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CHEMISTRY OF ANTITUMOR ISOQUINOLINEQUINONE ALKALOIDS: UNEXPECTED OXIDATIVE DEGRADATION OF SAFRAMYCIN S TO GENERATE SIMPLE ISOQUINOLINE ALKALOIDS, MIMOSAMYCIN AND MIMOCIN [1,2]

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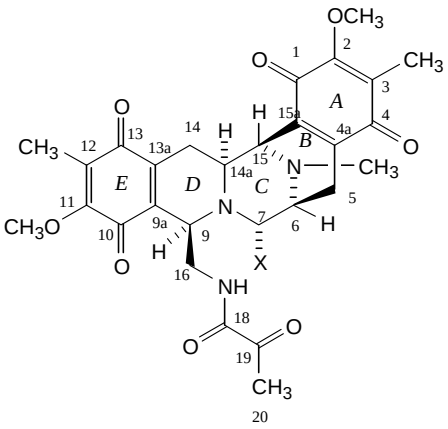
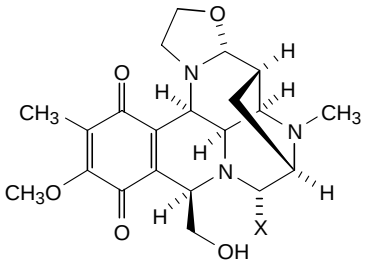
Abstract- Saframycin S, prepared by treating saframycin A with silver nitrate in aqueous acetonitrile, was transformed into simple isoquinolinequinones, mimosamycin and mimocin, by treatment with a catalytic amount of trifluoroacetic acid (TFA) in chloroform. The proposed mechanism for this oxidative degradation is presented.

Saframycins are a class of tetrahydroisoquinoline antibiotics that exhibits activity against gram-positive bacteria and several kinds of tumor. Over the last thirty years, a number of saframycin derivatives have been independently isolated from bacterial sources as well as marine sponges and tunicates.[3] Such natural products as saframycin S (**1b**), naphthyridinomycin (**2b**), and ecteinascidin 743 (**3b**) possess a

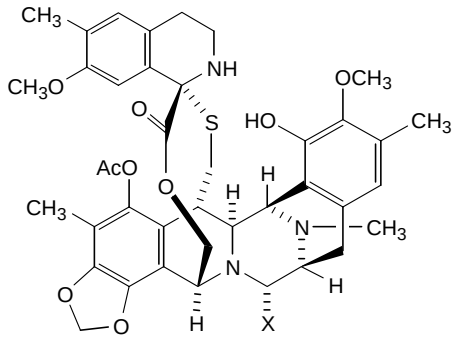
hydroxyl group at C-7[4] and display the most potent antitumor activity. However, one of the most intriguing problems is that this type of natural products is available only in very minute quantities (Figure 1). Arai and his group reported that the treatment of **1b** with sodium cyanide in phosphate buffer led to the formation of saframycin A (**1a**), which was also isolated as a natural product from a culture of *Streptomyces lavendulae*.[5] This simple yet extremely beneficial process has provided a major breakthrough for such highly unstable natural products. Indeed, cyanocycline A (**2a**) and ecteinascidin 770 (**3a**) were chosen as the principal targets for the evaluation of new anticancer drugs and for total synthesis.[6, 7] The presence of either a cyano or a hydroxyl group at C-7 is undoubtedly essential for good activity.[8]



