## Communications to the editor

# A NEW SAFRAMYCIN, SAFRAMYCIN R

Sir:

During the course of a study of minor components in the culture of *Streptomyces lavendulae* No. 314, another saframycin, saframycin R was obtained in a crystalline form. The antibiotic was assumed to have the same skeleton as other saframycins[1,2]. The only difference was that one of the carbonyl groups in the quinone rings was reduced to a hydroquinone derivative. The antibiotic, therefore, was designated as saframycin R. Saframycin R is unique in that it is as effective as saframycin A against L1210 cultured cells and murine tumors such as Ehrlich ascites tumor and P388 leukemia while the antimicrobial activity and acute toxicity to mice were reduced to one tenth that of saframycin A[3]. Furthermore, the antibiotic is interesting in that it possesses a side chain which is attached to the hydroxyl group of the benzoide ring and can be readily modified chemically.

The present communication describes the isolation, characterization and some of the biological activities of saframycin R.

*Streptomyces lavendulae* No. 314 was cultured in a submerged state in the medium (pH 7.0) containing glucose 0.5%, starch 0.5%, Polypeptone 1.0%, meat extract 0.5%, NaCl 0.3% and Adekanol 0.002% (Asahi Denka, Japan). After 20 hours incubation at 27°C, the pH of fermentation broth was controlled below 6.5 as described previously[4], and it was incubated for further 8 to 10 hours. NaCN was added to the culture filtrate to give a final concentration of 1 mM and it was incubated at 35°C for 30 minutes with shaking.

The culture filtrate (100 liters) was extracted twice with methylene chloride (40 liters) and the solvent layer was concentrated to syrup *in vacuo*. The extract thus obtained was dissolved in ethyl acetate (250 ml). The solution was counter extracted twice with 1 N HCl (200 ml). The aqueous layer was adjusted to pH 8.0 with ammonia in ice bath, and extracted three times with ethyl acetate (200 ml). The combined solvent extract was washed with 1 M NaCl sufficiently. A crude basic fraction (3.92 g) of saframycin R was obtained as a dark brown powder. This crude material was dissolved in 10 ml of a solvent mixture of benzene - ethyl acetate (4 : 1, v/v) and further purified on silica gel column chromatography. The column was developed successively with the solvent mixtures of benzene - ethyl acetate (4 : 1, 2 : 1, 1 : 1, v/v) and ethyl acetate. Saframycin R was eluted in the fractions of benzene - ethyl acetate (2 : 1 and 1 : 1, v/v). The fractions were purified by preparative thin-layer chromatography with silica gel plate (Merck) and solvent mixture of chloroform - methanol (10 : 1, v/v). Crude saframycin R was dissolved in a small amount of acetone and crystallized by the addition of benzene. After recrystallization from the solvent mixture of acetone - benzene, saframycin R (518 mg) was obtained as pale yellow needles.

It is soluble in methanol, acetone, ethyl acetate and dimethylsulfoxide, sparingly soluble in ether, benzene and chloroform, and isoluble in *n*-hexane and water. The antibiotic decomposed at 184 to 186°C. It gave positive reaction to Dragendorff and $FeCl_3$. The other physical constants of the antibiotic are as follows:

Saframycin R: Found C 60.05, H 5.54, N 8.63; Calcd. for $C_{31}H_{34}N_4O_{10}$: C 59.80, H 5.50, N 9.00; UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$) 270 (3.95); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$ 3400, 1770, 1685, 1660, 1620; $^1H$ NMR spectrum $\delta^{CDCl_3}$ (270 MHz in ppm from TMS) 6.30 (1H, t, –NH), 4.55 (2H, s, $CO–CH_2–OH$),

Fig. 1. $^1H$ NMR spectrum of saframycin R.

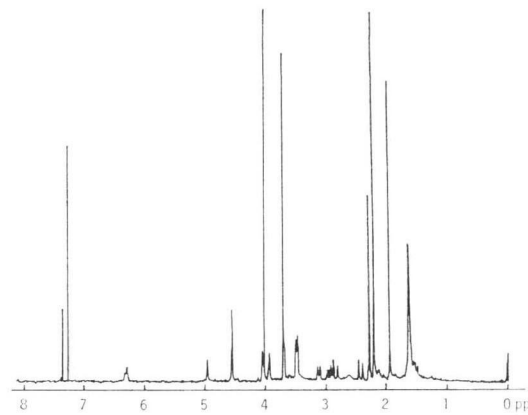


Table 1. $^{13}C$ NMR spectral data of saframycin R.

| a | b | | a | b | | a | b | |
|---|---|---|---|---|---|---|---|---|
| 195.8 | 196.7 | s | 128.4 | 129.0 | s | 54.6 | 56.1 | d |
| 185.5 | 187.1 | d°) | 122.1 | 123.2 | s | 54.4 | 56.0 | d |
| 180.0 | 182.4 | d°) | 118.0 | 119.7 | s | 41.6 | 41.8 | q |
| 171.6 | 172.9 | s | 117.0 | 119.2 | s | 40.5 | 41.5 | t |
| 160.5 | 162.4 | s | 116.8 | 118.7 | d°) | 24.4 | 25.8 | d°)-t |
| 155.9 | 157.5 | s | 61.1 | 61.4 | q | 24.3 | 24.5 | q |
| 149.1 | 151.2 | s | 61.0 | 61.4 | q | 20.8 | 22.1 | d°)-t |
| 148.4 | 149.3 | s | 60.8 | 61.2 | t | 9.2 | 9.8 | q |
| 141.8 | 143.0 | s | 59.0 | 60.1 | d | 8.7 | 8.7 | d°)-q |
| 135.8 | 137.2 | s | 56.8 | 58.2 | d | | | |
| 135.3 | 136.6 | s | 56.4 | 58.2 | d | | | |

