (1h)
revised structure
(= cribrostatin 4)

monomeric isoquinolinequinone natural product from marine sources.[11, 12] The synthesis of renieramycin H (cribrostatin 4) is in progress to evaluate its pharmacologic potency.

ACKNOWLEDGMENTS

We are grateful for financial support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan.

REFERENCES AND NOTES

¶ Presented at the 42nd Symposium on the Chemistry of Natural Products (Naha, Japan), November 6-8, **2000**.

1 a) J. M. Frincke and D. J. Faulkner, *J. Am. Chem. Soc*., 1982, **104**, 265 b) H. He and D. J. Faulkner, *J. Org. Chem.*, 1989, **54**, 5822. c) B. S. Davidson, *Tetrahedron,* 1992, **33**, 3721.

2 Reviews: a) T. Arai and A. Kubo, 'The Alkaloids,' Vol. 21, ed. by A. Brossi, Academic Press, Inc., New York, 1983, pp. 55-100. b) A. Kubo and N. Saito, 'Studies in Natural Products Chemistry,' Vol. 10, ed. by Atta-ur-Rahman, Elsevier, Inc., Amsterdam, 1992, pp. 77. c) T. Ozturk, 'The Alkaloids,' Vol. 53, ed. by G. A. Cordell, Academic Press, New York, 2000, pp. 119-238.

3 J. W. Lown, A. V. Joshua, and H. H. Chen, *Can. J. Chem.*, 1981, **59**, 2945.

4 Among renieramycins A-I, renieramycin G is the only one in which all of the carbon signals have been detected, and the only one for which the complete ^{1}H and ^{13}C NMR spectral assignments been reported by the application of ^{1}H-detected HMBC experiments.[1c]

5 P. S. Parameswaran, C. G. Naik, S. Y. Kamat, and B. N. Pramanik, *Indian J. Chem.*, 1998, **37B**, 1258.

6 N. Saito, H. Sakai, E. Takai, R. Muranaka, M. Itabashi, A. Kubo, K. Yazawa, and Y. Mikami, *Heterocycles*, 1997, **46**, 309.

7 For simplicity, IUPAC names and numbering of natural products are used in this paper.

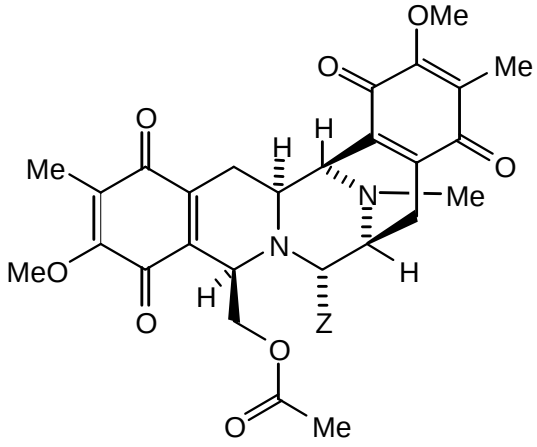
8 a) A. Kubo, N. Saito, Y. Kitahara, K. Takahashi, K. Yazawa, and T. Arai, *Chem. Pharm. Bull.*, 1987, **35**, 440. b) N. Saito, Y. Ohira, N. Wada, and A. Kubo, *Tetrahedron*, 1990, **46**, 7711. c) N. Saito, M.

Tanitsu, T. Betsui, R. Suzuki, and A. Kubo, *Chem. Pharm. Bull.*, 1997, **45**, 1120.

9 G. R. Pettit, J. C. Knight, J. C. Collins, D. L. Herald, R. K. Pettit, M. R. Boyd, and V. G. Young, *J. Nat. Prod.*, 2000, **63**, 793.

10 The molecular formula $C_{30}H_{30}N_2O_{11}$ (this formula is not consistent with our conclusion), which is based on an ion at *m/z* 594 (electron impact mass spectrometry: EIMS), still needs to be addressed. We believe that the HRMS measurement of the $(M + 2H)^+$ peak at *m/z* 580.2066 and the presence of the $(M + H)^+$ peak at *m/z* 579 let to the correct molecular formula of $C_{30}H_{30}N_2O_{10}$.

11 Recently, a new dimeric isoquinolinequinone named jorumycin was isolated from the mantle and mucus of the Pacific Nudibranch *Jorunna funebris*. A. Fontana, P. Cavaliere, S. Wahidulla, C. G. Naik, and G. Cimino, *Tetrahedron*, 2000, **56**, 7305. We have already prepared (+/-)-7-deshydroxyjorumycin (**5**) from the key intermediate in our saframycin synthesis.[12a, b]



jorumycin: Z = OH
compound 5: Z = H

12 a) N. Saito, R. Yamauchi, and A. Kubo, *Heterocycles*, 1991, **32**, 1203. b) For previous work on the synthesis of the renieramycins, see: T. Fukuyama, S. D. Linton, and M. M. Tun, *Tetrahedron Lett.*, 1990, **31**, 5989.