saframycins
A (**1a**): X = CN
S (**1b**): X = OH



cyanocycline A
2a: X = CN
naphthyridinomycin
2b: X = OH

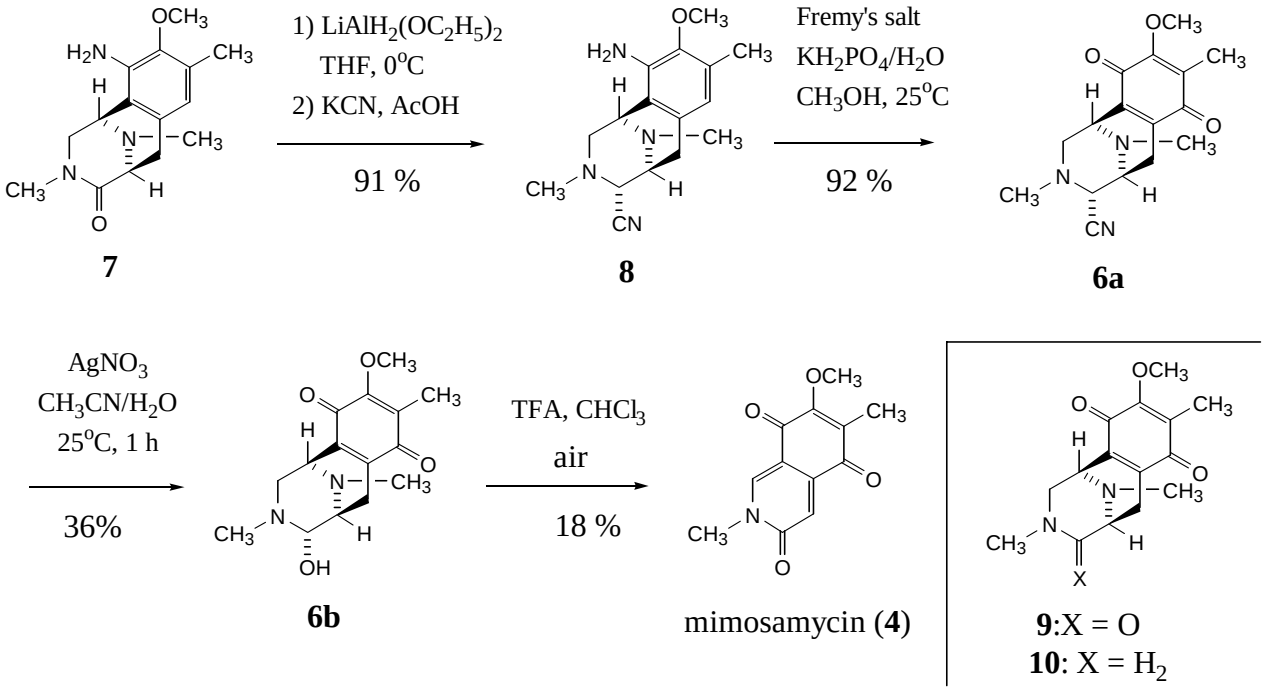


ecteinascidins
770 (**3a**): X = CN
743 (**3b**): X = OH

Figure 1

Mimosamycin (**4**) and mimocin (**5**) were isolated from *Streptomyces lavendulae* and showed antimicrobial activity particularly against mycobacteria.[9] Because mimosamycin and related compounds have been isolated not only from sponge but also from bryozoan, it is suggested that the unusual cross-phyletic distribution of isoquinolinequinone natural products is due to symbiotic microorganisms. These two simple isoquinolinequinone compounds have been prepared by synthesis;[10, 11] however, there are scant data on the biosynthesis of these two types of compounds. As part of that extended study, an

in-depth chemical analysis of saframycin S (**1b**) was undertaken, resulting in the preparation of mimosamycin (**4**) and mimocin (**5**) as oxidative degradation products.

