Structures of Two Remarkable Dimerization Products of Sarkomycin

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In 1953 UMEZAWA *et al.* discovered an antitumor antibiotic sarkomycin produced by *Streptomyces erythrochromogenes*.[1~3] Until the 1960's sarkomycin was used in Japan as a prescription drug against cancer. The structure is very simple, 2-methylene-3-oxocyclopentanecarboxylic acid,[4] but it is unstable. The stereochemistry which was reported by SATO *et al.*[5] was revised to *R* configuration by HILL *et al.*[6] in 1967. Sarkomycin was extracted with ethyl acetate from the culture filtrate and purified by countercurrent distribution with ethyl acetate-0.1 M sodium tartarate buffer (pH 4.2).[7] During the cold storage of the free acid syrup, the inactive crystalline product named crystal-Z was obtained,[8] and the gross structure was reported by MAEDA and KONDO.[7] Furthermore, crystal-Z was converted into another crystalline isomer, crystal-D, in a neutral aqueous solution with calcium carbonate.[8] In the 40 years since the first isolation of these remarkable dimerization products, no attempt has been registered to elucidate the stereostructures. In this paper the NMR spectral assignments of two dimerization products, crystal-Z (sarkomycin Z) and crystal-D (sarkomycin D), and the determination of these structures by X-ray crystallography are presented.

Table 1. ^{1}H and ^{13}C NMR data of sarkomycin Z.

Position	^{13}C	^{1}H (*J* Hz)
1	214.7	
2	78.6	
2-OH		5.43 br s
3	50.6	2.81 dd (5.8, 7.6)
4	20.7	1.91 dddd (5.8, 7.6, 7.6, 13)
		2.09 dddd (7.6, 7.6, 7.6, 13)
5	32.5	2.28 m
6 (COOH)	174.1	12.44 br (OH)
7	28.2	1.47 m
		1.54 m
8	22.3	1.42 m
		1.67 m
1′	217.5	
2′	51.3	2.24 m
3′	46.3	2.66 m
4′	24.5	1.81 m
		2.19 m
5′	36.7	2.16 m
		2.29 m
6′ (COOH)	175.7	12.44 br (OH)

Chemical shifts (δ ppm) were measured in DMSO-d_6.

Two dimerization products were prepared by the method described in our previous report.[7] Sarkomycin Z: recrystallization from methanol, mp 168~169°C (dec); $[\alpha]_D^{24}$ +30° (*c* 0.5, H_2O). Sarkomycin D: recrystallization from ethyl acetate, mp 183~185°C (dec); $[\alpha]_D^{25}$ −64° (*c* 0.5, H_2O). Mp's were obtained with a Yanagimoto melting point apparatus and were not corrected. Optical rotations were taken in a Perkin-Elmer 241 polarimeter.

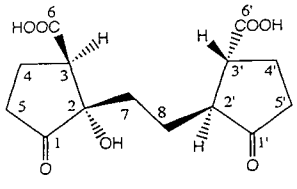
The ^{1}H and ^{13}C NMR data of sarkomycin Z and sarkomycin D are shown in Tables 1 and 2, respectively. The NMR spectra in DMSO-d_6 were determined with a JEOL JNM-A500 spectrometer using TMS as an internal standard. By extensive NMR studies employing ^{1}H-^{1}H-

Table 2. ^{1}H and ^{13}C NMR data of sarkomycin D.

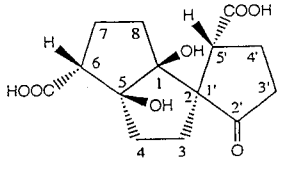
Position	^{13}C	^{1}H (*J* Hz)
1	88.8	
1-OH		4.68 s
2(1′)	62.3	
3	26.8	1.36 m
		1.83 m
4	32.3	1.82 m
		1.89 m
5	88.0	
5-OH		4.42 br s
6	52.8	2.78 dd (8.4, 8.7)
7	23.2	1.62 dddd (6.8, 8.7, 8.7, 12.5)
		1.78 m
8	34.5	1.71 ddd (6.8, 7.4, 13.2)
		1.99 ddd (5.8, 8.7, 13.2)
6-COOH	174.6	12.21 br (OH)
2′	219.5	
3′	35.8	2.23 m
		2.23 m
4′	22.6	1.94 m
		2.22 m
5′	48.3	3.00 m
5′-COOH	175.8	12.21 br (OH)

Chemical shifts (δ ppm) were measured in DMSO-d_6.

Fig. 1. Structures of sarkomycin Z and sarkomycin D.



Sarkomycin Z



Sarkomycin D

Table 3. Crystal data of sarkomycin Z and sarkomycin D.

