Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 48 (2007) 8628–8631
Tetrahedron Letters

Revised structure of kasarin, an antibacterial pyrazinone compound from the marine microorganism *Hyphomycetes* sp.

Masaki Kita,[a,†] Ryoka Miwa,[b] Triana Widianti,[b] Yoshikazu Ozaki,[b] Sachiko Aoyama,[b] Kaoru Yamada[b] and Daisuke Uemura[b,*]

[a]*Research Center for Materials Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602, Japan*

[b]*Department of Chemistry, Graduate School of Science, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8602, Japan*

Received 7 September 2007; revised 1 October 2007; accepted 5 October 2007
Available online 9 October 2007

Abstract—Kasarin is an antibacterial marine alkaloid that was isolated from the microorganism *Hyphomycetes* sp. Its structure was previously reported to be an azetinone compound **1**. However, detailed spectroscopic analysis of the natural compound and its related synthetic analogs revealed that the structure of kasarin should be revised to be a novel 5-malonyloxy-1-methoxy-pyrazin-2(*1H*)-one derivative **2**.

In our continuing search for bioactive compounds from marine symbiotic organisms,[1] an antibacterial marine alkaloid, kasarin, was isolated from the culture broth of the marine bacterium *Hyphomycetes* sp.[2] This microorganism has a symbiotic relationship with the zoanthid *Zoanthus* sp., from which several unique compounds, such as norzoanthamine, have been isolated.[3,4] Kasarin showed antibacterial activity against the marine bacterium *Rhodospirillium salexigens* SCRC 113 strain (12 mm, 0.7 mg/disc) and weak cytotoxicity against the P388 murine leukemia cell line ($IC_{50} = 34$ μg/mL). Its planar structure was determined to be an azetinone compound **1**.[2] However, based on the present detailed spectroscopic analysis, its structure may have to be revised.

Kasarin has a molecular formula of $C_{15}H_{23}N_3O_5$, as determined by HREIMS [*m/z* 325.1675 (M^+), Δ +0.7 mmu].[5] The ^1H, ^{13}C NMR, and HSQC spectra showed the presence of five methyl carbons including one methoxy carbon (δ_C 64.1), two methylene carbons, two methine carbons, two olefinic carbons (δ_C 135.0, 134.9), and four olefinic or carbonyl carbons (δ_C 168.0, 166.0, 161.5, 152.0). Detailed analysis of the 2D-NMR spectra (COSY, INADEQUATE, ^{13}C, and ^{15}N HMBC) allowed us to construct three partial structures (Fig. 1). The phase-sensitive DQF-COSY spectrum suggested carbon–carbon connectivities on an isopropyl group and a *sec*-butyl group. The HMBC correlations H7/C5, H7/C6, H8/C6, H10/C6, and H7/N1 suggested connectivity between C7, C5, and N1 atoms through C6 carbon. Although the ^{15}N HMBC correlation OCH_3/N4 was previously described, this was reassigned to OCH_3/N1. With respect to an isopropyl group, HMBC correlations H11/C3, H12/C3, and H11/N4 suggested the connectivity of C3 to C11 carbons and N4

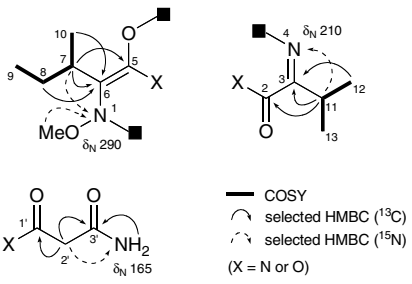
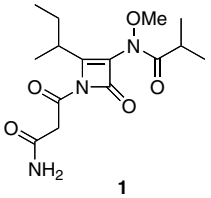


Figure 1. Partial structures of kasarin based on 2D-NMR correlations. ^{15}N chemical shifts were determined by the ^{15}N HMBC spectrum.[2]

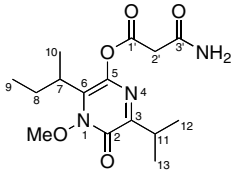
Keywords: Kasarin; Antibacterial marine alkaloid; Pyrazinone; Spectroscopic analysis; Structural revision.
* Corresponding author. Tel./fax: +81 52 789 3654; e-mail: uemura@chem3.chem.nagoya-u.ac.jp
† Present address: Department of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan.

0040-4039/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2007.10.024

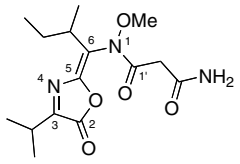
atom as previously mentioned. Moreover, the HMBC correlation H11/C2 was additionally assigned. Thus, it has been confirmed that a methoxy group is connected to N1, and that the quaternary carbonyl carbon C2 comes in direct contact with C3.



