

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

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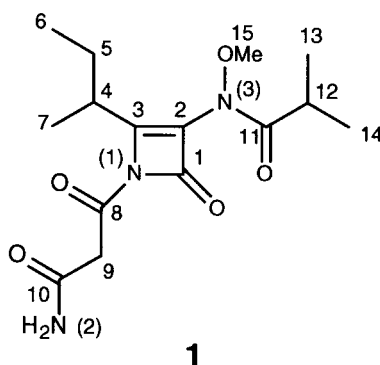
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**Abstract-** Kasarin (**1**), a novel azetinone compound, was isolated from a marine microorganism. The gross structure of kasarin was elucidated by spectroscopic analyses. Kasarin exhibited antibacterial activity and weak cytotoxicity.

In our continuing search for bioactive compounds from marine organisms,<sup>1</sup> 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,<sup>2</sup> which may be useful for the treatment of osteoporosis, have been isolated.



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

**Table 1** NMR Data of Kasarin

| position        | $^1\text{H}$                    | $^{13}\text{C}$      | HMBC             |
|-----------------|---------------------------------|----------------------|------------------|
| 1               |                                 | 152.0 s <sup>b</sup> |                  |
| 2               |                                 | 134.9 s              | H-4 <sup>c</sup> |
| 3               |                                 | 135.0 s              | H-4, H-5, H-7    |
| 4               | 2.96 tq (7.0, 7.0) <sup>a</sup> | 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 <sup>d</sup> | H-9              |
| 9               | 3.55 s                          | 41.1 t               |                  |
| 10              |                                 | 166.0 s <sup>d</sup> | 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 <sub>2</sub> | 6.93 br s, 6.22 br s            |                      |                  |

<sup>a</sup> Recorded at 400 MHz. Coupling constants (Hz) are in parentheses.

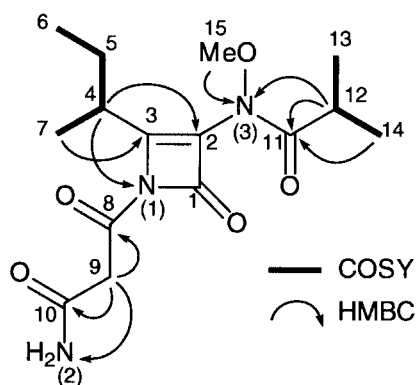
<sup>b</sup> Recorded at 100 MHz. Multiplicity was determined by DEPT experiments.

<sup>c</sup> Recorded at 600 MHz. Parameters were optimized for  $J_{\text{CH}} = 6$  Hz.

<sup>d</sup> Exchangeable.

The  $\text{CHCl}_3$ -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 ( $\text{CHCl}_3$ -MeOH, 95:5) to give kasarin (**1**, 22.8 mg) as a colorless oil.<sup>3</sup> This compound showed antibacterial activity (*Rhodospirillum salexigens* SCRC 113, 12 mm, 0.7 mg/disc) and weak cytotoxicity ( $\text{IC}_{50} = 34 \mu\text{g/mL}$ ) against P388 cells.

Kasarin (**1**) has a molecular formula of  $\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_5$ , which was determined by HREIMS [ $m/z$   $\text{M}^+$  325.1675,  $\Delta +0.7$  mmu]. The NMR data for **1** are summarized in Table 1. The  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and HSQC spectra of **1** showed the presence of five methyl carbons including one methoxy carbon ( $\delta_{\text{C}}$  64.1),

**Figure 1.** Partial Structures of Kasarin and Selected HMBC correlations

two methylene carbons, two methine carbons, two olefinic carbons ( $\delta_c$  135.0, 134.9), and four olefinic and/or carbonyl carbons ( $\delta_c$  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 ( $\delta_{H-9}$  3.55,  $\delta_{C9}$  41.1). The  $^1H$  NMR spectrum ( $\delta$  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<sup>4</sup> correlation H-9/N(2), and direct correlation between the  $NH$  proton ( $\delta$  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*-butyryl 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  $\beta$ -lactam functionality. Furthermore, the  $^{15}N$  HMBC correlation between H4 and N(1) suggested the connectivity between C3 and N(1). The remaining carbon ( $\delta$  152.0) was a  $\beta$ -lactam carbonyl carbon based on a consideration of the molecular formula. The high-field carbon chemical shift of C1 ( $\delta$  152.0) indicated the presence of a  $\alpha,\beta$ -unsaturated lactam (azetinone) structure. The carbon chemical shift of C8 ( $\delta$  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  $\beta$ -lactam compounds are found in nature, natural compounds with a monocyclic  $\beta$ -lactam are rare.<sup>5</sup> 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.

## ACKNOWLEDGEMENT

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3.  $[\alpha]_{\text{D}}^{26} +22^\circ$  ( $c$  0.30,  $\text{CHCl}_3$ )
4.  $^{15}\text{N}$  HMBC spectrum was recorded at 600 MHz. Parameters were optimized for  $J_{\text{NH}} = 6$  Hz.  $^{15}\text{N}$  chemical shifts: 290 [N (1)], 165 [N(2)], 210 [N(3)] (external standard:  $\text{NH}_4\text{NO}_3$ ).
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