	Sarkomycin Z	Sarkomycin D
Crystal data		
Empirical formula	$C_{14}H_{18}O_7$	$C_{14}H_{18}O_7$
Formula weight	298.29	298.29
Crystal color, habit	Colorless, prism	Colorless, prism
Crystal dimensions	0.06 × 0.25 × 0.25 mm	0.20 × 0.25 × 0.35 mm
Crystal system	Monoclinic	Monoclinic
Lattice parameter	a = 10.130(4) Å	a = 6.7225(9) Å
	b = 5.793(3) Å	b = 14.125(1) Å
	c = 12.974(3) Å	c = 6.9437(8) Å
	β = 110.41(2)°	β = 99.52(1)°
	V = 713.5(4) Å^3	V = 650.3(1) Å^3
Space group	$P2_1$	$P2_1$
Z value	2	2
D_{calc}	1.388 g/cm^3	1.523 g/cm^3
μ(CuKα)	9.56 cm^{-1}	10.49 cm^{-1}
Measurement		
Scan type	ω-2θ	ω-2θ
Scan rate	8.0°/minute (in θ) (up to 4 scans)	8.0°/minute (in θ) (up to 5 scans)
$2\theta_{max}$	130.2°	120.0°
No. of reflections measured	Total: 1437, unique: 1358	Total: 1112, unique: 1021
Structure solution		
No. observations (I > 2σ(I))	1180	1008
Residuals: R; Rw	0.047; 0.044	0.036; 0.044

Fig. 2. ORTEP drawing of sarkomycin Z.

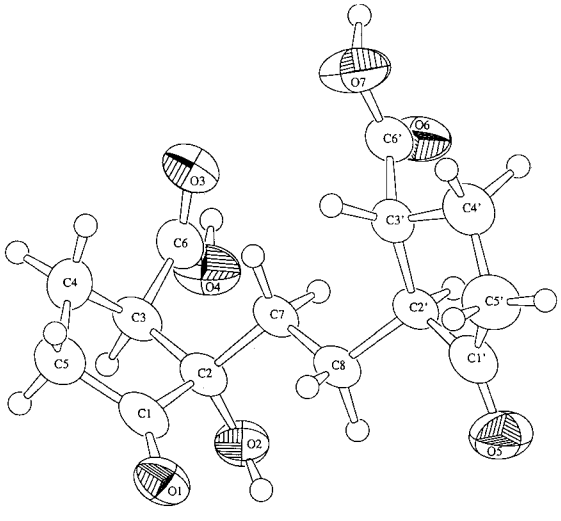
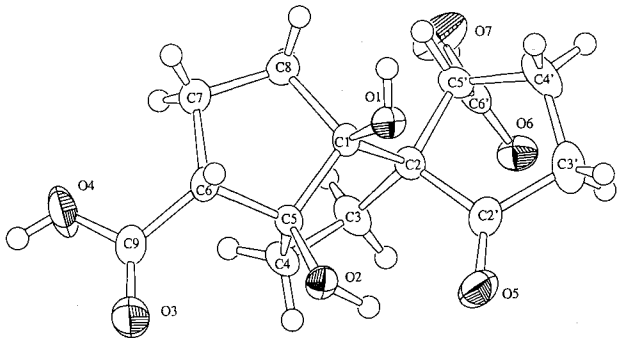


Fig. 3. ORTEP drawing of sarkomycin D.



COSY, DEPT, HMQC and HMBC experiments, the proposed structures of sarkomycin D were submitted, and the chemical shifts of these compounds were assigned.

In order to confirm the proposed structure of sarkomycin D and to establish the overall stereochemistry of sarkomycin Z, X-ray crystallographic analyses were performed. Based on the determination of *R* configuration at C-3 in sarkomycin,[6] the absolute structures of sarkomycin Z and sarkomycin D were assigned as (2*S*,2′*S*,3*R*,3′*R*)-2-hydroxy-2,2′-ethylenebis-(cyclopentanone-3-carboxylic acid) and (1*S*,2*R*,5*S*,6*R*,-5′*R*)-1,5-dihydroxy-2′-oxobicyclo[3.3.0]octane-2-spiro-1′-cyclopentane-6,5′-dicarboxylic acid, respectively (Fig. 1).

The X-ray crystallographic data of two dimerization products are shown in Table 3. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation (λ = 1.5418 Å). The structures were solved by direct methods (SHELXS86)[9] and refined by a full-matrix least-squares method. The ORTEP drawings of sarkomycin Z and sarkomycin D are shown in Figs. 2 and 3, respectively. The supplementary materials have been deposited at the Crystallographic Data Center, Cambridge, England.

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