Communications to the editor

NEW ANTIBIOTICS, SAFRAMYCINS A, B, C, D AND E

Sir:

In our preceding papers[1,2], the isolation, and physicochemical and biological properties of chlorocarcins A, B and C, satellite antibiotics derived from streptothricin-producing strain of *Streptomyces lavendulae,* were described. Antibiotic complex which is closely related to chlorocarcins, but without chlorine, was isolated from the culture broth of the same strain No. 314.

Five components in the complex were well characterized to date and designated as saframycins A, B, C, D and E. Saframycins B, C and D, and acetyl derivative of saframycin E were obtained in a crystalline form.

The isolation of saframycin complex was carried out by the same procedure as described for chlorocarcins only difference being that the counter extraction of the antibiotic containing solvent layer with 1 N NaOH was omitted. Further fractionation and purification of saframycin complex was achieved by column chromatography and preparative thin-layer chromatography.

Physicochemical properties of saframycins are described below;

Saframycin A: Slightly basic, yellow powder. m.p. 122~126°C: $[\alpha]_D^{20}$ +18.2° (*c* 0.9, MeOH): Anal. calcd. for $C_{29}H_{30}N_4O_8 \cdot 2/5H_2O$: C 61.14, H 5.41, N 9.83. Found: C 61.47, H 5.41, N 9.33. UV: λ_{max}^{MeOH} nm (log ε): 267 (4.34). λ_{min}^{MeOH} nm (log ε): 230 (3.88). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1716, 1685, 1660, 1615. NMR (100 MHz, $CDCl_3$): δ: 1.90 (3H, s), 1.98 (3H, s), 2.24 (3H, s), 2.30 (3H, s), 4.04 (6H, s), 6.65 (1H, bs) (Fig. 1). Mass: *m/e*: 562 (M^+), 462, 220 (base).

Saframycin B: Basic, orange yellow prisms. m.p. 108~109°C: $[\alpha]_D^{20}$ −54.4° (*c* 1.0, MeOH): Anal. calcd. for $C_{28}H_{31}N_3O_8$: C 62.56, H 5.81, N 7.82. Found: C 62.36, H 5.71, N 7.66. UV: λ_{max}^{MeOH} nm (log ε); 269 (4.35), 368 (3.13). λ_{min}^{MeOH} nm (log ε): 232 (3.86), 330 (3.10). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1720, 1690, 1660, 1620. NMR (100 MHz, $CDCl_3$): δ: 1.90 (3H, s), 1.98 (3H, s), 2.23 (3H, s), 2.28 (3H, s), 4.00 (6H, s), 6.28 (1H, bs) (Fig. 2). Mass: *m/e*; 537 (M^+), 437 (base), 220.

Saframycin C: Basic, orange needles. m.p. 143~146°C: $[\alpha]_D^{20}$ −20.8° (*c* 1.0, MeOH): Anal. calcd. for $C_{29}H_{33}N_3O_9$; C 61.36, H 5.86, N 7.40. Found: C 61.61, H 5.96, N 7.39. UV: λ_{max}^{MeOH} nm (log ε): 266.5 (4.32), 368 (3.19). λ_{min}^{MeOH} nm (log ε): 230 (3.86), 330 (3.16). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1720, 1685, 1655, 1615. NMR (100 MHz, $CDCl_3$): δ: 1.86 (3H, s), 2.00 (3H, s), 2.38 (3H, s), 2.44 (3H, s), 3.46 (3H, s), 3.96 (6H, s), 6.60

Fig. 1. NMR spectrum of saframycin A in $CDCl_3$.

