ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1985, p. 5–11
0066-4804/85/070005-07$02.00/0

Vol. 28, No. 1

Directed Biosynthesis of New Saframycin Derivatives with Resting Cells of *Streptomyces lavendulae*

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Received 10 December 1984/Accepted 27 March 1985

Saframycin A is an antitumor antibiotic produced by *Streptomyces lavendulae* 314 which falls into the category of the N-heterocyclic quinone group. Biosynthetically the quinone ring is derived from two tyrosine molecules which condense to generate the basic ring system of saframycin A. The side chain also has been found to derive from two amino acids, i.e., glycine and alanine. Supplementation by various amino acid analogs of the side chain produced three new saframycin derivatives with a replaced side chain. These three saframycins, designated Yd-1, Yd-2, and Y3, contained 2-amino-*n*-butyric acid, glycine, and alanine residues, respectively, in place of the normal N-terminal pyruvic acid in the side chain of saframycin A. Feeding experiments with ^{13}C-labeled dipeptide indicated that the amino acids are probably incorporated in the side chain as a dipeptide unit. It was also found that saframycin A is produced from saframycin Y3 by an enzymatic deamination reaction. Based on these results, saframycin biosynthesis in *S. lavendulae* is discussed.

Saframycins are antitumor antibiotics produced by *Streptomyces lavendulae*. The antibiotics are in the heterocyclic quinone antibiotic group, which includes mitomycin C (12) and streptonigrin (14). However, unlike those antibiotics, the saframycins contain two heterocyclic quinone moieties. Nine components, i.e., A, B, C, D, F, G, H, R, and S, have been isolated, their structures have been characterized, and their biological activities have been examined (Fig. 1; 3, 4, 7). Among these components, saframycin A has been shown to possess the highest biological activity and to be a potent antitumor agent against various experimental tumors (1).

The mode of action of saframycin A has been extensively studied. All the data so far obtained indicate that the major antitumor activity of the antibiotic is caused by covalent binding of duplex DNA (6, 9).

Alteration of the structure of antibiotics by means of an exogenously supplied precursor has been termed "directed biosynthesis" (10). Novel antibiotics have been prepared by the directed-biosynthesis method; in particular, this method has been applied to antitumor antibiotics such as actinomycins and bleomycins (10, 13).

Recently our biosynthesis studies on saframycin A demonstrated that the dimeric N-heterocyclic quinones which are common to saframycin group antibiotics were derived from two tyrosine molecules. Our attempted directed-biosynthesis experiments with various tyrosine analogs such as 3-nitrotyrosine, 3-aminotyrosine, *p*-aminophenylalanine, *p*-iodophenylalanine, *m*-fluorotyrosine, or *p*-fluorophenylalanine failed to produce new saframycin derivatives with the structural modifications in the quinones (K. Yazawa, K. Takahashi, Y. Mikami, and T. Arai, unpublished data). However, since these biosynthesis studies also indicated that the side chain arises from alanine and glycine (Fig. 2), the studies prompted us to attempt a means of preparing saframycin derivatives by a directed-biosynthesis method, which mainly involves structural alterations in the side chain.

This paper describes the method of directed biosynthesis and the isolation and characterization of the new saframycin derivatives, Y3, Yd-1, and Yd-2, which have replaced side chains. On the basis of the results, we also discuss the possible biosynthesis routes of the saframycins.

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MATERIALS AND METHODS

Microorganism. *S. lavendulae* 314, a saframycin producer, was used. The culture was maintained by transfer on yeast-starch agar (YSA medium) slants at 27°C.

Chemicals. [1-^{13}C]glycine (91 atom % ^{13}C) was purchased from the Radiochemical Centre, Amersham, England. Dotite Good buffer (MES [morpholineethanesulfonic acid]) was purchased from Wako Pure Chemicals Ltd., Osaka, Japan. Glycylglycine was from Nakarai Chemical Co., Ltd., Kyoto, Japan, and valylglycine, leucylglycine, serylglycine, prolylglycine, phenylalanylglycine, and tyrosylglycine were purchased from Sigma Chemical Co., St. Louis, Mo. Alanyl-[1-^{13}C]-glycine was synthesized by a modified version of the method of Wilcheck and Patchornik (15). Briefly, this synthesis was done by coupling carbobenzoxy-L-alanine with [1-^{13}C]-glycine benzyl ester by the dicyclohexylcarbodiimide method (15). Other dipeptides such as 2-amino-*n*-butyrylglycine, norvalylglycine, and norleucylglycine were also synthesized in the same manner and used for the incorporation studies. The enzymes L- and D-amino acid oxidase (EC 1.4.3.2 and EC 1.4.3.3, respectively) were purchased from Sigma. All other chemicals were of analytical grade and were obtained from Wako.