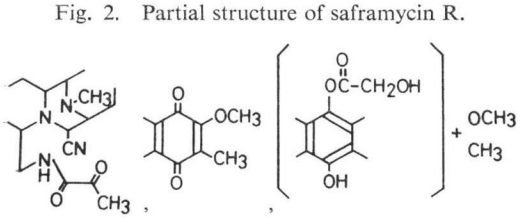
In parts per million downfield from $Me_4Si$.
a: in $CDCl_3$, b: in $CD_3OD$.
s: singlet, d: doublet, t: triplet, q: quartet.
°) small coupling constant.

Table 2. Comparison of antimicrobial spectra of saframycin R and A.

| Test organism | Safra-mycin R | Safra-mycin A | Test organism | Safra-mycin R | Safra-mycin A |
|---|---|---|---|---|---|
| *Staphylococcus aureus* FDA 209P | 1* | 0.1 | *Nocardia asteroides* | 10 | >100 |
| 〃 *aureus* Smith | 1 | 0.05 | *Escherichia coli* F1 | 100 | >100 |
| 〃 *albus* | 1 | 0.2 | *Salmonella typhimurium* | 1 | 50 |
| 〃 *citreus* | 1 | 0.4 | *Shigella dysenteriae* Shiga | 100 | 12.5 |
| *Streptococcus faecalis* | 10 | 3.12 | *Klebsiella pneumoniae* | 10 | 25 |
| 〃 *pyogenes* Cook | N.D.** | 0.025 | *Brucella abortus* | 10 | 1.6 |
| 〃 *pyogenes* 090R | N.D. | 0.05 | *Serratia marcescens* | >100 | 100 |
| 〃 *salivarius* | 100 | 12.5 | *Pseudomonas aeruginosa* | >100 | >100 |
| *Micrococcus luteus* | <1 | 0.05 | *Mucor mucedo* | >100 | >100 |
| *Bacillus subtilis* PCI 219 | 1 | 0.1 | *Saccharomyces cerevisiae* | >100 | 100 |
| 〃 *cereus* | 10 | 25 | *Rhodotorula glutinis* | >100 | 100 |
| *Corynebacterium diphtheriae* | <1 | 0.003 | *Aspergilus niger* | >100 | >100 |
| 〃 *xerosis* | 50 | 0.4 | 〃 *oryzae* | >100 | >100 |
| *Mycobacterium* sp. 607 | >100 | 50 | *Penicillium glaucum* | >100 | 100 |
| 〃 *phlei* | >100 | 50 | *Trichophyton mentagrophytes* | >100 | 100 |
| 〃 *avium* | >100 | 50 | *Candida albicans* 7N | >100 | >100 |

* MIC, μg/ml, ** Not determined.
Nutrient glucose (0.5%) agar for bacteria, and Sabouraud glucose (2%) agar for fungi were used.

Fig. 2. Partial structure of saframycin R.



4.05 (3H, s, Ar-$OCH_3$), 3.70 (3H, s, Ar-$OCH_3$), 2.28 (3H, s, N-$CH_3$), 2.20 (6H, s, Ar-$CH_3$ and $COCH_3$), 1.92 (3H, s, Ar-$CH_3$) (Fig. 1). $^{13}C$ NMR spectral data of saframycin R is shown in Table 1.

Chromatographic data of saframycin R are as follows. The Rf values were (on thin-layer chromatography with silica gel) 0.23 with ethyl ace-

tate - benzene (2 : 1, v/v), 0.32 with chloroform - acetone (1 : 1, v/v), 0.27 with chloroform - methanol (10 : 1, v/v), respectively.

The extinction coefficient at maximum UV absorption was reduced to one half in case of saframycin R compared to that of saframycin A[2] and only two signals of carbonyl of quinone rings were observed in the CMR spectrum. These data suggested that one of the quinone rings in the skeleton was reduced to hydroquinone in saframycin R.

Furthermore, the signals at $\delta$ 171.6 (s) and 60.8(t) suggested the presence of $-CO-CH_2OH$ group in the molecule. Considering that saframycin R is oxidized to form saframycin A, the above substitution was assumed to be at the hydroxyl group of hydroquinone ring. The partial structure of saframycin R is presented in Fig. 2.

The antimicrobial spectrum of saframycin R as determined by the agar streak method is shown in Table 2, in comparison with that of saframycin A. $ED_{50}$ of saframycin R against L1210 cultured cells was 0.004 $\mu$g/ml. $LD_{50}$'s for ICR mice by single injection were 29.5 mg/kg and 26.0 mg/kg, by intravenous and intraperitoneal administration respectively. The antibiotic proved to be active against Ehrlich ascites tumor and a significant prologation of mean survival time was observed in groups of mice receiving saframycin R at doses of 2.5 to 7.5 mg/kg for 10 consecutive days. The maximum increase of life span (T/C, %) was more than 260%.

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