1
proposed structure



kasarin (**2**)
revised structure

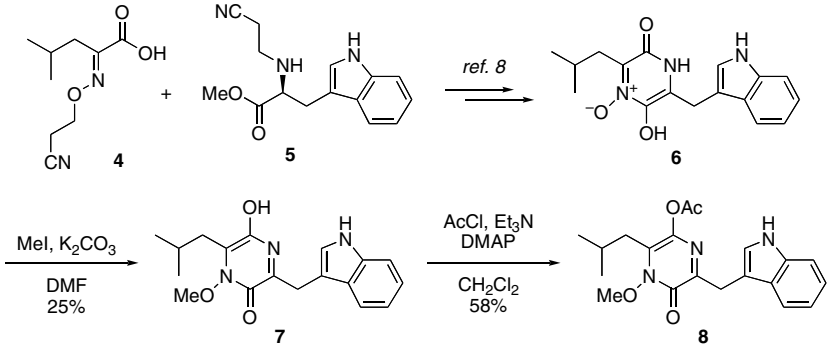


3

The characteristic methylene proton and carbon signals ($\delta_{H2'}$ 3.55, $\delta_{C2'}$ 41.1) and the HMBC correlations H2′/C1′ and H2′/C3′ indicated the presence of a malonyl group. The structure of the primary amide moiety in C3′ was elucidated based on the HMBC correlations NH_2/C3′, H2′/NH_2, and NH_2/NH_2. The presence of a malonyl amide group was supported by IR (3500 and 3380 cm^{-1}) and mass fragment ion [*m*/*z* 240 $(M-C_3H_3NO_2)^+$] data. The molecular formula suggested that kasarin has another heterocyclic ring in addition to the three partial structures as mentioned above. Thus, either the oxygenated pyrazinone **2** or oxazolone **3** was suggested as a proper structure of kasarin.

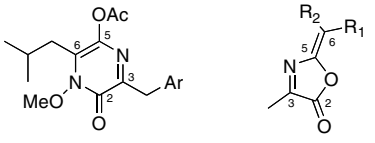
To compare the spectroscopic data (especially for the quaternary carbons in the heterocyclic rings), model compound **8** was synthesized (Scheme 1). Condensation of oxime acid **4** and a tryptophan derivative **5**[7a] followed by thiolate-mediated cyclization[7b] and the removal of the amide protecting group afforded pyrazine **6**.[7c,d] Methylation of **6** using an excess amount of iodomethane (2 equiv) provided the 1-methoxy-pyrazin-2(*1H*)-one derivative **7**. Finally, treatment of **7** with acetyl chloride in the presence of DMAP afforded the desired enol acetate **8**.[8]

Representative ^{13}C NMR, IR, and UV data of pyrazinone **8**, oxazolone derivatives **9**,[6] and natural kasarin are shown in Table 1. The IR bands of the lactone



Scheme 1.

Table 1. Comparison of spectroscopic data for natural kasarin and synthetic derivatives

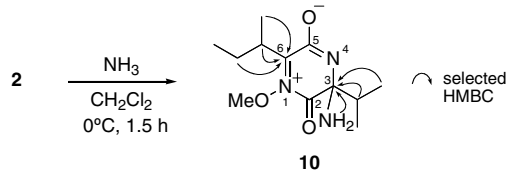


8
Ar = 3-indolyl

9*E* : R_1 = H, R_2 = Ph
9*Z* : R_1 = Ph, R_2 = H

Compound	^{13}C NMR (δ in $CDCl_3$)				IR[a] (cm^{-1})	UV[b] (nm)
	C2	C3	C5	C6		
Natural kasarin	152.0	161.5	134.9	135.0	1760, 1690, 1660	330
8	152.0	155.4	131.5	135.7	1765, 1690, 1664	330
9*E*[c]	163.3	159.9	153.7	111.2	1774, 1651	N.A.
9*Z*[c]	163.6	156.9	134.5	112.4	1785, 1659	N.A.

N.A., not applicable.
[a] In $CHCl_3$.
[b] In EtOH.
[c] See Ref. 6.