Media. YSA medium (pH 7.5) contained (percent composition, wt/vol) starch, 0.5%; yeast extract (Difco Laboratories, Detroit, Mich.), 0.1%; and agar, 1.5%. Seed and fermentation media of the following compositions were used. Glucose starch broth (GSB) seed medium (pH 7.0) contained glucose, 0.1%; starch, 1%; meat extract (Wako), 0.5%; NaCl, 0.3%; and anti-foam (Silicon KM-72F, Shinetsu Kagaku Co., Ltd., Fukui, Japan), 0.02%. GSB fermentation medium (pH 7.0) contained glucose, 0.5%; starch, 0.5%; meat extract, 0.5%; NaCl, 0.3%; and anti-foam (Adecanol LG-109, Asahidenka Co., Ltd., Tokyo, Japan), 0.002%.

Culture conditions. One loopful of spores from a culture grown on a YSA slant at 27°C for 7 days was inoculated into a Sakaguchi shake flask (500-ml volume; Iwashiya Seisakujo, Tokyo, Japan) containing 200 ml of GSB medium

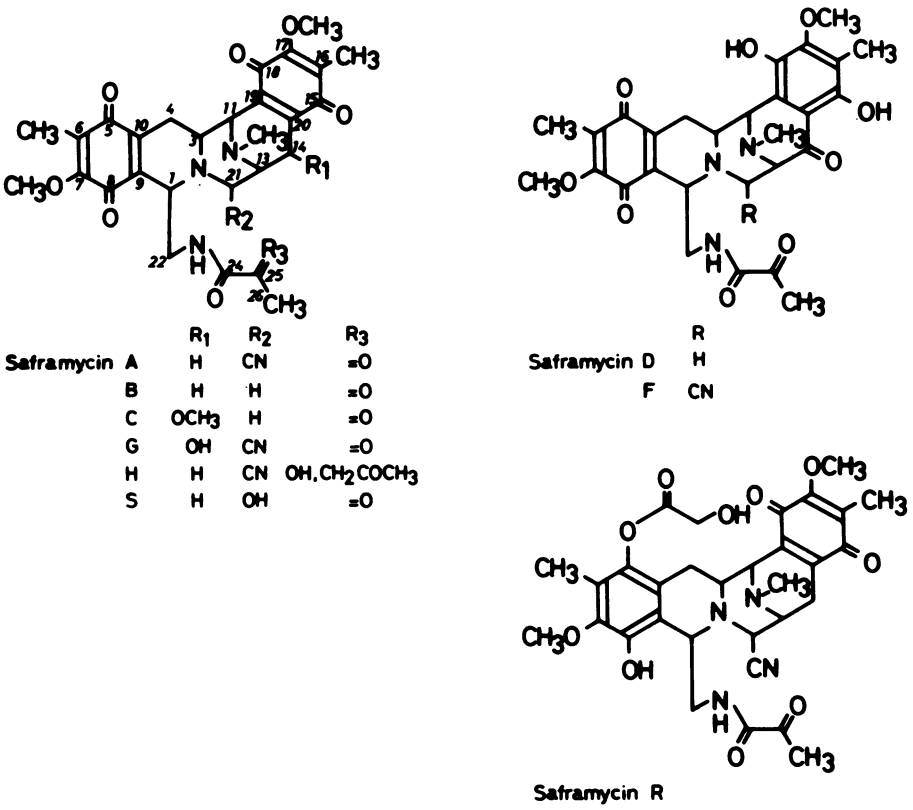

FIG. 1. Structures of saframycin group antibiotics.

and incubated by reciprocal shaking (250 strokes, 5.5 cm) at 27°C for 30 to 36 h. For preparation of the mycelia, the seed culture described above (0.02%) was inoculated into a 20-liter jar fermentor (model MSJ-U, Marubishi Co., Ltd., Tokyo, Japan) containing 10 liters of GSB fermentation medium and grown by agitation (350 rpm; aeration, 2.0 liter/vol per min).

Preparation of resting cells and directed biosynthesis of saframycins. After about 16 h of incubation in the 20-liter jar fermentor, the cells had a blue pigment (this blue pigmentation was associated with the initiation of saframycin production; 11). After an additional 1 h of incubation after the pigmentation of the cells, the cells were harvested by centrifugation at 8,000 × *g* for 15 min and washed twice with 0.01 M MES buffer (pH 5.7). The cells obtained from 40-ml cultures were then suspended in an Erlenmeyer shake flask (50 ml) containing 9 ml of 0.1 M MES buffer (pH 5.75) with 10 μM methionine and tyrosine. The concentration of cells in the buffer suspension was fourfold greater than in the fermentation medium from which the cells were recovered. The amino acids (1 mM in final concentration) were added to the buffer-cell suspension, and incubation was continued on the rotary shaker at 27°C for 6 h (300 rpm).

