SAFRACINS, NEW ANTITUMOR ANTIBIOTICS

II. PHYSICOCHEMICAL PROPERTIES AND CHEMICAL STRUCTURES

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The chemical structures of safracins A and B are proposed to be **1** and **2** respectively on the basis of their physicochemical properties and spectrometric studies.

Safracins A (**1**) and B (**2**), antitumor antibiotics, were isolated and purified from the culture broth of *Pseudomonas fluorescens* A2-2 as described in a previous paper.[1] The chemical and physicochemical properties, ^1H NMR and ^{13}C NMR spectra of both compounds suggest that they have structures very similar to saframycins[2,3,4] (Fig. 1).

The similarity between safracins and saframycins, metabolites of *Streptomyces lavendulae* No. 314, is striking; safracins contain an L-alanylamide side chain in place of the pyruvamide side chain of saframycins. Furthermore, FRINCKE *et al.*[5] reported that a kind of Sponge, *Reniera* sp., produced antimicrobial metabolites, renieramycins which have chemical structures similar to saframycins and safracins.

This report concerns the elucidation of chemical structures of **1** and **2** by comparing with saframycin B.

Dihydrochloride monohydrates of **1** and **2** were obtained as pale yellow needle crystals. **2** was rather unstable compared with **1**. Their physicochemical properties are summarized in Table 1.

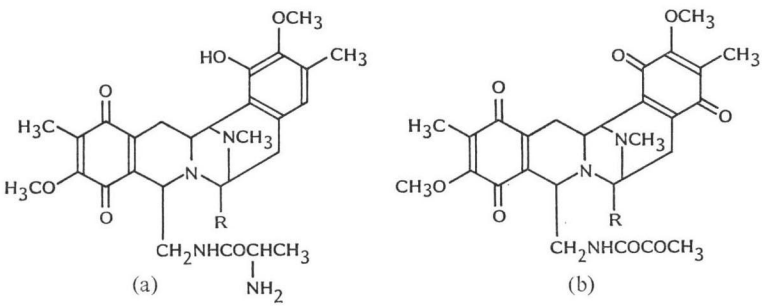
1 and **2** gave parent ion peaks at *m/z* 524 and 540 in the mass spectrometry respectively. The molecular formulas of **1** and **2** (base) were determined as $C_{28}H_{36}N_4O_6$ and $C_{28}H_{36}N_4O_7$ respectively based on elemental analysis and high resolution mass spectrometry. Both antibiotics gave positive reactions with DRAGENDORFF, ferric chloride and ninhydrin reagents. Titration of **1** with $HClO_4$ suggested that **1** had three basic nitrogens.

Table 1. Physicochemical properties of **1** and **2**.

	1	**2**
Molecular Formula	$C_{28}H_{36}N_4O_6$	$C_{28}H_{36}N_4O_7$
Elemental analysis[a]:		
Found	C 54.83, H 6.54, N 9.01	C 53.10, H 6.61, N 8.88
Calcd.	C 54.63, H 6.55, N 9.10	C 53.25, H 6.38, N 8.87
MS (*m/z*)	524 (M^+)	540 (M^+)
mp (°C)[a]	>300 (dec.)	>260 (dec.)
$[\alpha]_D^{20}$ (*c* 0.5, MeOH)[a]	−144°	−106°
IR_{max} KBr cm^{-1} [a]	1690, 1680, 1660, 1620	1690, 1680, 1660, 1620
UV λ_{max}^{MeOH} nm (log ε)[a]	271 (3.93)	270 (3.89)

[a] Dihydrochloride monohydrate of **1** and **2** were used for determinations.

Fig. 1. Structures of safracins A and B.



R=H Safracin A (**1**)
R=OH Safracin B (**2**)

R=H Saframycin B
R=OH Saframycin S

Fig. 2. Chemical transformations of **1**.