Scheme 2. Ammonolysis of kasarin.

and imine moieties in both compounds **9*E*** and **9*Z*** were nearly equal to those of natural kasarin. However, the chemical shifts of the lactone moiety (C2) of oxazolones **9** (δ 163.3, 163.6) differed substantially from those of the natural compound (δ 152.0). Meanwhile, all of the chemical shifts of the quaternary carbons C2, C3, C5, and C6 in **8** were almost identical to those of the natural compound. Furthermore, the IR spectrum of **8** (1765, 1690, and 1664 cm^{-1}) was nearly equal to that of the natural compound. The IR band at 1760 cm^{-1} in natural kasarin can be assigned as an enol acetate moiety. Similarly, the IR band between 1690 and 1660 cm^{-1} can be assigned as a pyrazinone moiety. The UV spectrum of **8** (ν_{max} 330 nm) was identical to that of natural kasarin, and was close to that of OPC-15161, a 5-methoxy-2(*1H*)-pyrazinone 4-oxide derivative (ν_{max} 354 nm).[9] Thus, the gross structure of kasarin was revised to be a 5-malonyloxy-1-methoxy-pyrazin-2(*1H*)-one derivative, as shown in **2**.

To obtain further structural information, the degradation of natural kasarin (**2**) was carried out (Scheme 2). Treatment of **2** with ammonia in CH_2Cl_2 at 0 °C gave a mixture of several degradation products, one of which was identified as compound **10** (~10% yield).[10,11] The degraded product **10** lost a malonyl group, whereas it still possessed a methoxy group. The HMBC correlations from the isopropyl group protons H11, H12, and H13, and from the amine protons NH_2 to C3 (δ_C 88.3) indicated that one molecule of ammonia was added to the imine carbon atom C3. Moreover, the chemical shift of the carbon C6 in **10** was significantly shifted to a lower field (δ 176.2) compared to **2**, which suggested the formation of an iminium cation moiety at C6. This result also strongly supported the notion that kasarin has an oxygenated pyrazinone ring structure.

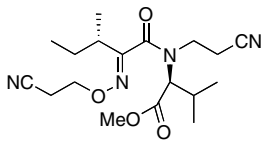
In summary, the structure of kasarin, an antibacterial marine alkaloid from *Hyphomycetes* sp., was revised to be a novel 5-malonyloxy-1-methoxy-pyrazin-2(*1H*)-one derivative **2**. Further biological studies on kasarin, including an exhaustive antibacterial spectrum analysis, are in progress. To determine its absolute stereochemistry, synthetic studies of kasarin are currently underway.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Creative Scientific Research (16GS0206) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. We are indebted to Ono Pharmaceutical Co., Ltd and the Naito Foundation for their financial support. We thank Drs. T. Iwashita and M. Ishiguro (Suntory Institute for Bioorganic Research) and Dr. H. Suda (Banyu Pharmaceutical Co., Ltd) for performing the NMR experiments and for their helpful discussions.

