UK-1, a Novel Cytotoxic Metabolite from *Streptomyces* sp. 517-02

III. Antibacterial Action of Demethyl UK-1

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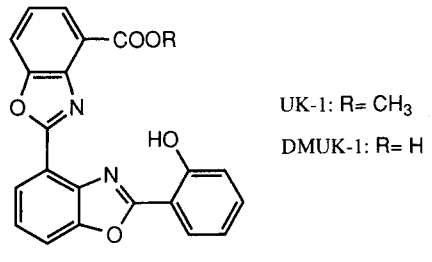
During the course of our screening program for new bioactive compounds, we have isolated a cytotoxic metabolite UK-1 from the acetone extracts of *Streptomyces* sp. 517-02[1]. UK-1 (Fig. 1) is structurally unique bis-(benzoxazole) in which the 2-position of one benzoxazole is joined to the 4-position of a second benzoxazole ring[2,3]. UK-1 did not show any antimicrobial activity, but its alkaline hydrolysate, demethyl UK-1 (DMUK-1, Fig. 1), was active against some bacteria. This paper presents the antibacterial action of DMUK-1.

DMUK-1 was prepared as follows: To a solution of UK-1 (50 mg) in 3 ml of pyridine was added 3 ml of 1 N NaOH. The mixture was then stirred gently at room temperature for 6 hours. After acidification with hydrochloric acid, the reaction mixture was extracted with chloroform. The extract was evaporated *in vacuo*. After washing the residue with *n*-hexane-chloroform (1 : 1), DMUK-1 (30 mg) was obtained as a white powder. The absorption based on a carboxy group appeared at ν_{max} 2500~3100 and 1690 cm^{-1} in the IR spectrum of DMUK-1, and the signal of a methoxycarbonyl group disappeared in the ^1H NMR. The ^1H and ^{13}C NMR data and some physicochemical properties of DMUK-1 were previously reported[2].

The MIC of DMUK-1 was measured by the serial 2-fold agar dilution method in 3% nutrient agar at 30°C for bacteria and in Sabouraud dextrose agar at 25°C for fungi. As shown in Table 1, DMUK-1 completely inhibited the growth of *Staphylococcus aureus* NCTC 8530 at 3.13 μg/ml, *Micrococcus luteus* IFO 3333 at 12.5 μg/ml and *Pseudomonas aeruginosa* IFO 3080 at 6.25 μg/ml. However, other bacteria and fungi tested were insensitive to DMUK-1 up to 100 μg/ml.

Fig. 1. UK-1 and DMUK-1.



UK-1: R= CH_3

DMUK-1: R= H

The mode of antibacterial action of DMUK-1 on *S. aureus* NCTC 8530 was examined. A 20-hour culture of *S. aureus* was diluted with 3% nutrient broth to give approximately 3×10^7 cells/ml. After a 1-hour incubation with shaking at 30°C, known concentrations of DMUK-1 were added to the cell suspensions, which were then incubated again with shaking. Portions of the cultures were withdrawn at specified intervals to measure the O.D. at 660 nm and CFU per ml. The growth effects of DMUK-1 measured in terms of turbidity and cell viability are shown in Fig. 2. When exponetially growing cells were exposed to DMUK-1 at 3.13 μg/ml, weak growth inhibition was observed. At 12.5 μg/ml of DMUK-1, there was a drastic reduction in the viable cell number after 4 hours of exposure, indicating that the growth inhibition by DMUK-1 was bacteriocidal. But no reduction in the turbidity was observed, indicating that no cell-lysis occurred.

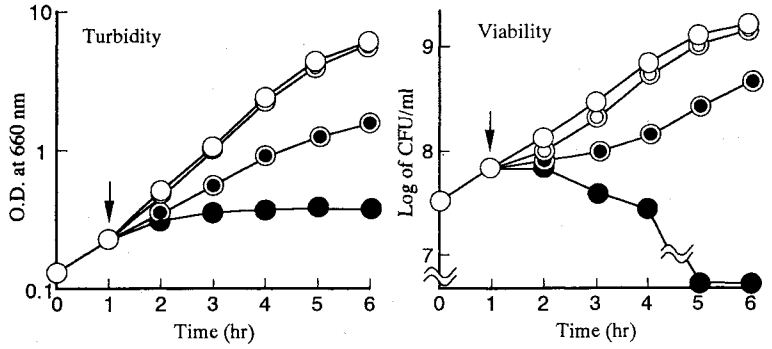
The effect of DMUK-1 on incorporation of radioactive precursors into the 5% trichloroacetic acid-insoluble fraction of *S. aureus* cells are shown in Fig. 3. In the control cultures, the incorporation of radioactive L-isoleucine, uridine, thymidine and *N*-acetyl-D-glucosamine started instantaneously and the counts of each fraction linearly increased. Irrespective of the above radioactive precursors, the rate of incorporation dropped with increasing concentrations of DMUK-1. These effects are not specific to the synthesis of one macromolecule and is presumed to be a secondary effect of other damage caused by DMUK-1.

DMUK-1 did not induce an appreciable amount of leakage of 260 nm-absorbing materials and potassium

Table 1. Antimicrobial activity of DMUK-1.