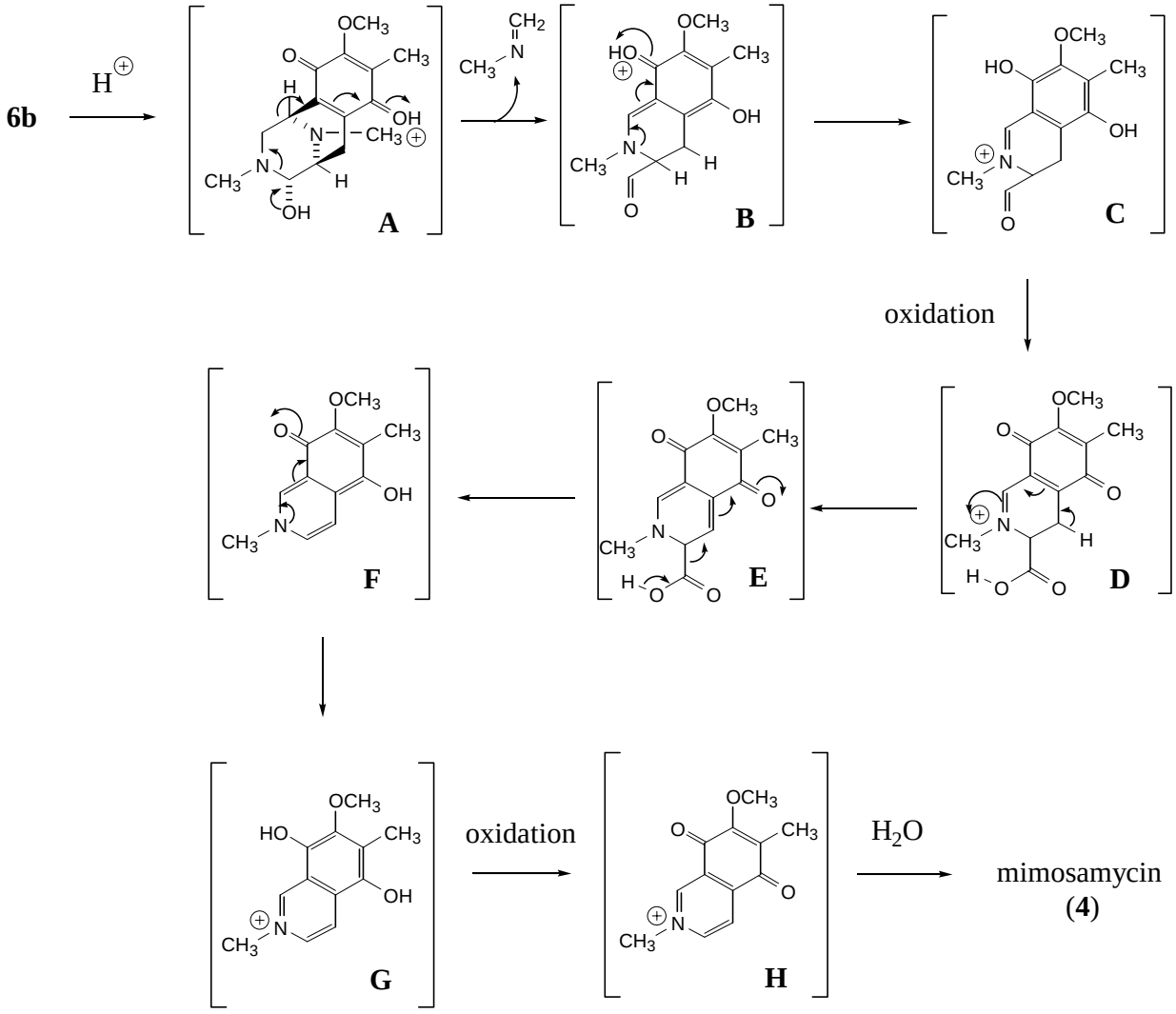


Scheme 1

As a model compound, we selected the *p*-quinone (**6a**)[12] that was prepared in 84% overall yield from the readily available aniline derivative (**7**)[13] by partial reduction followed by KCN treatment and oxidation of the resulting α-amino nitrile compound (**8**) (mp 146-147°C) with Fremy's salt (Scheme 1). Treatment of **6a** with a large excess of silver nitrate in aqueous acetonitrile at room temperature for 1 h gave the α-amino alcohol (**6b**)[14] in quantitative yield; however, the isolation yield was surprisingly low (36%) because **6b** was unstable and generated the amide (**9**)[15] (14%) during purification by flash column chromatography. The ^{13}C NMR signal of the carbinolamine-containing carbon (C-7) of **6b** was shifted downfield to δ 85.8 ppm compared to that of **6a** at δ 61.0 ppm. The observed NOE between 5-Hβ (δ 2.13) and 7-H (δ 4.10) revealed the relative stereochemistry at C-7. Treatment of **6b** with a catalytic amount of TFA in chloroform at room temperature for 3 days afforded mimosamycin (**4**) in 18% yield,