In some experiments, L- or D-cycloserine (1 mM in final concentration) was added to the reaction mixture to inhibit the saframycin synthesis.

For the preparative isolation of products, the same washed cells from 4 liters of culture as described above were suspended in a 2-liter fermentor (model MS-1000, Marubishi) containing 1 liter of 0.01 M MES buffer (pH 5.75). Various amino acids (as described above) were added to the cells, and the reaction mixture was incubated for an additional 6 h at 27°C by agitation (350 rpm; aeration, 2.0 liters/vol per min).

Isolation and purification of products. The reaction mixture described above was adjusted to pH 7, and a final concentration of 1 mM NaCN was added to the filtrate. The filtrate was then incubated for 30 min at 35°C. By this treatment, decyano-saframycins, which are unstable biosynthetic precursors, are converted into stable saframycins (2). The saframycin fraction was extracted with ethyl acetate. The solvent layer was then counterextracted twice with 10% acetic acid solution. The aqueous layers were combined and adjusted to pH 8.0 to 9.0 with concentrated ammonium hydroxide. The aqueous layer was reextracted with ethyl acetate, and the resultant extract was washed with deionized water and concentrated in vacuo to dryness. The dried residue was applied to Silica Gel 60 F254 (E. Merck, Darmstadt, Federal Republic of Germany) column chromatography, and saframycins were eluted first with ethyl acetate-benzene (1:1) and then with ethyl acetate-methanol (9:1) as solvents. When required, saframycins were further purified by the combination of silica gel preparative thin-layer chromatography (TLC) with a solvent system of chloroform-ethanol (1:1) and a silica gel column.

Deamination of saframycins Y3 and Yd-1. Cells from the 20-liter jar fermentor mentioned above were washed three times with 0.01 M MES buffer, and 5 g (wet weight) of cells was suspended in 15 ml of 0.01 M MES buffer. The cells were disrupted by a sonicator (Matsumoto Seisakujo, Tokyo, Japan) and then centrifuged at 30,000 × *g* for 15 min. The supernatants were used as cell extract enzyme preparation. To 4 ml of the cell extract in a 10-ml Erlenmeyer shake flask was added 0.4 ml of 13 mM EDTA–0.6 μM saframycin

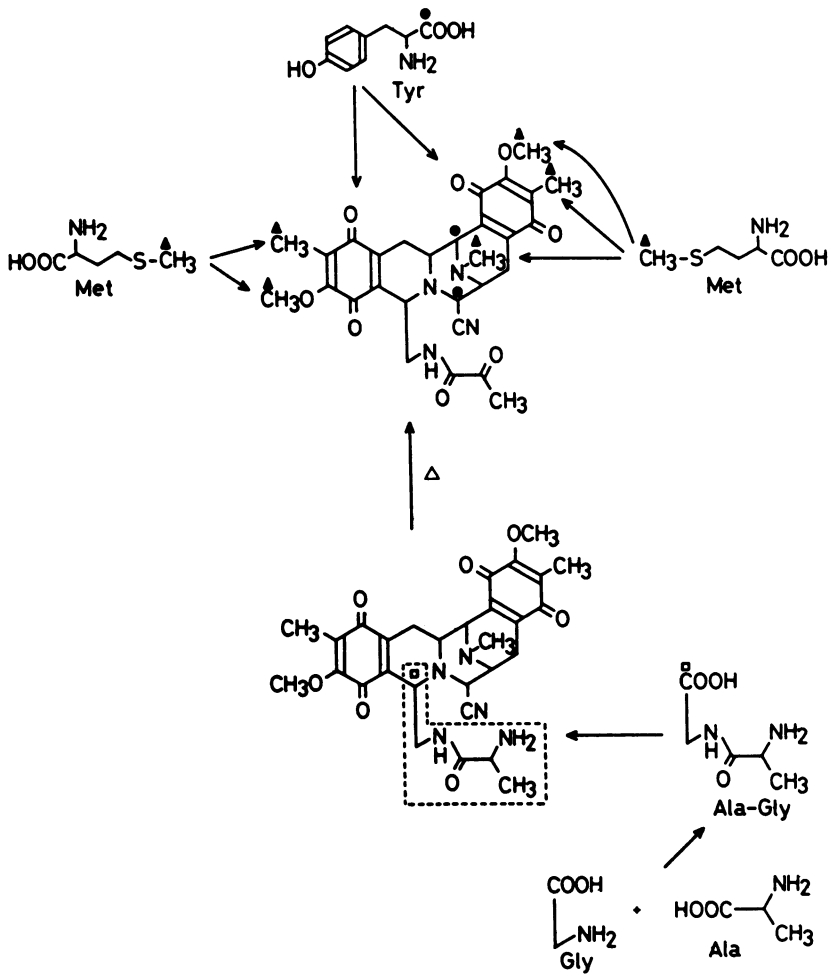

FIG. 2. Possible biosynthetic routes to saframycin A. △, Enzymic deamination reaction at C-25 site.