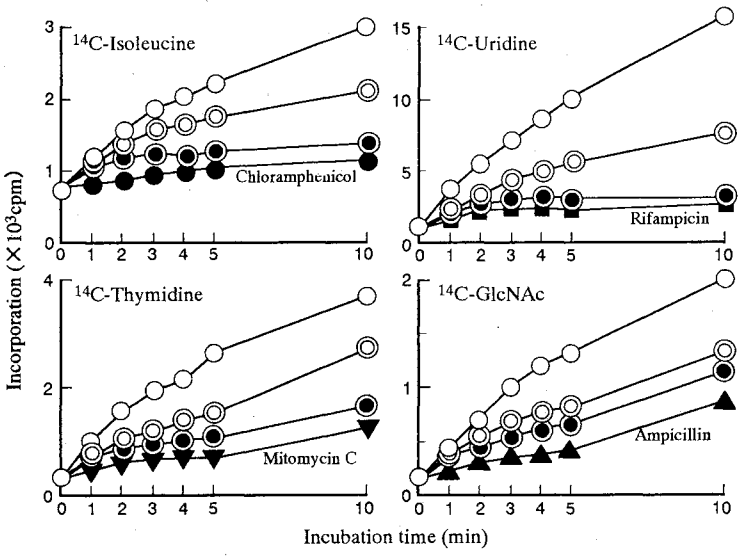
Microorganism	MIC (μg/ml)
Escherichia coli IFO 3992	>100
Pseudomonas aeruginosa IFO 3080	6.25
Proteus vulgaris IFO 3851	>100
Bacillus subtilis IFO 3007	>100
Staphylococcus aureus NCTC 8530	3.13
Micrococcus luteus IFO 3333	12.5
Saccharomyces cerevisiae IFO 0203	>100
Candida albicans IFO 1061	>100
Rhodotorula rubra IFO 0001	>100
Aspergillus niger ATCC 6275	>100
Rhizopus javanicus IFO 5441	>100
Penicillium chrysogenum IFO 4626	>100
Mucor mucedo IFO 7684	>100

Fig. 2. Effect of DMUK-1 on growth of *Staphylococcus aureus* NCTC 8530.



The arrows indicate the time of addition of DMUK-1 in the following concentrations (μg/ml): ○, 0; ◎, 0.78; ◉, 3.13; ●, 12.5.

Fig. 3. Effect of DMUK-1 on incorporation of radioactive precursors into acid-insoluble fraction of *Staphylococcus aureus* NCTC 8530 cells.



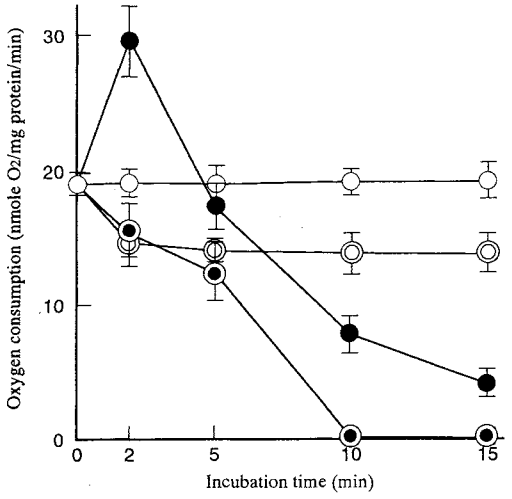
DMUK-1 was added at 0 minute in the following concentrations (μg/ml): ○: 0, ◎: 0.78, ◉: 3.13. Macromolecule synthesis inhibitors were also added at 0 minute in the following concentrations (μg/ml): ●: chloramphenicol at 12.5, ■: rifampicin at 1.56, ▼: mitomycin C at 0.1, ▲: ampicillin at 0.1.

ions from *S. aureus* cells (data not shown). These results suggested that DMUK-1 would interfere with the energy metabolism like several respiratory-inhibiting antibiotics[4~6], because energy is required for the active uptake of various compounds and for the biosynthesis of macromolecules.

The effects of DMUK-1 on the oxygen consumptive activity of *S. aureus* cells polarographically measured are shown in Fig. 4. DMUK-1 strongly inhibited the respiration; almost complete inhibition was noticed at 12.5 μg/ml within 10 minutes. Interestingly, 50 μg/ml of DMUK-1 was slightly stimulatory after 2 minutes of exposure, suggesting that DMUK-1 would have an uncoupling activity at high concentrations. The bacterial aerobic respiratory systems have greater diversity for the electron transfer pathway than for the mitochondrial respiratory systems, depending on the natural habitats of the bacteria and their modes of aerobic metabolism[7]. DMUK-1 may show a growth inhibitory activity for some bacteria.

On the basis of these results, it is reasonable to assume that certain bacterial respiratory systems are the principal site of action of DMUK-1, and other effects are secondary, although further detailed studies are needed.

Fig. 4. Effect of DMUK-1 on oxygen consumptive activity of *Staphylococcus aureus* NCTC 8530 cells.



DMUK-1 was added at 0 minute in the following concentrations (μg/ml); ○: 0, ◎: 3.13, ◉: 12.5, ●: 50. Each plot is the mean of triplicate determinations with the standard deviation indicated by a vertical bar.

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