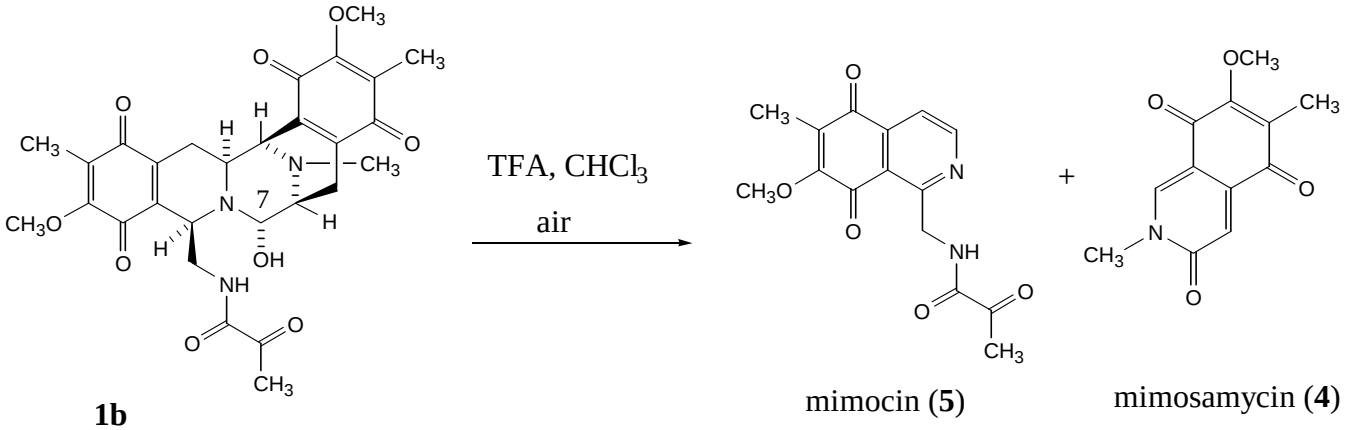
along with **9** and **10**[15] in 29.5% and 25.7% yields, respectively. The synthetic mimosamycin (mp 223-224^{o}C) was identical with an authentic sample in terms of TLC behavior and spectroscopic properties.

Although the mechanism of the oxidative degradation is not clear, one possible explanation for the cleavage of C14a-C15 bond is the protonation of the quinone ring to generate the hydroquinone intermediate (**C**). The following steps including oxidative decarboxylation are unclear at this stage (Scheme 2). Since the α-amino alcohol (**6b**) is a masked aldehyde, the formation of **9** and **10** would take place *via* a competitive Cannizzaro type disproportionation reaction.[16]



Scheme 2

Encouraged by the results of model studies, we applied this strategy to the transformation of **1b** into mimosamycin (**4**) and mimocin (**5**). The transformation of **1a** into **1b** with 0.1 M H_2SO_4 at 120°C for 40 min was reported to give in 40% yield;[5] however, the best result was obtained by reacting of **1a** with 25 equiv. of silver nitrate in aqueous acetonitrile at 40°C for 1 h, which gave rise to a reaction mixture containing only a small amount of side products from which **1b** could be isolated in 83% yield after flash column chromatography. The lack of published data for the natural product (**1b**), particularly MS spectra, prompted us to confirm the structure independently. Saframycin S was obtained as a fairly unstable dark yellow amorphous powder, $[\alpha]^{25}_{D}$ –56.5° (*c* 0.1, $CHCl_3$). High-resolution FABMS spectral measurement of the $(M + H - H_2O)^+$ ion at *m/z* 536.22025 (Δ 0.8 mmu) and observation of the $(M + H)^+$ ion at *m/z* 554 indicated a molecular formula of $C_{28}H_{31}N_3O_9$. HMBC, HMQC, and NOESY spectral experiments of **1b** enabled the unambiguous assignment of almost all of the quaternary carbons and the proposed structure (Table 1). An observed NOE between 5-Hβ and 7-H revealed the relative stereochemistry at C-7. The oxidative degradation of **1b** with a catalytic amount of TFA in chloroform under reflux for 3 h gave **4** and **5** (mp 189-191°C) in 16.7% and 16.2% yields, respectively (Scheme 3).



