SANDRAMYCIN, A NOVEL ANTITUMOR ANTIBIOTIC PRODUCED BY A *Nocardioides* sp.

II. STRUCTURE DETERMINATION

JAMES A. MATSON*, KIMBERLY L. COLSON, GILBERT N. BELOFSKY and BONNIE B. BLEIBERG

Bristol-Myers Squibb, Pharmaceutical Research Institute,
5 Research Parkway, P.O. Box 5100, Wallingford, CT. 06492, U.S.A.

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The structure of sandramycin, a novel antitumor antibiotic, was established by spectroscopic analysis and chiral chromatography of its acid hydrolysate. It was determined to be a cyclic decadepsipeptide with a two-fold axis of symmetry and 3-hydroxyquinaldic acid as an appended chromophore.

Sandramycin is a new antitumor antibiotic produced by a *Nocardioides* sp. (ATCC 39419). Its production, isolation, characterization and biological properties were reported in a previous paper[1]. The details of the structure determination and the assignment of chirality are described in this paper.

Results and Discussion

Because of the similarity in their antitumor activity, in their isolation pathway and in their chromatographic behavior, sandramycin was initially thought to be identical to luzopeptin A (formally called BBM-928)[2~5]. However, direct comparison of their UV and ^{1}H NMR quickly dispelled this hypothesis. Their UV spectra were quite different and the ^{1}H NMR revealed at least three differences between them. Proton coupling in the upfield methyl signals of sandramycin suggested that the β-hydroxy-*N*-methylvaline residue of luzopeptin A was missing and perhaps replaced by *N*-methylvaline. A different chromophore was evident by the different UV spectra and by an additional aryl proton and missing aryl methoxy group in the ^{1}H NMR of sandramycin. Additional NMR resonances in the region of δ 1.4 to 2.0 and the lack an acetyl group in sandramycin also suggested a difference at the tetrahydropyridazine residue of luzopeptin A. The identification and connection of the residues in sandramycin was accomplished by analysis of ^{1}H and ^{13}C NMR data and degradation studies.

Residue Identification

The ^{1}H and ^{13}C NMR data of sandramycin are shown in Tables 1 and 2. From the ^{1}H-^{1}H COSY and long-range ^{1}H-^{1}H COSY spectra[6,7] the amino acids serine, glycine, sarcosine, *N*-methylvaline and pipecolic acid were readily identified by their respective spin systems as shown in Fig. 1. Similarly, the aromatic chromophore, 3-hydroxyquinaldic acid, was identified from the ^{1}H-^{1}H COSY and ^{1}H-^{13}C heteronuclear multiple-bond correlation (HMBC)[8] spectra of sandramycin with the couplings shown in Fig. 2.

Amino Acid Sequence

Nuclear Overhauser effect[9] and ^{1}H-^{13}C HMBC experiments were used to determine the

Table 1. ^{1}H NMR spectral data for sandramycin in $CDCl_3$[a].

δ_H	$J_{H,H}$ (Hz)	Observed ^{1}H NOE to:	δ_H	$J_{H,H}$ (Hz)	Observed ^{1}H NOE to:
11.71 bs, 1H			3.98 m, 1H		
9.50 d, 1H	4.4	5.19	3.96 m, 1H		
8.45 d, 1H	4.4	4.36, 3.98, 5.49	3.68 d, 1H	12.8	4.36
7.76 m, 1H			3.50 d, 1H	16.2	3.04
7.65 dd, 1H	3.4, 5.4		3.04 s, 3H		9.50, 7.76, 4.79, 0.71
7.60 s, 1H			2.87 s, 3H		
7.44 dd, 2H	3.0, 6.1		1.97 m, 1H	6.4	
5.49 d, 1H	6.9	8.45	1.73 m, 2H		8.45, 3.98
5.46 d, 1H	16.7		1.59 m, 2H		5.49
5.19 m, 1H	5.9	9.50, 4.36	1.47 m, 2H		
4.90 d, 1H	11.3		0.85 d, 3H	6.4	
4.79 d, 1H	10.8	0.71	0.71 d, 3H	6.4	4.79, 3.04, 1.97
4.36 d, 2H	11.3	3.68			

[a] Measured at 500 MHz with a drop of DMSO-d_6 added for line sharpening.

Table 2. Heteronuclear NMR spectral data for sandramycin in $CDCl_3$[a].