Y3 or Yd-1–0.15 M alanine or pyruvate (sodium salt). The reaction mixture was incubated at 37°C for 6 h. After adjustment of the pH to 7.0, the reaction mixture was treated with sodium cyanide as stated above, and the saframycin fraction was extracted to recover conversion products.

Detection of the products and spectroscopic measurements. The products on TLC plates were monitored with a dual-wavelength chromatogram scanner (CS-910, Shimazu Seisakujo, Kyoto, Japan). Melting points were determined on a melting point apparatus (model MP-21, Yamato, Japan) and were uncorrected. Optical rotations were measured on a DIP-4 spectrometer (Jasco, Japan). UV absorption spectra were determined on a Hitachi 323 spectrophotometer (Hitachi, Japan), and infrared (IR) absorption spectra were taken on an IRA-2 spectrometer (Jasco). Nuclear magnetic resonance (NMR) spectra were determined on a JNM-PFT-100 (JEOL, Japan) with tetramethylsilane as an internal standard. Mass spectra were determined on a GSM-01SG-2 double-focusing mass spectrometer (JEOL) by the field desorption method. Amino acids were determined by an automatic amino acid analyzer (Hitachi).

Structure determination. All the new saframycins, Y3, Yd-1, and Yd-2, were assigned structures consistent with their elemental analysis and IR, UV, and ^{1}H and ^{13}C NMR spectra (Table 1), although the ^{13}C NMR spectra of saframycin Yd-2 could not be measured due to lack of sufficient material. Information on ^{1}H and ^{13}C NMR spectra of saframycin A is available in the literature (4, 8, 11). The features in the spectra critical to the structure assignments of the antibiotics are as follows. Saframycin Y3: basic, yellow powder; mp 143 to 146°C; $[\alpha]_D^{22}$ −46.1 (c 0.07, MeOH); elemental analysis $C_{29}H_{33}N_5O_7$; field desorption mass, *m/z* 563 (M^+); UV max (MeOH) 268 nm (log ε 4.27), 340 nm (sh), UV min (MeOH) 232 nm (log ε 3.77); IR ($CHCl_3$) 3380, 1655, 1615, 1515. Saframycin Yd-1: basic, yellow powder, mp 124 to 127°C; $[\alpha]_D^{22}$ −43.5 (c 1.0, MeOH); elemental analysis $C_{30}H_{35}N_5O_7$; field desorption mass, *m/z* 577 (M^+); UV max (MeOH) 269 nm (log ε 4.26), UV min (MeOH) 233 nm (log ε 3.77); IR ($CHCl_3$) 3380, 1660, 1615, 1515. Saframycin Yd-2: basic, yellow powder; mp 144 to 148°C; elemental analysis $C_{28}H_{31}N_5O_7$; field desorption mass, *m/z* 549 (M^+), UV max (MeOH) 268 nm (log ε 4.23), UV min (MeOH) 233 nm (log ε 3.77); IR ($CHCl_3$) 3380, 1660, 1615, 1515.

RESULTS

Production of new saframycins Y3 and Yd-1. Our recent studies on the action mechanisms of saframycin A indicated a significant role for the side chain in the activity (K. Kishi, K. Yazawa, K. Takahashi, Y. Mikami, T. Arai, M. Namikoshi, S. Iwasaki, and S. Okuda, unpublished data). Therefore, our first experiments in directed biosynthesis aimed to alter the side chain of saframycins. In addition to tyrosine and methionine, alanine and glycine were added to saframycin-producing *S. lavendulae* resting cells. The

TABLE 1. Assignments of ^{13}C and ^{1}H NMR signals of saframycins Y3, Yd-1, and Yd-2 in $CDCl_3$, with tetramethylsilane as the internal reference