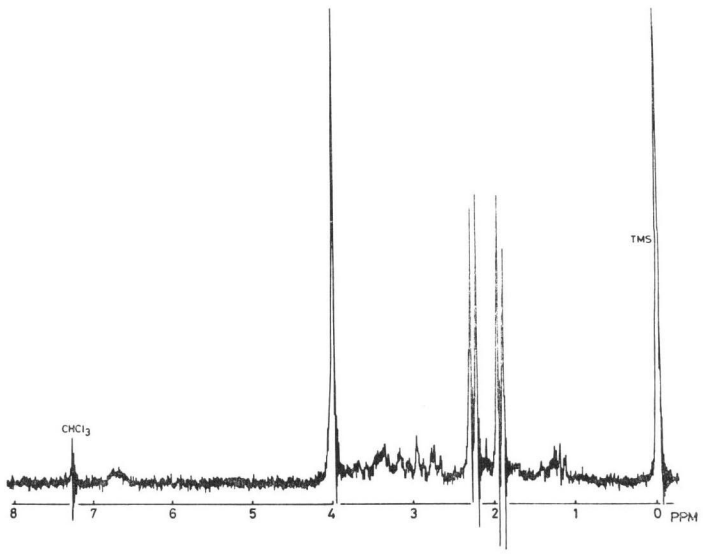


Fig. 2. NMR spectrum of saframycin B in $CDCl_3$.

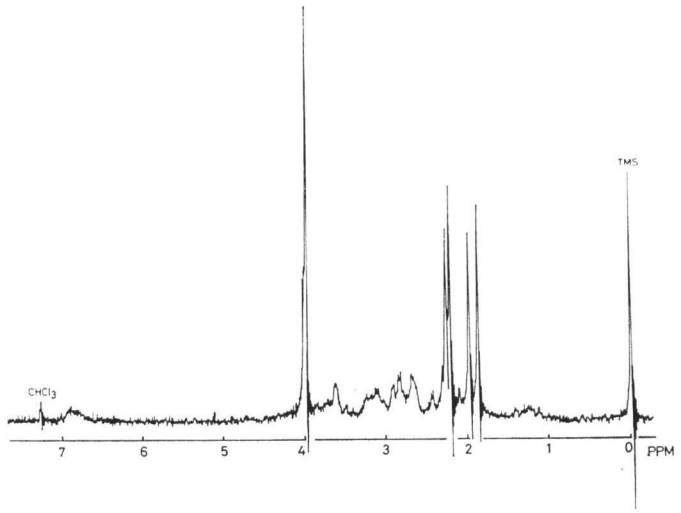
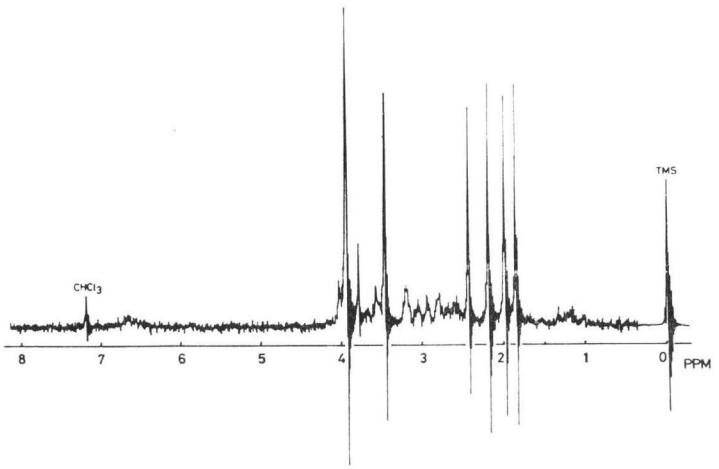


Fig. 3. NMR spectrum of saframycin C in $CDCl_3$.



(1H, bs) (Fig. 3). Mass: *m/e*; 567 (M^+), 467, 220, 218 (base).

Saframycin D: Basic, yellow needles. m.p. 150~154°C: $[\alpha]_D^{20}$ +141.0° (*c* 1.0, MeOH): Anal. calcd. for $C_{28}H_{31}N_3O_9$: C 60.75, H 5.65, N 7.59. Found: C 60.48, H 5.68, N 7.59. UV: λ_{max}^{MeOH} nm (log ε): 243 (4.14), 274 (4.24), 369 (3.75). λ_{min}^{MeOH} nm (log ε); 231 (4.08), 253 (4.06), 319 (3.30). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3400, 1720, 1685, 1660, 1630. NMR (100 MHz, $CDCl_3$): δ: 1.91 (3H, s), 2.17 (3H, s), 2.28 (3H, s), 2.45 (3H, s), 3.97 (3H, s), 4.06 (3H, s) (Fig. 4). Mass: *m/e*; 553 (M^+), 453, 236 (base), 220.