1b mimocin (**5**) mimosamycin (**4**)

Scheme 3

Table. NMR Spectral Assignments of Saframycin S (**1b**) (all data were recorded in $CDCl_3$).

atom No.	^{13}C NMR δ_{mult}.		^{1}H NMR (mult. integral, *J* (Hz))		correlation from C No.	NOEs correlation
1	182.5	s				
2	156.0	s			3-Me, 2-OMe	
3	127.8	s			3-Me	
4	185.6	s			5-Hα, 3-Me	
4a	135.1	s			5-Hα, 5-Hβ, 15-H	
5	20.9	t	2.74	(dd, 1H, 21.1, 7.6)		5-Hβ, 6-H, N-Me
			2.13	(d, 1H, 21.1)		5-Hα, 7-H, N-Me
6	57.4	d	3.24	(br d, 1H, 7.6)	5-Hα, 15-H, N-Me	5-Hα, 7-H, N-Me
7	82.0	d	4.40	(d, 1H, 2.0)	5-Hα, 5-Hβ, 9-H	5-Hβ, 6-H
9	52.6	d	4.36	(ddd, 1H, 4.0, 2.3, 2.0)		16-H2
9a	141.1	s			14-Hα, 14-Hβ	
10	181.0	s				
11	155.6	s			11-OMe, 12-Me	
12	129.6	s			12-Me	
13	186.9	s			12-Me	
13a	137.3	s			9-H, 14-Hα, 14-Hβ	
14	25.3	t	2.77	(dd, 1H, 17.2, 2.6)		14a-H, 14-Hβ
			1.25	(ddd, 1H, 17.2, 11.6, 2.3)		14-Hα
14a	50.4	d	3.18	(ddd, 1H, 11.6, 2.6, 2.3)	14-Hβ, 15-H	14-Hα, 15-H
15	54.1	d	2.97	(d, 1H, 2.3)	N-Me	14a-H, N-Me
15a	141.0	s			5-Hα, 5-Hβ	
16	41.0	t	3.72	(ddd, 1H, 13.9, 7.6, 2.0)		9-H, 16-H, NH
			3.21	(ddd, 1H, 13.9, 4.0, 1.0)		9-H, 16-H, NH
18	160.1	s			16-H_2, 19-Me	
19	196.7	s			19-Me	
20	24.2	q	2.24	(s, 3H)		
3-Me	8.6	q	1.88	(s, 3H)		
12-Me	8.7	q	1.90	(s, 3H)		
N-Me	41.4	q	2.30	(s, 3H)		5-Hα, 6-H, 15-H
2-OMe	60.8	q	4.01	(s, 3H)		
11-OMe	61.0	q	4.01	(s, 3H)		
NH			6.77	(dd, 1H, 7.6, 1.0)		
OH			survived			

In summary, we have obtained experimental evidence that mimosamycin (**4**) and mimocin (**5**) may be generated from saframycin S (**1b**) by oxidative degradation. These observations represent the first convincing demonstration that all of the simple isoquinolinequinones from marine organisms are

degradation products and/or artifacts of isolation procedures. More detailed studies of oxidative degradation are necessary including disproportionation. Experiments are under way using another type of model compound to fully elucidate these mechanistic possibilities.