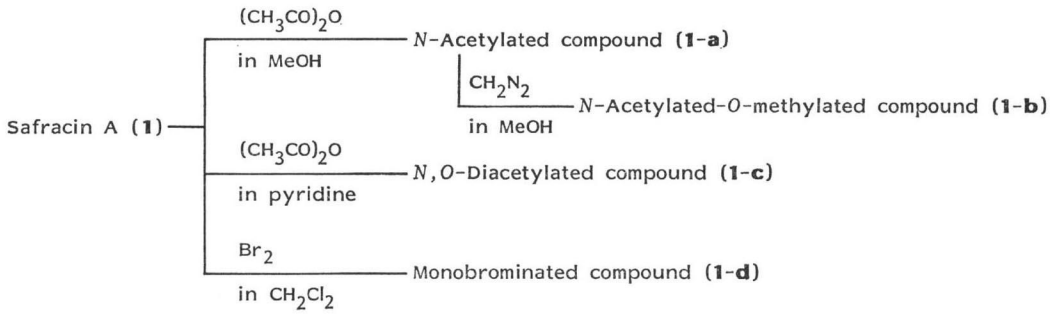
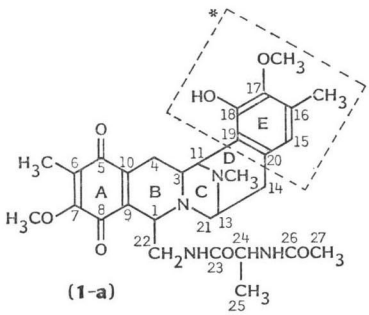
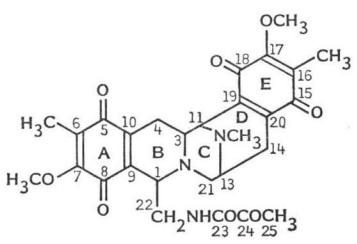


Table 2. Assignment of ^{13}C NMR chemical shifts for **1-a**[a] and saframycin B.

Carbon No.	δ ppm in $CDCl_3$			
	1-a		Saframycin B[b]	
5, 15	185.0	120.3	185.7 or 187.0	
6, 16	127.4	127.4	127.7 or 129.2	
7, 17	156.0	147.9	155.5 or 156.1	
8, 18	181.5 *	143.7	181.3 or 182.8	
9, 19	136.2	117.9	136.3 or 136.6	
10, 20	142.0	132.1	141.6 or 142.8	
6, 16-Me	8.6	15.9	8.6	8.6
7, 17-*O*-Me	60.7	60.7	60.9	60.9
N-Me	41.4		41.2	
1, 11	52.9	48.7	52.2 or	54.8
3, 13	56.9	56.9	56.9 or	57.4
4	27.0		25.6	
14	24.8		22.7	
21	59.6		58.7	
22	41.2		40.4	
23	172.5		160.1	
24	58.0		196.5	
25	19.1		24.2	
26	168.9			
27	23.0			



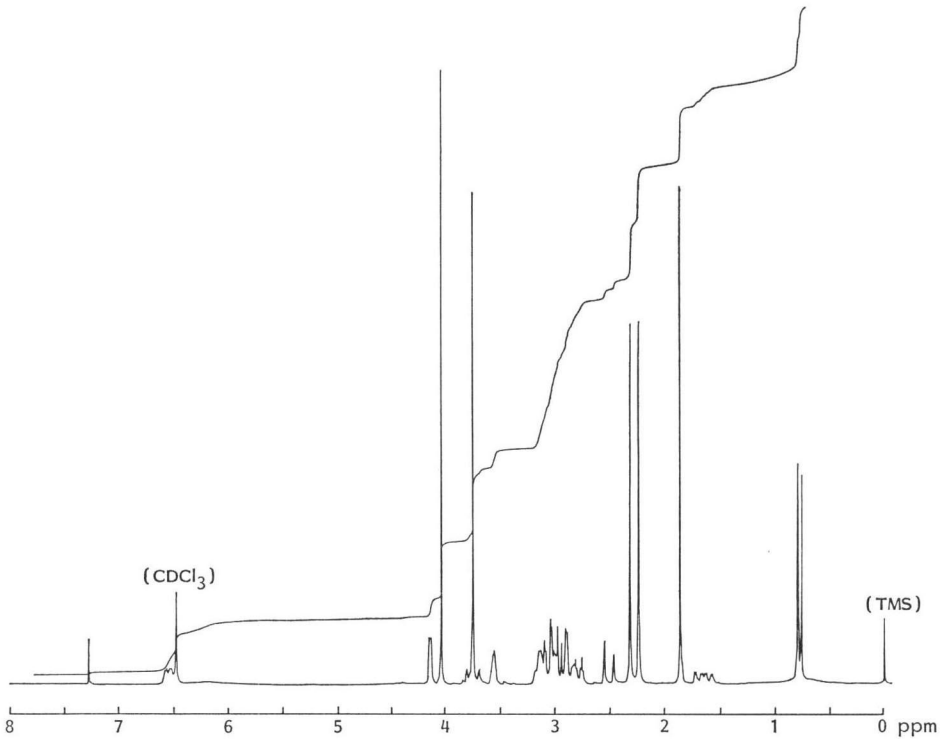
(**1-a**)