Saframycin E: Basic, yellow powder. m.p. 146~148°C: $[\alpha]_D^{20}$ −37.3° (*c* 0.53, MeOH): Anal. calcd. for $C_{28}H_{33}N_3O_9 \cdot H_2O$: C 58.63, H 6.15, N 7.33. Found: C 58.52, H 5.89, N 7.36. UV: λ_{max}^{MeOH} nm (log ε): 272 (4.10), 368 (2.98). λ_{min}^{MeOH} nm (log ε): 241 (3.84), 340 (2.96). IR: ν_{max}^{KBr} cm^{-1}: 3380, 1720, 1685, 1655, 1620. NMR (100 MHz, d_5-pyridine): δ: 1.83 (3H, s), 2.17 (3H, s), 2.30 (3H, s), 2.48 (3H, s), 3.82 (3H, s), 3.95 (3H, s), 5.22 (1H, s) (Fig. 5).

Saframycin E was not isolated in crystalline form, but crystalline saframycin E acetyl derivative was obtained by treating saframycin E base with acetic anhydride in pyridine. It was determined triacetate from NMR spectrum [δ: 2.12

Fig. 4. NMR spectrum of saframycin D in $CDCl_3$.

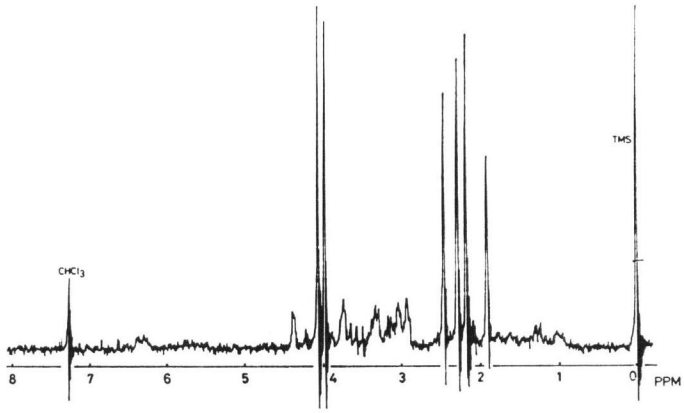
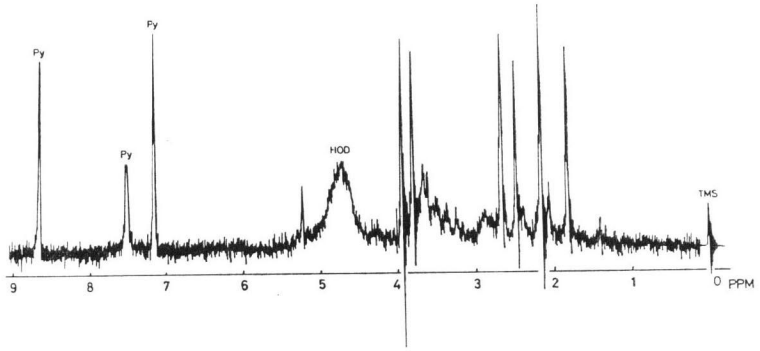


Fig. 5. NMR spectrum of saframycin E in d_5-pyridine.



(3H × 2, s), 2.46 (3H, s)]. Mass spectrum of saframycin E was not detectable. However, its molecular weight was determined as 555 from the mass spectrum of saframycin E triacetate which shows molecular ion peak at 681. NMR spectra of saframycins are shown in Figs. 1 ~ 5.

All the saframycins are soluble in lower alcohols, chloroform or acetone, slightly in ether, and insoluble in *n*-hexane or water. They give a positive DRAGENDORFF reaction, while EHRLICH and $FeCl_3$ reactions are negative.

Their antimicrobial spectra are compared in Table 1. They are only active on gram-positive bacteria. Saframycin A showed the highest activity and saframycin D and E the lowest.