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REFERENCES AND NOTES

1 This paper is dedicated to the 30th Anniversary of Heterocycles.
2 Dedicated also to Professor Norio Aimi (Chiba University) on the occasion of his 65th birthday.
3 For a general review, see: 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 Product Chemistry,' Vol. 10, ed. by Atta-ur-Rahman, Elsevier, Inc., Amsterdam, 1992, pp. 77-145. c) T. Ozturk, 'The Alkaloids,' Vol. 53, ed. by G. A. Cordell, Academic Press, New York, 2000, pp. 119-238. J. D. Scott and R. M. Williams, *Chem. Rev*., 2002, **102**, 1669.
4 For simplicity, IUPAC names and numbering of saframycins are employed for all the model compounds in this paper.
5 a) T. Arai, K. Takahashi, K. Ishiguro, and K. Yazawa, *J. Antibiot.*, 1980, **33**, 951. b) Y. Mikami, K. Yokoyama, H. Tabeta, K. Nakagaki, and T. Arai, *J. Pharm. Dyn.*, 1981, **4**, 282.
6 Isolation and structure determination of cyanocycline A: a) M. J. Zmijewski and M. Goebel, *J. Antibiot*., 1982, **55**, 524. b) T. Hayashi, T. Noto, Y. Nawata, H. Okazaki, M. Sawada, and K. Ando, *J. Antibiot*., 1982, **35**, 771. c) T. Hayashi and Y. Nawata, *J. Chem. Soc., Perkin Trans. 2*, 1983, 335. d) S. J. Gould, W. He, and M. C. Cone, *J. Nat. Prod.*, 1993, **56**, 1239. Total synthesis: e) D. A. Evans, C. R. Illig, and J. C. Saddler, *J. Am. Chem. Soc.*, 1986, **108**, 2478. f) T. Fukuyama, L. Li, and R. K. Frank, *J. Am. Chem. Soc*., 1987, **109**, 1587. g) T. Fukuyama, *Adv. Heterocycl., Nat. Prod. Synth.*, 1992, 189.
7 Isolation and structure determination of ecteinascidin 770: a) K. L. Rinehart, T. G. Holt, N. L. Fregeau, J. G. Stroh, P. A. Kieffer, F. Sun, L. H. Li, and D, G. Martin, *J. Org. Chem.*, 1990, **55**, 4512. b) K. Suwanborirux, K. Charupant, S. Amnuoypol, S. Pummangura, A. Kubo, and N. Saito, *J. Nat. Prod.*, 2002, **65**, 935. Total synthesis: c) E. J. Corey, D. Y. Gin, and R. S. Kania, *J. Am. Chem. Soc.*, 1996, **118**, 9202. d) A. Endo, A. Yanagisawa, M. Abe, S. Tohma, T. Kan, and T. Fukuyama, *J. Am. Chem. Soc*., **2002**, 124, 6552.
8 K. E. Rao and L. W. Lown, *Biochemistry*, 1992, **31**, 12076.
9 Isolation and structure determination of mimosamycin from *Streptomyces lavendulae*: a) T. Arai, K. Yazawa, Y. Mikami, A. Kubo, and K. Takahashi, *J. Antibiot.*, 1976, **29**, 398; Y. Mikami, K. Yokoyama, A. Omi, and T. Arai, *J. Antibiot.*, 1976, **29**, 408; H. Fukumi, F. Maruyama, K. Yoshida,

M. Arai, A. Kubo, and T. Arai, *J. Antibiot.*, 1978, **31**, 847; T. Hata, H. Fukumi, S. Sato, K. Aiba, and C. Tamura, *Acta Crystallog. Sec. B.*, 1978, **B34**, 2899; Mimosamycin and related compounds have also been identified in marine organisms, see: J. M. Frincke and D. J. Faulkner, *J. Am. Chem. Soc*., 1982, **104**, 265; T. C. Makee and C. M. Ireland, *J. Nat. Prod.*, 1987, **50**, 754; H. He and D. J. Faulkner, *J. Org. Chem.*, 1989, **54**, 5822; B. S. Davidson, *Tetrahedron Lett.*, 1992, **33**, 3721; G. R. Pettit, J. C. Collins, D. L. Herald, D. L. Doubek, M. R. Boyd, J. M. Schmidt, J. N. A. Hooper, and L. P. Tackett, *Can. J. Chem.*, 1992, **70**, 1170; Y. Venkateswarlu, M. V. R. Reddy, K. V. N. S. Srinivas, and J. V. Rao, *Indian J. Chem., Sect. B*, 1993, **32B**, 704; M. Kobayashi, S. R. Rao, R. Chavakula, and N. S. Sarma, *J. Chem. Res.*, 1994, 282; R. A. Edrada, P. Proksch, V. Wray, R. Christ, L. Witte, R. and W. M. Van Soest, *J. Nat. Prod.*, 1996, **59**, 973; P. S. Parameswaran, C. G. Naik, S. Y. Kamat, and B. N. Pramanik, *Indian J. Chem., Sect. B*, 1998, **37B**, 1258; P. Ramesh, N. S. Reddy, and Y. Venkateswarlu, *J. Nat. Prod.*, 1999, **62**, 780; M. A. Rashid, K. R. Gustafson, and M. R. Boyd, *J. Nat. Prod*., 2001, **64**, 1249. b) Isolation of mimocin: A. Kubo, S. Nakahara, R. Iwata, K. Takahashi, and T. Arai, *Tetrahedron Lett.*, 1980, **21**, 3207.