Carbon no.	Chemical shifts δ (ppm)[a]				
	Saframycin Y3		Saframycin Yd-1		Saframycin Yd-2 proton
	Carbon	Proton	Carbon	Proton	
1	56.8 (d)	3.94 (bs)	56.8 (d)	3.91 (bs)	3.39 (bs)
3	52.6 (d)	3.15 (d-d-d)	53.6 (d)	3.14 (d-d-d)	3.14 (d-d-d)
4	25.4 (t)	2.88 (d-d)	25.4 (t)	2.86 (d-d)	2.88 (d-d)
		1.37 (d-d-d)		1.35 (d-d-d)	1.38 (d-d-d)
5, 15	185.7 (s), 186.4 (s)		185.5 (s), 186.4 (s)		
6, 16	127.0 (s), 128.4 (s)		127.2 (s), 128.3 (s)		
7, 17	155.5 (s), 155.1 (s)		156.6 (s), 155.3 (s)		
8, 18	180.4 (s), 182.0 (s)		180.8 (s), 182.5 (s)		
9, 19	136.4 (s), 135.9 (s)		136.5 (s), 135.9 (s)		
10, 20	139.7 (s), 141.7 (s)		139.7 (s), 141.7 (s)		
11	54.3 (d)	4.07 (bs)	54.2 (d)	4.03–4.05[b]	4.05 (bs)
13	54.5 (d)	3.45 (d-d)	54.5 (d)	3.45 (d-d)	3.44 (d-d)
14	21.6 (t)	2.80 (d-d)	21.5 (t)	2.81 (d-d)	2.82 (d-d)
		2.27 (d)		2.32 (d)	2.31 (d)
21	58.2 (d)	4.00 (d)	58.1 (d)	4.01 (d)	4.00 (d)
CN	116.8 (s)		116.8 (s)		
$=C-CH_3$	8.5 (q), 8.6 (q)	1.92 (s)	8.5 (q), 8.7 (q)	1.89 (s)	1.90 (s)
		1.88 (s)		1.93 (s)	1.93 (s)
$=C-OCH_3$	61.0 (q), 61.1 (q)	4.03 (s)	61.0 (q) × 2	4.03 (s)	4.03 (s) × 2
		4.06 (s)		4.05 (s)	
$N-CH_3$	41.7 (q)	2.33 (s)	41.6 (q)	2.32 (s)	2.32 (s)
	$R=CH_2NHCOCH(NH_2)CH_3$		$R=CH_2NHCOCH(NH_2)CH_2CH_3$		$R=CH_2NHCOCH_2NH_2$
CH_2	39.9 (t)	3.83 (d-d-d)	39.9 (t)	3.83 (d-d-d)	3.82 (d-d-d)
		3.00 (d-d-d)		3.06 (d-d-d)	3.10 (d-d-d)
NH		7.23 (d-d)		7.23 (d-d)	7.23 (d-d)
CO	174.5 (s)		174.7 (s)		2.97 (d)
$CH(NH_2)$	50.1 (d)	3.27 (q)	56.0 (d)	3.05 (t)	3.11 (d)
CH_3	21.3 (q)	0.92 (d)			
CH_2CH_3			27.9 (t)	1.06 (m), 1.48 (m)	
			10.3 (q)	0.74 (t)	

[a] d, m, q, s, and t show multiplicity. R indicates the side chain attached at C-1 carbon as shown in Fig. 1.
[b] Not determined.

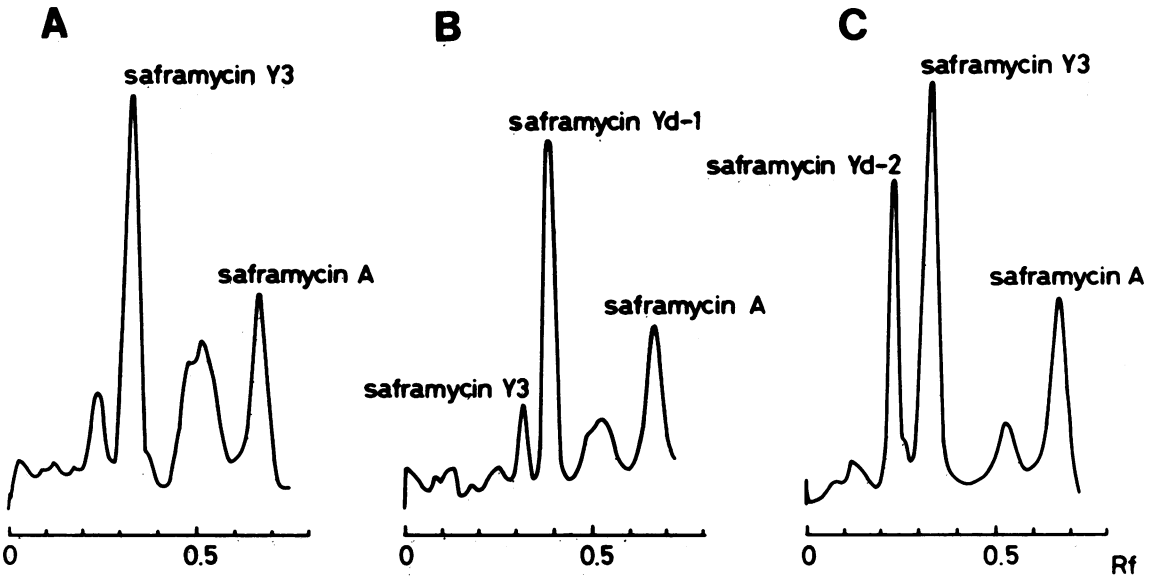

FIG. 3. TLC patterns of the fermentation products of the directed biosynthesis method employing resting cells of *S. lavendulae* 314. (A) In addition to tyrosine and methionine, alanine and glycine were added. (B) 2-Amino-*n*-butyric acid was added. (C) Glycylglycine was added.

