## NOTES

# BIOLOGICAL ACTIVITIES OF ALBONOURSIN

KAZUTAKA FUKUSHIMA, KATSUKIYO YAZAWA and TADASHI ARAI

Department of Antibiotics,
Institute of Food Microbiology,
Chiba University,
Narashino, Chiba, Japan

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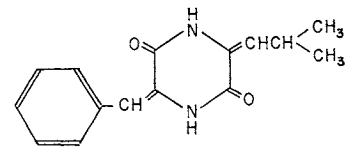
In the course of the studies of metabolites derived from *Actinomyces tumemacerans* strain 1NM1 P-42[1], we obtained three secondary metabolites in crystalline form, these were designated antibiotics P-42-1[2], P-42-2 and P-42-3, respectively.

Antibiotic P-42-2 was isolated by ethyl acetate extraction of filtered broth at pH 7.0, followed by silica gel column chromatography and crystallization from methanol. By comparing the physical properties of the antibiotic P-42-2 with those of albonoursin, it was proved that antibiotic P-42-2 is identical with albonoursin.

The isolation of albonoursin was first reported by G. S. ROSENFELD *et al.* in 1963[3], and its chemical structure was determined to be 3-benzylidene-6-isobutylidene-2,5-dioxopiperazine (I) by A. S. KHOKHLOV *et al.* in 1963[4]. It has been reported that albonoursin does not exhibit any antibiotic activities.[3] In the present paper, we report that albonoursin does exhibit antibacterial activity and, furthermore, inhibits the growth of transplantable solid tumors in mice.

The antimicrobial activities of albonoursin (or the antibiotic P-42-2) as determined by the agar-streak method are summarized in Table 1. The medium is extremely important for demonstration of the antimicrobial activity of albonoursin. We found that, with a usual medium such as nutrient agar, albonourisin did not show any antimicrobial activity at a concentration of over 100 mcg/ml; however, with a minimal medium[5,*], it inhibited the growth of some species of *Bacillus* at concentrations ranging from 6.0 mcg/ml to 100 mcg/ml. Albonoursin is not active against fungi.



(I)

Table 1. Antimicrobial spectrum of albonoursin

| Test organisms | MIC (mcg/ml) |
|---|---|
| *Bacillus subtilis* PCI 219 | 100 |
| *Bacillus cereus* | 25 |
| *Bacillus brevis* AH-29 | 6 |
| *Bacillus firmus* $H_2d$ | 50 |
| *Bacillus polymyxa* AX-7 | 50 |
| *Bacillus circulans* CN-2924 | 25 |
| *Bacillus pumilis* K-4 | >100 |
| *Serratia marcescens* | >100 |
| *Sarcina lutea* | >100 |
| *Mycobacterium* sp. 607 | >100 |
| *Mycobacterium avium* | >100 |
| *Escherichia coli* $F_1$ | >100 |
| *Pseudomonas aeruginosa* | >100 |
| *Shigella dysenteriae* | >100 |
| *Proteus vulgaris* | >100 |
| *Salmonella typhosa* | >100 |
| *Klebsiella pneumoniae* | 25 |

Table 2. Effect of albonoursin on EHRLICH carcinoma (solid form)

| Dose (mg/kg/day) | No. of death | Body weight change (g) | Tumor inhibition | |
|---|---|---|---|---|
| | | | weight (g) | ratio (%)* |
| 12.5 | 0/4 | 20 +10.9 | 1.22 | 49 |
| 6.3 | 0/4 | 19.5+11.0 | 1.46 | 34 |
| 3.2 | 0/4 | 20 +10.7 | 1.24 | 43 |
| 1.6 | 0/4 | 20 +11.0 | 1.36 | 38 |
| 0.8 | 0/4 | 19.5+11.5 | 1.00 | 54 |
| Control | 0/4 | 20.0+11.3 | 2.20 | |

* Inhibition ratio: [1-(average tumor weight of treated/not treated)]×100

The effect of albonoursin on the solid form of EHRLICH ascites carcinoma is given in Table 2. The treatment was started intraperitoneally 24 hours after subcutaneous transplantation of $1\times10^6$ tumor cells at inguinal regions of DDY mice. The degree of inhibition of tumor growth was assessed

by measuring tumor weight 14 days after transplantation of the tumor. Although the results in Table 2 do not show a dose-dependent relationship between albonoursin and tumor inhibition, the inhibition ratio obtained was about 35~50 %. No toxicity was observed with albonoursin at 50 mg/kg in mice.

## References

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* The chemically defined minimal medium had the following composition, per liter : $NH_4Cl$, 5 g; $NH_4NO_3$, 1 g; $Na_2SO_4$, 2 g; $K_2HPO_4$, 3 g; $KH_2PO_4$, 1 g; $MgSO_4$ 0.1 g; glucose, 5 g; $CaCl_2$, trace; and trace element solution, 1 ml.