10 Total synthesis of mimosamycin, see: a) H. Fukumi, H. Kurihara, T. Hata, C. Tamura, A. Kubo, and T. Arai, *Tetrahedron Lett.*, **1977**, 3825; H. Fukumi, H. Kurihara, and H. Mishima, *Chem. Pharm. Bull.*, 1978, **26**, 2175. b) A. McKillop and S. P. Brown, *Synth. Commun*., 1987, **17**, 857. c) K. A. Parker, A. Kathlyn, and D. A. Casteel, *J. Org. Chem*., 1988, **53**, 2847. d) B. Keteleyn and N. D. Kimpe, *J. Org. Chem.*, 2000, **65**, 635.

11 Total synthesis of mimocin, see: a) ref. 9b. b) K. Matsuo, M. Okumura, and K. Tanaka, *Chem. Lett.*, 1982, 1339; K. Matsuo, M. Okumura, and K. Tanaka, *Chem. Pharm. Bull.*, 1982, **30**, 4170. c) A. Kubo, Y. Kitahara, S. Nakahara, and R. Numata, *Chem. Pharm. Bull.*, 1983, **31**, 341; A. Kubo, Y. Kitahara, S. Nakahara, R. Iwata, and R. Numata, *Chem. Pharm. Bull.*, 1988, **36**, 4355. d) N. Saito, N. Kawakami, E. Yamada, and A. Kubo, *Chem. Pharm. Bull.*, 1989, **37**, 1493.

12 a) Y. Koizumi, A. Kubo, K. Suwanborirux, and N. Saito, *Heterocycles*, 2002, **57**, 2345. b) H. Kurihara, H. Mishima, and M. Arai, *Heterocycles*, 1986, **24**, 1549.

13 N. Saito, Y. Obara, M. Azumaya, and A. Kubo, *Chem. Pharm. Bull*., 1992, **40**, 2620.

14 This compound cannot be stored even as dry powder under nitrogen atmosphere in a refrigerator overnight: IR ($CHCl_3$) 3520, 3290, 1650, 1625 cm^{-1}; ^1H NMR δ (270 MHz, $CDCl_3$) 1.96 (3H, s, 3-CH_3), 2.13 (1H, d, *J* = 20.8 Hz, 5-Hβ), 2.25 (1H, dd, *J* = 11.6, 1.0 Hz, 14a-H), 2.27 and 2.28 (each 3H, s, NCH_3), 2.63 (1H, dd, *J* = 20.8, 7.6 Hz, 5-Hα), 2.93 (1H, dd, *J* = 11.6, 3.6 Hz, 14a-H), 3.09 (1H, ddd, *J* = 7.6, 1.7, 1.5 Hz, 6-H), 3.73 (1H, br s, 15-H), 4.00 (3H, s, OCH_3), 4.10 (1H, br s, 7-H); ^{13}C NMR δ (67.5 Hz, $CDCl_3$) 8.7 (q, 3-CH_3), 20.0 (t, C-6), 41.0 (q, NCH_3), 41.3 (q, NCH_3), 51.0 (d, C-15), 57.7 (d, C-6), 60.9 (q, OCH_3), 85.8 (d, C-7), 129.0 (s, C-3), 136.8 (s, C-4a), 140.5 (s, C-15a), 155.3 (s, C-2), 182.6 (s, C-1), 187.3 (s, C-4); MS *m/z* (%) 292 (M^+, 20), 234 (17), 221 (19), 220 (100), 219 (16), 218 (21), 206 (12), 205 (26), 204 (21), 190 (35), 176 (16), 58 (12); High-resolution EIMS Calcd for $C_{15}H_{20}N_2O_4$: 292.1423. Found: 292.1419.

15 N. Saito, Y. Ohira, N. Wada, and A. Kubo, *Tetrahedron*, 1990, **46**, 7711.

16 In the chemistry of quinocarcin A, a unified mechanism for this type of redox self-disproportionation was reported by Professor Williams, see: R. M. Williams, T. Glinka, M. E. Flanagam, R. Gallegos, H. Coffman, and D. Pei, *J. Am. Chem. Soc.*, 1992, **114**, 733.