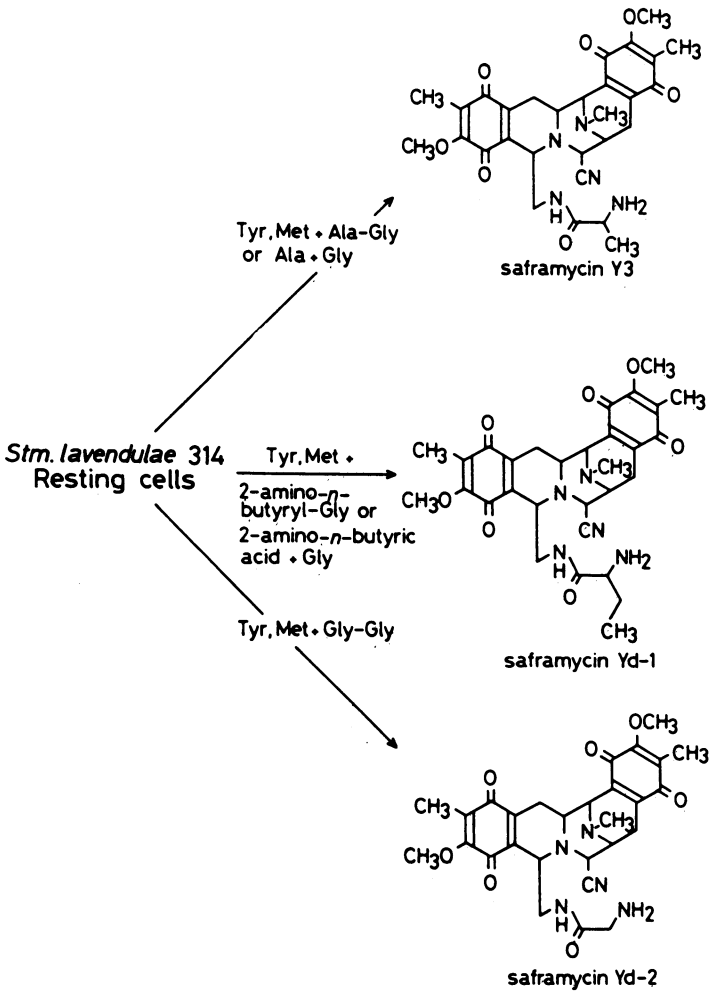

FIG. 4. Scheme of directed biosynthesis of new saframycin derivatives employing resting cells of *S. lavendulae* 314. Supplemented substrates are indicated for each experiment. Tyrosine and methionine were commonly added to all reaction mixtures for the synthesis of the heterocyclic quinone ring of the saframycins.

former two amino acids were shown to contribute to the synthesis of the basic rings and to methyl and methoxy substituents on the quinone ring of saframycin A; the latter two amino acids contribute to the synthesis of the saframycin side chain (Fig. 2).

When these amino acids were added to the resting cells, a strong new peak was observed on the TLC pattern in addition to the normal saframycin A peak produced by this organism (Fig. 3A). The new peak component, designated saframycin Y3, was isolated and purified from the reaction mixture described above. Studies on its physicochemical data showed that saframycin Y3 is a new saframycin group antibiotic containing an alanine residue in place of a pyruvate in saframycin A. The total structure was determined to be as shown in Fig. 4. To obtain other new saframycin derivatives, similar experiments were conducted with the following amino acid analogs: valine, leucine, isoleucine, serine, threonine, cystine, 2-amino-isobutyric acid, 2-amino-*n*-butyric acid, sarcosine, *N*-acetylglycine, taurine, ethionine, norvaline, and norleucine. These amino acids were supplied to the resting cells of saframycin-producing strains. Among the added amino acids, only 2-amino-*n*-butyric acid produced an additional new peak on the TLC pattern (Fig. 3B). The new product, designated saframycin Yd-1, was isolated and purified. By using detailed physicochemical information, it was determined that the antibiotic contained 2-amino-*n*-butyric acid in place of the C-terminal amino acid, alanine, of saframycin Y3 (Fig. 4).