C/H	δ ^{13}C[b]	δ ^{1}H	Observed heteronuclear correlations to δ ^{1}H	C/H	δ ^{13}C[b]	δ ^{1}H	Observed heteronuclear correlations to δ ^{1}H
10	172.4 s		8.45, 5.49	2	61.8 d	4.79	1.97
1	169.3 s		4.90, 4.79, 4.36	11	52.4 d	5.49	
7	169.0 s		5.46, 4.36, 3.96, 3.50, 2.87	18	50.5 d	5.19	4.90
				5	49.2 t	5.46, 3.50	
17	167.6 s		9.50, 5.49, 3.98	15	43.7 t	3.98, 3.68	5.49
4	167.3 s		5.46, 3.50, 3.04, 4.79	8	41.7 t	4.36, 3.96	
26	166.1 s		9.50, 5.19, 4.90	24	34.8 q	2.87	5.46, 3.50
3′	153.6 s		7.60	23	30.0 q	3.04	4.79
8a′	141.1 s		7.76, 7.65, 7.60, 7.44	14	28.6 t	1.59, 1.73	
2′	134.4 s		7.60	20	26.1 d	1.97	4.79
4a′	131.9 s		7.76, 7.65, 7.44	13	24.8 t	1.73, 1.47	5.49
5′	129.1 d	7.76	7.44	12	20.0 t	1.59, 1.47	5.49
7′	128.5 d	7.44	7.76	21	19.3 q	0.85	4.79, 1.97
6′	127.1 d	7.44	7.65	22	18.5 q	0.71	4.79, 1.97
8′	126.3 d	7.65	7.44	3′-OH		11.71	
4′	120.4 d	7.60	7.65	25-NH		9.50	
19	62.1 t	4.90, 4.36		9-NH		8.45	

[a] Measured at 500 MHz with drop of DMSO-d_6 added for line sharpening.
[b] s: Singlet, d: doublet, t: triplet, q: quartet.

amino acid sequence. A NOE observed between the serine β-proton (19-H) at δ 4.36 and the ε-proton of pipecolic acid (15-H) at δ 3.68 evidenced the attachment of these residues. A NOE observed between the α-proton (11-H) of pipecolic acid at δ 5.49 and glycine amide proton (9-H) at δ 8.45 demonstrated the connection between these two amino acids. Long range couplings to the glycine carbonyl (C-7) at δ 169.0 from the glycine geminal protons (8-H) at δ 3.96 and δ 4.36 and the sarcosine *N*-methyl protons (24-H) at δ 2.87 established their connection. The attachment of sarcosine to *N*-methylvaline was determined by a NOE between an α-proton (5-H) of sarcosine and the *N*-methyl protons (23-H) of valine. These interactions and sequence are illustrated in Fig. 3.

Cyclic Depsipeptide Structure

The molecular formula of sandramycin was established as $C_{60}H_{76}O_{16}N_{12}$ as reported earlier[1]. This

Fig. 1. ^{1}H-^{13}C long range couplings detected in the amino acids of sandramycin by HMBC.

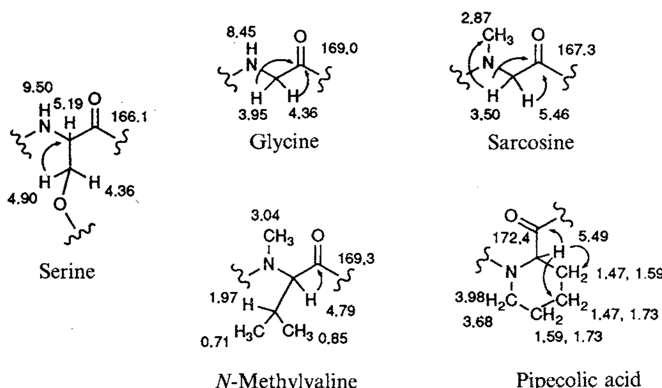


Fig. 2. ^{1}H-^{13}C long range coupling of sandramycin chromophore, 3-hydroxyquinaldate, as detected by HMBC.

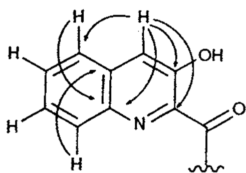
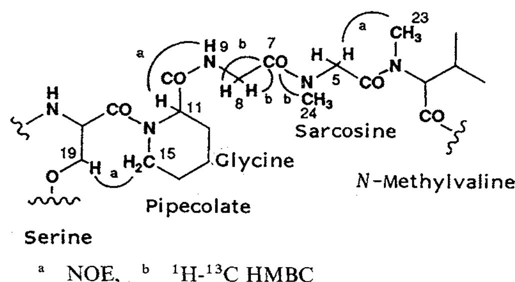


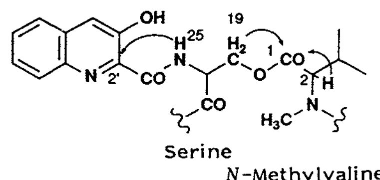
Fig. 3. Amino acid sequence and NOE data.



ᵃ NOE, ᵇ ^{1}H-^{13}C HMBC

formula requires that the structure of sandramycin have twenty nine degrees of unsaturation. Fourteen of the twenty nine degrees were defined with the substructural elements identified and the partial sequence of the core. Since the molecular formula has twice the number of the carbon and proton atoms observed in the NMR spectra, sandramycin had to be a symmetrical dimer. The remaining degree of unsaturation could only be accounted for by a macrocyclic structure.