References and notes

1. (a) Kita, M.; Kondo, M.; Koyama, T.; Yamada, K.; Matsumoto, T.; Lee, K.-H.; Woo, J.-T.; Uemura, D. *J. Am. Chem. Soc.* **2004**, *126*, 4794; (b) Kita, M.; Ohishi, N.; Washida, K.; Kondo, M.; Koyama, T.; Yamada, K.; Uemura, D. *Bioorg. Med. Chem.* **2005**, *13*, 5253; (c) Washida, K.; Koyama, T.; Yamada, K.; Kita, M.; Uemura, D. *Tetrahedron Lett.* **2006**, *47*, 2521; (d) Kita, M.; Ohishi, N.; Konishi, K.; Kondo, M.; Koyama, T.; Kitamura, M.; Yamada, K.; Uemura, D. *Tetrahedron* **2007**, *63*, 6241; (e) Kita, M.; Roy, M. C.; Siwu, E. R. O.; Noma, I.; Takiguchi, T.; Itoh, M.; Yamada, K.; Koyama, T.; Iwashita, T.; Uemura, D. *Tetrahedron Lett.* **2007**, *48*, 3423; (f) Kita, M.; Roy, M. C.; Siwu, E. R. O.; Noma, I.; Takiguchi, T.; Yamada, K.; Koyama, T.; Iwashita, T.; Wakamiya, A.; Uemura, D. *Tetrahedron Lett.* **2007**, *48*, 3429.
2. Suenaga, K.; Aoyama, S.; Xi, W.; Arimoto, H.; Yamaguchi, K.; Yamada, K.; Tsuji, T.; Yamada, A.; Uemura, D. *Heterocycles* **2000**, *52*, 1033.
3. (a) Kuramoto, M.; Hayashi, K.; Yamaguchi, K.; Yada, M.; Tsuji, T.; Uemura, D. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 771; (b) Kuramoto, M.; Hayashi, K.; Fujitani, Y.; Yamaguchi, K.; Tsuji, T.; Yamada, K.; Ijuin, Y.; Uemura, D. *Tetrahedron Lett.* **1997**, *38*, 5683; (c) Fukuzawa, S.; Hayashi, Y.; Uemura, D.; Nagatsu, A.; Yamada, K.; Ijyuin, Y. *Heterocycl. Commun.* **1995**, *1*, 207.
4. Recent reviews for bioactive nitrogenous marine metabolites: (a) Gerwick, W. H.; Tan, L. T.; Sitachitta, N. *The Alkaloids* **2001**, *57*, 75; (b) Kuramoto, M.; Arimoto, H.; Uemura, D. *Mar. Drugs* **2004**, *1*, 39; (c) Kita, M.; Uemura, D. *Chem. Lett.* **2005**, *34*, 454; (d) Kita, M.; Uemura, D.. In *Topics in Heterocycles*; Eguchi, S., Ed.; Springer, 2006; Vol. 6, pp 157–179.
5. *Spectral data for* **2**: $[\alpha]_D^{26}$ +22 (*c* 0.30, $CHCl_3$); IR ($CHCl_3$) 3500, 3380, 1760, 1690, 1660, 1580 cm^{-1}; UV (EtOH) λ_{max} 330 nm (ε 2100); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.93 (br s, 1H, N*H*), 6.22 (br s, 1H, N*H*), 4.04 (s, 3H, O*Me*), 3.55 (s, 1H, H2′), 3.36 (tt, $J = 6.9$, 6.9 Hz, 1H, H11), 2.96 (tq, $J = 7.0$, 7.0 Hz, 1H, H7), 1.64 (m, 2H, H8), 1.25 (d, $J = 7.0$ Hz, 3H, H10), 1.13 (d, $J = 6.9$ Hz, 6H, H12 and H13), 0.84 (t, $J = 7.3$ Hz, 3H, H9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.0 (C3′), 166.0 (C1′), 161.5 (C3), 152.0 (C2), 135.0 (C6), 134.9 (C5), 64.1 (O*Me*), 41.1 (C2′), 33.7 (C7), 30.7 (C11), 27.5 (C8), 18.2 (C10), 12.6 (C9), 19.9 (C12), 19.9 (C13).
6. Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1351.
7. (a) Tone, H.; Matoba, K.; Goto, F.; Torisawa, Y.; Nishi, T.; Minamikawa, J. *Org. Process Res. Dev.* **2000**, *4*, 312; (b) Shinhama, K.; Matoba, K.; Torisawa, Y.; Minamikawa, J. *Tetrahedron* **2000**, *56*, 7427; (c) Kita, Y.; Akai, S.; Fujioka, H.; Tamura, Y.; Tone, H.; Taniguchi, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 875; (d) Kita, Y.; Akai, S.; Fujioka, H.; Tamura, Y.; Tone, H.; Taniguchi, Y. *Tetrahedron Lett.* **1991**, *32*, 6019.
8. Thiolate-mediate cyclization of a valyl-isoleucyl dipeptide analog **11** was unsuccessful due to the steric repulsions of the side-chain methyl groups.



11

9. Nakano, Y.; Kawaguchi, T.; Sumitomo, J.; Takizawa, T.; Uetsuki, S.; Sugawara, M.; Kido, M. *J. Antibiot.* **1991**, *44*, 52.
10. *Spectral data for* **10**: MS (FAB) 255 $(M+H)^+$, 277 $(M+Na)^+$; ^1H NMR (C_6D_6, 750 MHz) δ 8.25 (br s, 1H, N*H*), 6.60 (br s, 1H, N*H*), 3.34 (s, 3H, O*Me*), 2.78 (m, 1H, H7), 2.10 (tt, $J = 6.8$, 6.8 Hz, 1H, H11), 1.80 (m, 1H, H8a), 1.42 (m, 1H, H8b), 1.06 (d, $J = 6.8$ Hz, 3H, H10), 0.85 (d, $J = 6.8$ Hz, 3H, H12), 0.82 (t, $J = 7.0$ Hz, 3H, H9), 0.57 (d, $J = 6.8$ Hz, 3H, H13); ^{13}C NMR (C_6D_6, 175 MHz, based on HSQC and HMBC spectra) δ 176.2 (C6), 88.3 (C3), 63.9 (O*Me*), 35.7 (C11), 35.1 (C7), 27.2 (C8), 16.8 (C12), 16.5 (C10), 15.4 (C13), 11.7 (C9). Chemical shifts of the two carbonyl carbons (C2 and C5) could not be determined.
11. Compound **10** was isolated as a single stereoisomer, and its C3 epimer was not detected in the degraded products mixture.