Incorporation of preformed dipeptide into the side chain of saframycins. To determine whether alanine and glycine were incorporated into the side chain before or after the dipeptide formation, preformed dipeptide was added to the reaction mixture. For this experiment, chemically synthesized alanyl-[1-^{13}C]glycine was supplied to the reaction mixture of the resting cells in a 1.0-liter jar fermentor. After 6 h of incubation, the reaction mixture was treated with sodium cyanide to generate saframycin Y3 from its unstable saframycin Y3 precursor. Then saframycin Y3 was extracted and successively purified as described previously to yield 25 mg of the labeled antibiotic. As shown in the ^{13}C NMR spectrum of the product (Fig. 5), most of the labeled dipeptide was incorporated into saframycin Y3, and, moreover, only one signal due to the C-1 carbon (δ 56.8) at the starting point of the side chain was enriched. The enrichment was about threefold natural abundance. Under the conditions described above, the utilization of several potential dipeptide precursors composed of glycine as a C-terminal amino acid and one additional amino acid in the N-terminal position was

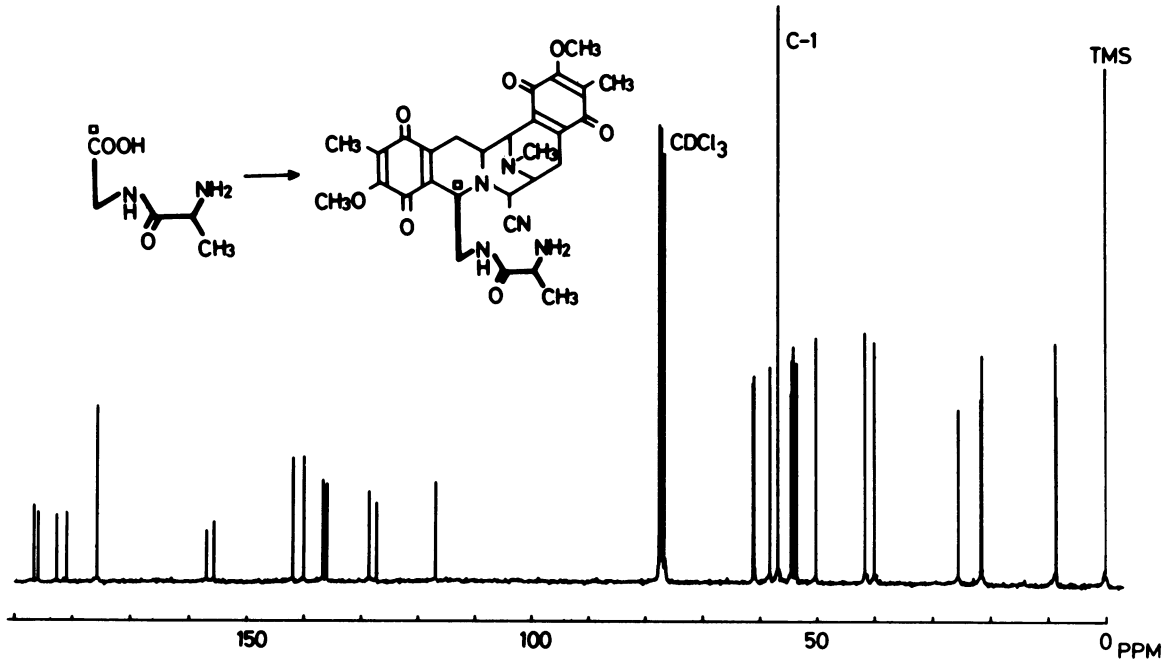

FIG. 5. ^{13}C NMR spectra at 67 MHz of alanyl-[1-^{13}C]glycine-labeled saframycin A in $CDCl_3$. Chemical shifts are in parts per million from tetramethylsilane (TMS). The enrichment of C-1 was threefold natural abundance. The concentration of ^{13}C was determined by using neighboring signals as internal standards.

evaluated. When 10 dipeptide side chain analogs such as glycylglycine, valylglycine, leucylglycine, serylglycine, prolylglycine, phenylalanylglycine, tyrosylglycine, 2-amino-*n*-butyrylglycine, norvalylglycine, and norleucylglycine were supplied to the resting cells, new peaks on the TLC patterns were observed with glycylglycine (Fig. 3C), 2-amino-*n*-butyrylglycine, and alanylglycine. The new product from *N*-2-amino-*n*-butyrylglycine was identified as saframycin Yd-1. Studies on the remaining product with glycylglycine showed that it is a new saframycin group antibiotic, and it was designated saframycin Yd-2. The physicochemical data indicate that the side chain alanylglycine of saframycin Y3 was replaced by glycylglycine in saframycin Yd-2. These results of directed biosynthesis are summarized in Fig. 4.

Deamination of saframycins Y3 and Yd-1. The fact that increased production of saframycin Y3 by the addition of alanine and glycine or alanylglycine to the resting cells was coupled with a rise in saframycin A (carbonyl type of saframycin Y3) strongly suggested a close biosynthetic relationship between the two antibiotics.