Saframycin B

[a] **1-a**: *N*-Acetylated compound of **1**.
[b] Reference 6.

Fig. 3. ^{1}H NMR spectrum of **1** (base) in $CDCl_3$ (200 MHz).



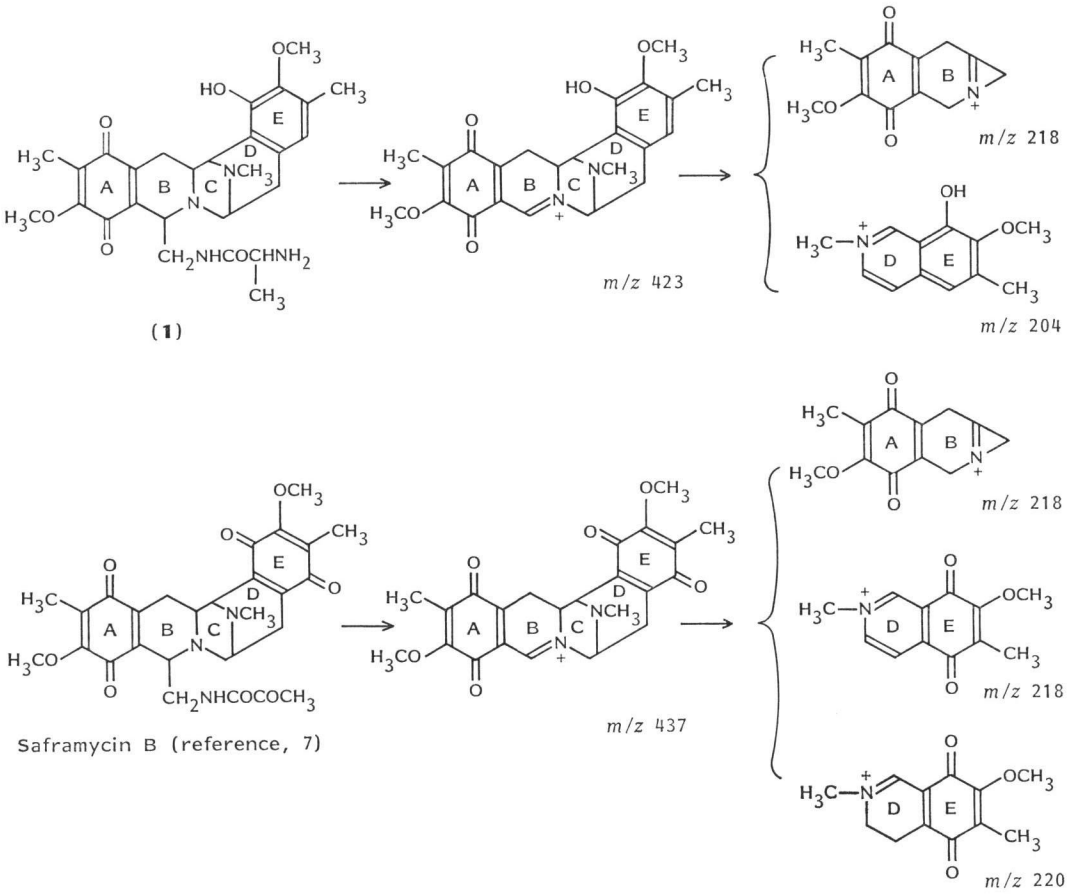
In order to confirm the presence of an amino group, a phenolic hydroxyl group and an aromatic proton in **1**, chemical modifications were carried out as shown in Fig 2. **1** gave an *N*-acetylated compound (**1-a**) with acetic anhydride in methanol, and an *N,O*-diacetylated compound (**1-c**) in pyridine. **1-a** further reacted with diazomethane in methanol resulting in an *O*-methylated compound (**1-b**). **1** was converted to a monobromo compound by bromine in methylenechloride. An alanine residue was detected as DNP-alanine by acid hydrolysis of DNP-**1**.

