NOTES

# AJI9561, a New Cytotoxic Benzoxazole Derivative Produced by *Streptomyces* sp.

SEIICHI SATO, TAKAYUKI KAJIURA, MISATO NOGUCHI, KENJI TAKEHANA, TSUYOSHI KOBAYASHI and TAKASHI TSUJI*

Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki 210-8681, Japan

(Received for publication July 13, 2000)

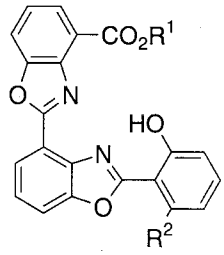
In our search for new antitumor agents, a new cytotoxic metabolite, AJI9561 (**1**) was isolated from the mycelium extract of *Streptomyces* sp. AJ9561, and the structure of **1** was determined to be a new bezoxazole derivative related to UK-1 (**2**) (Fig. 1)[1,2]. Herein, we report fermentation, isolation, structure elucidation, and cytotoxic activity of AJI9561.

Strain AJ9561 was isolated from a soil sample collected at Chiba Prefecture, Japan. The strain was identified to be *Streptomyces* sp. by the International Streptomyces Project (ISP) procedure[3]. A seed medium containing soluble starch 1.0%, glucose 0.5%, NZ-case (Humco) 0.3%, yeast extract (Difco) 0.1%, tryptone 0.5%, $KH_2PO_4$ 0.1%, $MgSO_4$ 0.05%, and $CaCO_3$ 0.1% (pH 7.0) was inoculated with a slant culture of strain AJ9561, and incubated at 28°C on a rotary shaker (180 rpm) for 96 hours. The resultant seed culture was transferred to fifty 500 ml Erlenmeyer flasks containing 100 ml of a producing medium composed of glucose 1.0%, malt extract (Difco) 0.05%, Pharmamedia (Traders) 1.0%, yeast extract 0.1%, and $CaCO_3$ 0.2% (pH 7.2). The fermentation was carried out at 28°C on a rotary shaker (180 rpm) for 96 hours.

The culture broth of *Streptomyces* sp. AJ9561 (5 liters) was filtered to obtain mycelium, which was extracted with acetone (3 liters) at room temperature. The extract was concentrated *in vacuo* to an aqueous suspension, which was extracted with EtOAc (500 ml×3). The organic layers were combined and dried *in vacuo* to give brown oil (3.5 g). The residue was subjected to a Diaion HP-20 column (150 ml). After washing with 30% aqueous acetone, the column was eluted with 80% aqueous acetone. The fraction was concentrated to dryness, and the residue (1.5 g) was applied to a silica gel column (3.0 i.d.×14 cm) eluting with mixture of $CHCl_3$ and MeOH containing 1% formic acid. The fraction eluted with $CHCl_3$/MeOH (95 : 5) (96 mg) was chromatographed on a Sephadex LH-20 column (1.3 i.d.× 24 cm) eluting with $CHCl_3$/MeOH (1 : 1). The active fractions were combined and evaporated *in vacuo* to give yellow solid (38 mg). Further purification was achieved by preparative HPLC (Inertsil ODS-3, 1.0 i.d.×15 cm, flow rate of 1.8 ml/minute) with a linear gradient from 60% to 90% aqueous acetonitrile containing 0.1% formic acid to yield AJI9561 (**1**) (21 mg).

AJI9561 (**1**) was obtained as a white powder, and was positive to the $FeCl_3$ reagent. The physico-chemical properties of **1** are shown in Table 1. The UV spectrum of **1** showed absorption maxima at 250, 272, 281, 311, 319, 328, 347, and 361 nm. The molecular formula of **1** was established to be $C_{22}H_{14}O_5N_2$ by HR FAB-MS. The $^1H$ NMR spectrum of **1** (Table 2) showed a methyl proton ($\delta$ 2.86), nine aromatic protons ($\delta$ 6.85~8.37), and two deuterium exchangeable protons ($\delta$ 11.70 and 12.91). Methylation of **1** by diazomethane afforded a monomethyl ester, which was treated with acetic anhydride in pyridine to give a monoacetate **3** (96%). The $^1H$ and $^{13}C$ NMR spectra of **3** are also in Table 2. In the $^1H$ NMR spectrum, two additional methyl signals were observed at $\delta$ 2.24 and 4.08, respectively, and the exchangeable protons were disappeared. These results suggest that a carboxyl group and a phenol hydroxyl group are contained in **1**. Various

Fig. 1.




$R^1$=H, $R^2$=Me AJI9561 (**1**)
$R^1$=Me, $R^2$=H UK-1 (**2**)

* Corresponding: takashi_tsuji@ajinomoto.com

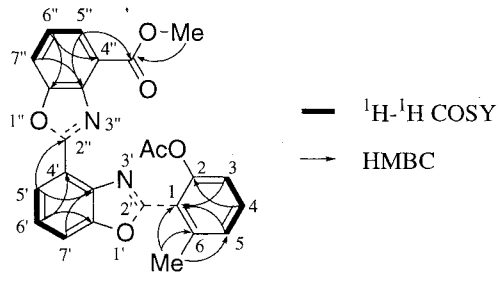
Table 1. Physico-chemical properties of **1**.