Although saframycins show similar ultraviolet (UV) and infrared (IR) absorption spectra to those of chlorocarcins, they are differentiated from the latter in their detailed characteristics besides the chlorine contents. In nuclear magnetic resonance (NMR) spectrum, saframycin A lacks the peak at δ 1.23 (3H, s) which is eminent in chlorocarcin A. More significant difference in NMR spectrum was noticed between saframycin B and chlorocarcin B. Thus a single peak at δ 4.00 (2 × 3H) is observed with saframycin B instead of two singlets δ 4.04 (3H) and 4.08 (3H) with chlorocarcin B. According to these instrumental analyses, saframycin C is assumed to be O-methyl derivative of saframycin B.

As discussed earlier, the antibiotic which shows two maxima near 270 and 370 nm in UV absorption spectra has not been described in the literature. The above physicochemical properties as well as other characteristics seems to warrant distinguishing saframycins from already known antibiotics.

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Table 1. Antimicrobial spectra of saframycins

Test organism	MIC (mcg/ml)				
	A	B	C	D	E
Staphylococcus aureus FDA 209P	0.1	12.5	25.0	25.0	100.0
Staphylococcus aureus Smith	0.05	1.56	6.25	50.0	50.0
Staphylococcus albus	0.1	12.5	25.0	50.0	100.0
Staphylococcus citreus	0.1	12.5	25.0	50.0	100.0
Streptococcus faecalis	6.25	>100.0	>100.0		>100.0
Streptococcus pyogenes Coock*	0.78	12.5	25.0		>100.0
Streptococcus pyogenes 090R*	6.25	25.0	12.5		25.0
*Streptococcus salivarius**	6.25	100.0	>100.0		>100.0
Sarcina lutea	0.05	1.56	6.25	50.0	12.5
Bacillus subtilis PCI 219	0.1	25.0	25.0	50.0	100.0
Bacillus cereus	12.5	100.0	100.0	100.0	25.0
Corynebacterium diphtheriae	<0.003	0.4	3.125	0.195	100.0
Corynebacterium xerosis	<0.003	12.5	25.0	6.25	25.0
Mycobacterium sp. 607	12.5	100.0	>100.0	50.0	100.0
Mycobacterium phlei	25.0	50.0	>100.0	50.0	25.0
Mycobacterium avium	12.5	100.0	>100.0	50.0	100.0
Nocardia asteroides	6.25	50.0	50.0	50.0	25.0
Escherichia coli F_1	50.0	>100.0	>100.0	>100.0	>100.0
Salmonella typhimurium	100.0	>100.0	>100.0	>100.0	>100.0
Shigella dysenteriae Shiga	25.0	>100.0	>100.0	>100.0	>100.0
Klebsiella pneumoniae	6.25	12.5	50.0	100.0	50.0
Brucella abortus	6.25	50.0	50.0	25.0	25.0
Serratia marcescens	>100.0	>100.0	>100.0	>100.0	>100.0
Pseudomonas aeruginosa	>100.0	>100.0	>100.0	>100.0	>100.0
Mucor mucedo	>100.0	>100.0	>100.0	>100.0	>100.0
Saccharomyces cerevisiae	>100.0	>100.0	>100.0	>100.0	>100.0
Rhodotorula glutinis	>100.0	>100.0	>100.0	>100.0	>100.0
Aspergillus niger	>100.0	>100.0	>100.0	>100.0	>100.0
Aspergillus oryzae	>100.0	>100.0	>100.0	>100.0	>100.0
Penicillium glaucum	50.0	>100.0	>100.0	>100.0	>100.0
Trichophyton mentagrophytes	>100.0	>100.0	>100.0	>100.0	>100.0
Candida albicans 7N	>100.0	>100.0	>100.0	>100.0	>100.0

Agar dilution streak method.
Medium: 0.5% glucose nutrient agar, 37°C, 24 or 48 hours for bacteria.
1% glucose SABOURAUD agar, 27°C, 48 or 72 hours for fungi.
* Blood agar, 37°C, 24 hours.

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