Fig. 4. Chromophore attachment and dimerization site.



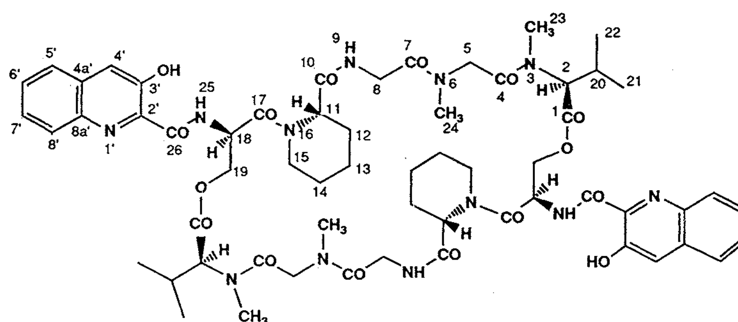
The solid arrows indicate ^{1}H-^{13}C long range coupling.

The sites of dimerization and the attachment of the aromatic chromophore were revealed by ^{1}H-^{13}C HMBC and COLOC[10)] results as shown in Fig. 4. The couplings to the carbonyl (C-1) at δ 169.3 from the serine β-protons (19-H at δ 4.36 and δ 4.90) and the *N*-methylvaline α-proton (2-H at δ 4.79), and an IR absorption band at $1748\,cm^{-1}$ showed that two core units were attached end to end through an ester bond at these residues. The site of attachment of the remaining 3-hydroxyquinaldic acid was assured by observing a ^{13}C-^{1}H long range coupling between the serine amide proton (25-H) at δ 9.50 and the quinoline carbon (C-2′) at δ 134.4. From the data described above, the structure of sandramycin was determined to be as shown in Fig. 5.

Chirality of Amino Acids

A hydrolysate of sandramycin was examined by GC and HPLC to establish the configuration of the

Fig. 5. The structure of sandramycin.



optically active amino acids present. The GC chiral analysis indicated that the configurations of the amino acids as pentafluoropropionyl isopropyl esters were D-serine and L-pipecolic acid. Sarcosine and glycine separated from all peaks of interest. *N*-Methyl-D,L-valine did not separate under the GC conditions. An HPLC chiral analysis was devised to separate *N*-methyl-D,L-valine. It was determined that *N*-methyl-L-valine was present. All other compounds present separated from the peaks of interest.

Experimental

Spectroscopic Studies

FAB-MS was measured on a Kratos MS-50 spectrometer. IR spectra were recorded on a Beckmann IR4240 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-500 or a Bruker AM-300 in $CDCl_3$ with a drop of DMSO-d_6 (for line sharpening). Long range heteronuclear correlations were determined with HMBC and COLOC experiments acquired at various mixing times (0.06～0.09 s) at 297 K and 317 K. Nuclear Overhauser effects were determined using the difference method at 297 K.

Hydrolysis of Sandramycin

A sealed tube containing 14.4 mg of sandramycin in 1 ml of 6 N HCl was heated to 110°C for 18 hours. After cooling, the reaction mixture was neutralized to pH 7 with Amberlite IRA-400 (OH) ion exchange resin, filtered and concentrated *in vacuo* to yield 13.4 mg of hydrolysate.

Chiral GC of Derivatized Hydrolysate and Reference Amino Acids

Volatile pentafluoropropionyl isopropyl esters of reference and hydrolysate amino acids were analyzed using a Perkin-Elmer Sigma 2000 gas chromatograph with a flame ionization detector by the following method: Column—Supelco Chirasil-Val capillary (50 m × 0.25 mm); flow rate 18 ml/minute, hydrogen; injector 250°C; oven program—initial 85°C for 15 minutes then increased at 2°C/minute to 200°C and hold 10 minutes; detector 250°C. Approximate retention times of derivatized amino acids: *N*-Methylvaline 35 minutes; glycine 35.6 minutes; D-serine 40 minutes; L-serine 41 minutes; D-pipecolic acid 47.8 minutes; L-pipecolic acid 48.1 minutes.

Chiral HPLC of Hydrolysate and Reference Amino Acids

Hydrolysate and reference amino acids were assayed using the following HPLC method: Column—150 × 4.6 mm, 5 µm, copper/L-proline column, prepared in-house, *in situ*[11]; mobile phase 95% 1 mM copper acetate (pH adjusted to 5 with dilute NaOH) - 5% acetonitrile; flow rate 0.7 ml/minute; detector 240 nm; diluent—MeOH to dissolve sample, then mobile phase. Approximate retention times: *N*-Methyl-D-valine 11.5 minutes; *N*-methyl-L-valine 16.6 minutes.

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