## ROSEOFLAVIN, A NEW ANTIMICROBIAL PIGMENT FROM *STREPTOMYCES*

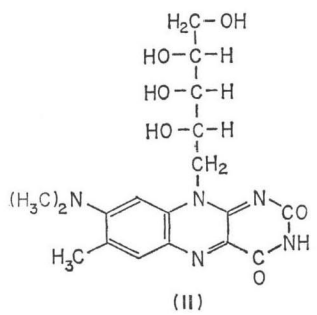
Sir:

In our screening program for antibiotic-producing organisms, we found that *Streptomyces* strain No. 768 isolated from a Philippine soil by SHINOBU (unpublished), produced a pink or reddish orange pigment, which showed antimicrobial activity against Gram-positive bacteria. The pigment was isolated as dark red needle crystals. The compound was determined to be a new flavin compound and was named "Roseoflavin".

*Streptomyces* strain No. 768 was cultured at 30°C for 80~120 hours in a starch, soybean meal, meat extract and salt medium. The activity in the filtered broth was adsorbed on diatomaceous earth (Celite FC), and eluted with hot 5 % pyridine. After concentration and cooling of the eluate, crude crystals were obtained, which was purified by cellulose-column chromatography to give dark red needle crystals of roseoflavin. It discolors in solution upon exposure to light because of its photosensitive property.

Roseoflavin showed antimicrobial activity against Gram-positive bacteria as shown in Table 1. However, the MIC against *Staphylococcus aureus* obtained by a serial agar dilution method was quite different from that by the broth dilution method. Such variability of MIC might be attributed to natural resistant strain. The $LD_{50}$ of roseoflavin in mice by intraperitoneal injection and oral administration was 400 mg/kg and >3,000 mg/kg respectively.

The molecular formula of roseoflavin was $C_{18}H_{23}N_5O_6$ from elemental analysis, mass spectrum, and PMR spectrum of the acetylated compound. Roseoflavin was oxidized with sodium periodate and the product was reduced with sodium borohydride, and then acetylated. After recrystallization from ethyl acetate-methanol, X-ray crystallographic analysis demonstrated that this compound is 7-methyl-8-dimethylamino-10-(2′-acetoxyethyl) isoalloxazine[1]. PMR spectrum of acetylated roseoflavin was consistent with this structure, *i.e.* one H was assigned to N-H, 3 to C-$CH_3$, 6 to $N(CH_3)_2$, 12 to $(CO-CH_3)_4$, 2 to aromatic H, and 7 were unassignable. From this structure and molecular formula, roseoflavin was deduced to be 7-methyl-8-dimethylamino-10-pentitylisoalloxazine [I]. Of eight isomers of I, four D-isomers, those are, D-ribityl, D-arabityl, D-xylityl, and D-lyxityl isoalloxazines were synthesized by condensation of 2-dimethylamino-4-pentitylaminotoluenes with violuric acid in methanol. Physicochemical



(II)

Table 1. Minimal inhibitory concentration of roseoflavin by dilution method

| Test organism | Medium* | MIC (mcg/ml) |
|---|---|---|
| *Staphylococcus aureus* FDA209P | I | 1.25 |
| *Staphylococcus aureus* (PC, SF-R) | I | 0.25 |
| *Staphylococcus aureus* (PC, SM, SF, EM-R) | I | 3.13 |
| *Staphylococcus aureus* (PC, EM, TC, SF-R) | I | 6.25 |
| *Bacillus subtilis* PCI-219 | I | 1.56 |
| *Bacillus cereus* | I | 12.5 |
| *Bacillus cereus* var. *mycoides* | I | 0.25 |
| *Sarcina lutea* ATCC-9341 | I | 0.25 |
| *Escherichia coli* | I | > 50 |
| *Pseudomonas aeruginosa* | I | > 50 |
| *Proteus vulgaris* | I | > 50 |
| *Klebsiella pneumoniae* | I | > 50 |
| *Mycobacterium phlei* | II | > 50 |
| *Aspergillus niger* | III | > 50 |
| *Aspergillus oryzae* | III | > 50 |
| *Piricularia oryzae* | IV | >100 |
| *Pellicularia sasakii* | IV | >100 |
| *Trichophyton beigelii* | III | >100 |
| *Saccharomyces cerevisiae* | III | > 50 |

* Medium I: Nutrient broth. II: DUBOS's broth. III: SABOURAUD's agar. IV: Sucrose-potato agar.

properties of roseoflavin itself and of acetylated derivative were compared with those of four D-isomers of **I** and of the acetylated derivatives, respectively. Ultraviolet-visible absorption spectrum of an aqueous solution (absorption maxima, 223, 258, 314, and 506 nm) corresponded to those of four isomers. Melting point (monohydrate, 274~276°C), specific rotatory power ($[\alpha]_D$, −315° in 0.1 M NaOH), and Rf-value in thin-layer chromatography of roseoflavin coincided with those of 7-methyl-8-dimethylamino-10-D-ribityliso-alloxazine [**II**] and not with those of the other three D-pentityl isomers. Moreover, melting point (279~280°C), specific rotatory power ($[\alpha]_D$ +264° in chloroform), and PMR spectrum of acetylated roseoflavin also coincided with those of tetraacetylated derivative of **II**, and not with those of the other three D-pentityl isomers. The antimicrobial activity of **II** also corresponded to that of roseoflavin, and those of other three isomers did not.

Thus, roseoflavin was identified as 7-methyl-8-dimethylamino-10-D-ribitylisoalloxazine (Fig. 1).

According to a personal communication **II** was synthesized independently in the laboratory of Prof. P. HEMMERICH of University of Konstanz (unpublished).

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## Reference

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