ISOLATION AND STRUCTURE OF KASARIN, A NOVEL AZETINONE COMPOUND, ISOLATED FROM A MARINE MICROORGANISM*

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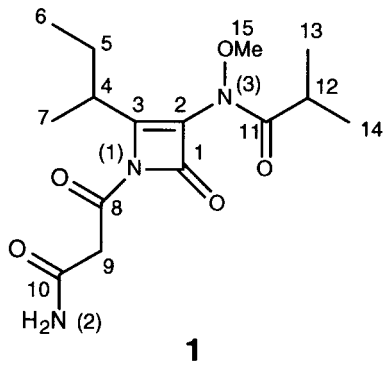
Abstract- Kasarin (**1**), a novel azetinone compound, was isolated from a marine microorganism. The gross structure of kasarin was elucidated by spectroscopic analyses. Kasarin exhibited antibacterial activity and weak cytotoxicity.

In our continuing search for bioactive compounds from marine organisms,[1] we isolated a novel azetinone compound, kasarin (**1**), from the cultured broth of a *Hyphomycetes* sp. separated from the zoanthid *Zoanthus* sp. collected at Amami Island, Kagoshima Prefecture, Japan, from which many biological active compounds such as norzoanthamines,[2] which may be useful for the treatment of osteoporosis, have been isolated.



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* Dedicated to Professor Teruaki Mukaiyama in celebration of his 73rd birthday.

Table 1 NMR Data of Kasarin

position	^1H	^{13}C	HMBC
1		152.0 s[b]	
2		134.9 s	H-4[c]
3		135.0 s	H-4, H-5, H-7
4	2.96 tq (7.0, 7.0)[a]	33.7 d	H-5, H-6, H-7
5	1.64 m	27.5 t	H-4, H-6, H-7
6	0.84 t (7.3)	12.6 q	H-4, H-5
7	1.25 d (7.0)	18.2 q	H-4, H-5
8		168.0 s[d]	H-9
9	3.55 s	41.1 t	
10		166.0 s[d]	H-9
11		161.5 s	H-12, H-13, H-14
12	3.36 sept (6.9)	30.7 d	H-13, H-14,
13	1.13 d (6.9)	19.9 q	H-12
14	1.13 d (6.9)	19.9 q	H-12
15	4.04 s	64.1 q	
NH_2	6.93 br s, 6.22 br s		

[a] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses.
[b] Recorded at 100 MHz. Multiplicity was determined by DEPT experiments.
[c] Recorded at 600 MHz. Parameters were optimized for J_{CH} = 6 Hz.
[d] Exchangeable.

The $CHCl_3$-MeOH (3:1) extract of the cultured broth (0.5 L) of this microorganism was partitioned between EtOAc and water. The EtOAc-soluble material was separated by thin layer chromatography ($CHCl_3$–MeOH, 95:5) to give kasarin (**1**, 22.8 mg) as a colorless oil.[3] This compound showed antibacterial activity (*Rhodospirillium salexigens* SCRC 113, 12 mm, 0.7 mg/disc) and weak cytotoxicity (IC_{50} = 34 μg/mL) against P388 cells.

Kasarin (**1**) has a molecular formula of $C_{15}H_{23}N_3O_5$, which was determined by HREIMS [*m/z* M^+ 325.1675, Δ +0.7 mmu] . The NMR data for **1** are summarized in Table 1. The ^1H NMR, ^{13}C NMR, and HSQC spectra of **1** showed the presence of five methyl carbons including one methoxy carbon (δ_c 64.1),

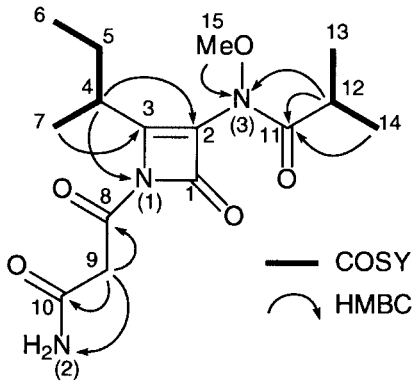

Figure 1. Partial Structures of Kasarin and Selected HMBC correlations

two methylene carbons, two methine carbons, two olefinic carbons (δ_c 135.0, 134.9), and four olefinic and/or carbonyl carbons (δ_c 168.0, 166.0, 161.5, 152.0). Detailed analysis of the phase-sensitive DQF-COSY spectrum allowed us to construct two partial structures (Figure 1), an isopropyl group (C12-C14) and a *sec*-butyl group (C4-C7). The HMBC correlations H14/C11, H7/C3, H4/C3, and H4/C2 suggested the connectivities C11-C12 and C2-C3-C4. The presence of a malonyl group in **1** was revealed by correlations in the HMBC spectrum (H-9/C8 and H-9/C10) as well as by characteristic NMR signals ($\delta_{H\text{-}9}$ 3.55, δ_{C9} 41.1). The ^{1}H NMR spectrum (δ 6.93 and 6.22) and IR bands at 3500 and 3380 cm^{-1} showed the presence of an amide NH_2 group, which was connected to the malonyl group based on the ^{15}N HMBC[4] correlation H-9/N(2), and direct correlation between the NH proton (δ 6.93) and N(2). This was supported by the MS spectrum [*m/z* 240 (M^+–$C_3H_3NO_2$)]. The ^{15}N HMBC correlations, H-15/N(3) and H-12/N(3), suggested that the methoxy group (C15) was connected to N(3), which was bonded to the *iso*-butyroyl group. The IR spectrum of **1** showed bands at 3500, 3380, 1690, and 1660 cm^{-1} that were assigned to an amide functionality and a band at 1760 cm^{-1} that was assigned to a β-lactam functionality. Furthermore, the ^{15}N HMBC correlation between H4 and N(1) suggested the connectivity between C3 and N(1). The remaining carbon (δ 152.0) was a β-lactam carbonyl carbon based on a consideration of the molecular formula. The high-field carbon chemical shift of C1 (δ 152.0) indicated the presence of a α,β-unsaturated lactam (azetinone) structure. The carbon chemical shift of C8 (δ 168.0) suggested that C8 was an amide carbonyl; C8 must be connected to N(1). Although connectivity between C2 and N(3) could not be established, such connectivity was obvious based on the molecular formula of **1**. Thus, the gross structure of kasarin was determined to be as shown in formula (**1**).

Although many β-lactam compounds are found in nature, natural compounds with a monocyclic β-lactam are rare.[5] Kasarin has a very unique azetinone structure. Further biological studies, including of the antibacterial spectrum of kasarin, are in progress. To determine its stereochemistry, synthetic studies of kasarin are currently underway.

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3. $[\alpha]_D^{26}$ +22 ° (*c* 0.30, $CHCl_3$)
4. ^{15}N HMBC spectrum was recorded at 600 MHz. Parameters were optimized for J_{NH} = 6 Hz. ^{15}N chemical shifts: 290 [N (1)], 165 [N(2)], 210 [N(3)] (external standard: NH_4NO_3).
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