| | |
|---|---|
| Appearance | White powder |
| Melting point | 251-252°C |
| Molecular formula | $C_{22}H_{14}O_5N_2$ |
| HR FAB-MS$(M+H)^+$ | |
| Found | 387.0983 |
| Calcd. | 387.0981 |
| UV $\lambda_{max}^{MeOH}$ nm(ε) | 250(27700), 272(23800), 281(24900), 311(37500), 319(38100), 328(37200), 347(27000), 361(21600) |

Table 2. $^1H$ and $^{13}C$ NMR spectral data of **1** and **3**.

| | **1** | **3** | |
|---|---|---|---|
| | $^1H$ | $^1H$ | $^{13}C$ |
| 1 | - | - | 120.5 |
| 2 | - | - | 150.4 |
| 3 | 7.06, d, *J*=8.0 | 7.09, d, *J*=8.0 | 121.1 |
| 4 | 7.36, t, *J*=8.0 | 7.46, t, *J*=8.0 | 131.8 |
| 5 | 6.85, d, *J*=8.0 | 7.25, d, *J*=8.0 | 128.7 |
| 6 | - | - | 140.8 |
| 2' | - | - | 161.5 |
| 3'a | - | - | 139.9 |
| 4' | - | - | 118.7 |
| 5' | 8.37, d, *J*=7.6 | 8.48, dd, *J*=1.2, 8.0 | 125.9 |
| 6' | 7.59, t, *J*=7.6 | 7.55, t, *J*=8.0 | 125.3 |
| 7' | 7.88, d, *J*=7.6 | 7.78, dd, *J*=1.2, 8.0 | 113.9 |
| 7'a | - | - | 151.2 |
| 2" | - | - | 162.8 |
| 3"a | - | - | 141.4 |
| 4" | - | - | 122.3 |
| 5" | 8.21, d, *J*=8.0 | 8.06, dd, *J*=1.2, 8.0 | 127.3 |
| 6" | 7.58, t, *J*=8.0 | 7.45, t, *J*=8.0 | 124.8 |
| 7" | 7.94, d, *J*=8.0 | 7.89, dd, *J*=1.2, 8.0 | 115.3 |
| 7"a | - | - | 151.7 |
| Me | 2.86, s | 2.62, s | 21.3 |
| OH | 12.91, brs | - | - |
| $CO_2H$ | 11.70, br | - | - |
| $CO_2Me$ | - | - | 166.0 |
| $CO_2$*Me* | - | 4.08, s | 52.5 |
| COMe | - | - | 169.8 |
| CO*Me* | - | 2.24, s | 21.0 |

Fig. 2.




2D-NMR analyses including $^1H$-$^1H$ COSY, NOESY, HMBC, and HMQC spectra of **3** revealed the presence of three 1, 2, 3-trisubstituted benzene rings. The chemical shifts of C-3′a ($\delta$ 139.9) and C-3″a ($\delta$ 141.4) were suitable for placement of nitrogen atoms, and those of C-2 ($\delta$ 150.4), C-7′a ($\delta$ 151.2), and C-7″a ($\delta$ 151.7) were suitable for placement of oxygen atoms, respectively. In addition, the signal of H-5 ($\delta$ 7.25) was observed at lower field than that in **1**. It was deduced that the hydroxyl group at C-2 was acetylated. Two remaining carbons ($\delta$ 161.5 and $\delta$ 162.8) were located on C-2′ and C-2″, respectively, by considering degree of unsaturation. These results indicate the presence of two benzoxazole rings (Fig. 2). The HMBC correlation between H-5′ and C-2″ connected C-4′ and C-2″. Furthermore, observation of a weak correlation peak between the methyl proton at C-6 ($\delta$ 2.62) and C-2′ together with the molecular formula assembled the structure of **3**. From above, the structure of AJI9561 (**1**) is established to be a new bezoxazole derivative related to a

known antitumor agent, UK-1 (**2**)[1,2].

AJI9561 (**1**) was evaluated for antitumor activity against cancer cell lines *in vitro*. Cells were treated with **1** for 72 hours in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), and cell viability was evaluated by MTT assay. AJI9561 showed cytotoxic activity against both Jurkat and P388 cells with $IC_{50}$ values of 0.88 $\mu$M and 1.63 $\mu$M, respectively. Inhibitory activity against DNA topoisomerase II of UK-1 was reported by REYNOLDS *et al*[4]. AJI9561 may inhibit the enzyme to exhibit cytotoxic activity.

Acknowledgements

We thank UNO TAGAMI, REIKO YUJI, NAOKO OHTSU, and JUNKO SHINOZAKI for measurement of NMR and mass spectra, and skillful technical assistance. We are also grateful to Dr. TOSHIHIKO ANDO and SEIKICHI KOBARU for helpful discussions.

## References

1) UEKI, M.; K. UENO, S. MIYADOH, K. ABE, K. SHIBATA, M. TANIGUCHI & S. OI: UK-1, a novel cytotoxic metabolite from *Streptomyces* sp. 517-02. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties. J. Antibiotics 46: 1089~1094, 1993
2) SHIBATA, K.; M. KASHIWADA, M. UEKI & M. TANIGUCHI: UK-1, a novel cytotoxic metabolite from *Streptomyces* sp. 517-02. II. Structural elucidation. J. Antibiotics 46: 1095~1100, 1993
3) SHITLING, E. B. & D. GOTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
4) REYNOLDS, M. B.; M. R. DELUCA & S. M. KERWIN: The novel bis(benzoxazole) cytotoxic natural product UK-1 is a magnesium ion-dependent DNA binding agent and inhibitor of human topoisomerase II. Bioorg. Chem. 27: 326~337, 1999