^{1}H NMR of **1** are shown in Fig. 3. The presence of six methyl groups is indicated by the signals at δ 0.8 (3H, d), 1.9 (3H, s), 2.25 (3H, s), 2.3 (3H, s), 3.75 (3H, s), and 4.0 (3H, s). Signals for an amide and an aromatic proton appeared at δ 6.55 (1H, d) and 6.5 (1H, s) respectively.

The ^{13}C NMR of **1-a** was measured, since **1-a** is more soluble in $CDCl_3$ than **1**. The assignment of chemical shifts for **1-a** by comparing with saframycin B[6] is shown in Table 2. The signals for C_5, C_{15} (δ 185.7 and 187.0) and for C_8, C_{18} (δ 181.3 and 182.8) in the paraquinone rings of saframycin B, though not clearly assigned, changed considerably with **1-a**. One signal disappeared for each of these pairs while two new signals appeared, a doublet at δ 120.3 and a singlet at δ 143.7. The new doublet indicates a nonsubstituted aromatic carbon, and the new singlet indicates a hydroxylated aromatic carbon, presumably adjacent to the methoxylated aromatic carbon indicated by δ 147.9. Therefore, in **1** one of the paraquinone rings of saframycin B (A or E) is reduced to a monophenol ring.

The signal δ 6.50 for the aromatic proton of **1** disappeared in the ^{1}H NMR spectrum of the monobrominated compound (**1-d**), while a new signal at δ 116.5 appeared in the ^{13}C NMR spectrum of **1-d**, suggesting that the bromo group was substituted at a *para* position of the phenolic hydroxyl group, C_5, C_8, C_{15} or C_{18}.

Fig. 4. Mass spectral fragmentation of **1** and saframycin B.



The proposed structures for the mass fragmentations of **1** at *m/z* 423, 218 and 204, made by comparing with saframycin B,[7] are shown in Fig. 4. These structures suggest that the phenolic ring of **1** is E ring.

In the ^{13}C NMR spectrum of **1-a**, the signals at δ 24.2 and 196.5 which were assigned to pyruvic acid in saframycin B were absent, while the new signals at δ 19.1 and 58.0 appeared, indicating that pyruvic acid residue of saframycin B was substituted with an alanine residue in **1**.

The proposed structure for **1**, based on the above results together with the assumption that **1** is an analogue of saframycin B, is shown in Fig. 1.

In the ^{13}C NMR of **2**, the triplet at δ 58.8 for C_{21} disappeared, while a new doublet at δ 83.3 was observed. Similar chemical shifts between saframycins B and S have been reported.[7] Therefore, in **2** the proton on C_{21} of **1** was substituted with a hydroxyl group.

Recently the complete structure and stereochemistry of **1** was established by UEDA *et al.* (personal communication) using X-ray crystallographic analysis of the monobrominated compound (**1-d**). The results will be reported elsewhere.

Experimental

^{1}H NMR spectra were recorded on a JNM FX-100 (100 MHz) or a JNM FX-200 (200 MHz). ^{13}C NMR spectra were recorded on a JNM FX-100 (25 MHz). Mass spectra were measured on a JMS OISG-2. Optical rotation was determined on a Jasco DIP-181. IR spectra were obtained on a Jasco IRA-2. UV were recorded on a Hitachi-32.

Nonaqueous Titration (Detection of Basic Nitrogen)

1 (base, 175 mg) dissolved into acetic acid (*ca.* 50 ml) was titrated with 0.01 N $HClO_4$. The consumption of 6.6 ml (2 mol equiv) and 10 ml (3 mol equiv) of 0.01 N $HClO_4$ was observed.