When saframycin Y3 was incubated with the cell extracts of *S. lavendulae* and the resultant product was monitored by TLC, enzymatic conversion of saframycin Y3 to saframycin A, but not the reverse, was observed. The recovery of the new product was 70 to 80%. The activity was completely abolished by heating the extract at 100°C for 10 min, but the addition of NAD or NADH did not affect the reaction. Consequently it was concluded that conversion of saframycin Y3 to saframycin A was an irreversible reaction and that saframycin Y3 was a direct biosynthetic intermediate of saframycin A.

Configuration of the alanine moiety of the saframycin side chain. Studies were directed toward establishing the precursor configuration of the alanine moiety in the saframycin Y3 side chain. We hydrolyzed saframycin Y3, and the hydrolysates were treated with D- or L-amino acid oxidase to determine the alanine configuration. The amino acid pattern obtained by the automatic amino acid analysis of the hydrolysates showed that alanine in saframycin Y3 is L-form. When L- or D-cycloserine was added to the above-mentioned reaction system, saframycin Y3 synthesis was completely inhibited by L-cycloserine but not by D-cycloserine.

DISCUSSION

The strong antitumor activity of the saframycins has prompted us to prepare a number of analogs. Although the possible structural variations are limited, directed biosyntheses have the advantage that exogenously supplied substrates are specifically incorporated in place of a normal precursor into possible active sites. The advantages were more apparent with resting-cell systems than with growing-cell systems.

Among the three new antibiotics which were found by directed biosynthesis, the existence of saframycin Y3 in an ordinal culture broth, although in only a trace amount, was very recently confirmed. Our finding that the resting cells supplemented with either alanine and glycine or alanylglycine are stimulated to produce a net yield of saframycin Y3 of two to five times that of ordinal cultures suggests that the exogenously added amino acids serve as a directing force in the biosynthesis route of saframycin Y3.

Saframycins Y3 and Yd-1 were produced with the addition of either a free two-amino-acid mixture or dipeptides. Saframycin Yd-2, however, was detected only with the addition of a dipeptide, glycylglycine, but not with the addition of a single amino acid, glycine alone. This phenomenon is in contrast to the situation with saframycins Yd-1 and Y3 and may be because the enzyme systems responsible for the formation of dipeptide do not specifically recognize glycine as forming glycylglycine.

Very good incorporation of a preformed dipeptide unit into the side chains of saframycins was observed with a ^{13}C-labeled dipeptide. These results strongly suggest that the

^{13}C-labeled dipeptide. These results strongly suggest that the saframycin Y3 side chain came from the corresponding dipeptide units of alanylglycine. Although the possibility existed that some preformed dipeptides were hydrolyzed and reincorporated into the side chain, this possibility might be excluded by the following facts: (i) incorporation of ^{13}C-labeled dipeptide in saframycin Y3 was very good; (ii) inhibition of saframycin Y3 synthesis by L-cycloserine was observed in the presence of alanine and glycine but not in the presence of the dipeptide alanylglycine; and (iii) saframycin Yd-2 was produced only by the addition of a dipeptide, glycylglycine, but not by the addition of its single amino acid, glycine.

The Corey-Pauling-Koltun space-filling model for the saframycin A-DNA adduct (T. Arai, K. Kishi, Y. Mikami, M. Namikoshi, S. Iwasaki, and S. Okuda, unpublished data) suggested that the modification at the C-25 position on the side chain of saframycin A with a substituent should result in changes in the activity, because the side chain carbonyl group should fit along the minor groove of duplex DNA in the model. Therefore, saframycin derivatives with a modified side chain could be of interest not only for their potential value as antitumor agents, but also as probes of the role of the side chain in the interaction between saframycin antibiotics and DNA.

Attempts at formation of a modified antibiotic have been made by the addition of precursors to a fermentation medium (13). In most cases, however, modified antibiotics showed lower activities than normal products, but some of them exhibited improved characteristics such as lower toxicities or higher antibiotic activities than normal products. Our preliminary studies on biological activity suggested that saframycins Yd-1 and Y3 showed higher antitumor activity than saframycin A against various murine tumors such as L1210 leukemia, although the activity of saframycin Yd-2 was drastically reduced (K. Takahashi, Y. Mikami, K. Yazawa, and T. Arai, unpublished data). The latter result also confirmed the importance of the side chain in the exhibition of biological activity as stated above.

All these saframycins possess an amino functional group in their side chain and are more soluble than other saframycins (3) in water. Therefore, the newly discovered saframycin derivatives were expected to have different pharmacodynamic properties.

Studies on the conversion of saframycin Y3 to saframycin A by the cell-free systems indicate that saframycin Y3 is a direct biosynthetic intermediate of saframycin A, although further enzymatic studies, including studies of the specificity of the deaminase, are necessary.

The time course of the various events in the assembly of saframycin molecules is now being studied.

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