N-Acetylation of **1**

Acetic anhydride (0.5 ml) was added to 50 mg of **1** (base) dissolved in 5 ml of MeOH. After standing for 60 minutes at room temperature, ice was added to the reaction mixture and MeOH was evaporated *in vacuo*. The product was extracted with EtOAc, washed with H_2O, and the EtOAc was evaporated. 45 mg of yellow powder (**1-a**) was obtained. *m/z* 566 (M^+); ^{1}H NMR in $CDCl_3$ (100 MHz) δ 1.80 (3H, s, NHCOCH_3).

N,O-Diacetylation of **1**

Acetic anhydride (1 ml) was added to 50 mg of **1** (base) dissolved in 3 ml of pyridine. After standing for 24 hours at room temperature, ice was added to the reaction mixture and the product was extracted with EtOAc, washed with 0.1% $NaHCO_3$ followed by H_2O and dried with Na_2SO_4. Brown oil was obtained by evaporation of EtOAc. The oil was chromatographed on a column of silica gel (50 g), eluting with $CHCl_3$ - MeOH (95: 5). 30 mg of yellow oil (**1-c**) was obtained. *m/z* 608 (M^+); ^{1}H NMR in $CDCl_3$ (100 MHz) δ 1.70 (3H, s, NHCOCH_3), 2.48 (3H, s, PhOCOCH_3).

Methylation of **1-a**

Excess diazomethane was added to 50 mg of **1-a** dissolved in 5 ml of MeOH, and the mixture was allowed to stand for 24 hours at room temperature. After evaporating the solvent, 52 mg of yellow oil (**1-b**) was obtained. *m/z* 580 (M^+); ^{1}H NMR in $CDCl_3$ δ 3.88 (s, PhOCH_3).

Bromination of **1**

700 mg bromine in 3 ml of $CHCl_3$ was added to 470 mg of **1** (base) dissolved in 7 ml of CH_2Cl_2, under cooling with ice, and the mixture was allowed to stand for 2 hours. The reaction mixture was washed with 3% $NaHCO_3$ followed by H_2O. $CHCl_3$ was evaporated *in vacuo*. 400 mg of yellowish brown oil was obtained.

This oil was purified on a column of silica gel, eluting with $CHCl_3$ - benzene - MeOH (3: 1: 0.3). The yellow powder obtained was recrystallized from benzene. Yellow crystals (253 mg) (**1-d**) were obtained. mp 111~113°C (dec.); *m/z* 602 (M^+); ^{13}C NMR in $CDCl_3$ (25 MHz) δ 116.5 (s, C_{15}).

Detection of Alanine in **1**

2 ml of 3% Na_2CO_3 and 1 ml of 5% methanolic solution of 2,4-dinitrofluorobenzene (DNP) were added to a solution of 50 mg of **1** (base) dissolved in 1 ml of MeOH, under cooling with ice, and stirred for 3 hours. The precipitated yellow crystals were filtered and washed with isopropyl ether. 43 mg of DNP-**1** was obtained. 4 mg of DNP-**1** obtained above was hydrolyzed with 1 ml of 6 N HCl in a sealed tube at 110°C for 16 hours. The hydrolysate was developed on a TLC (silica gel) using the solvent, 1-butanol - AcOH - H_2O (4: 1: 1). The spot was detected at Rf 0.7, indicating DNP-alanine.

Note Added in Proof

Safracins A and B were originally designated as Y-16482β and Y-16482α respectively in Japan Kokai (patent) 82-18,633 (Jan. 30, 1982) and EP 55,299 (July 7, 1982). Later these designations were changed to Y-16601 and Y-16760 respectively in oral presentations at the Annual Meetings of the Agricultural Chemical Society of Japan and the Pharmaceutical Society of Japan. In February of this year MEYERS *et al.* reported that EM5519 was identical to Y-16482α (safracin B)[8]. Recently

TSUJI *et al.* reported at the 233rd Meeting of Japan Antibiotics Research Association the discovery of quinonamines A and B very similar to safracins A and B. The chemical structures of these quinonamines A and B were identical to safracins B and A respectively. But the complete stereochemical identity of these compounds is still unknown.

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