Short Communication

Cosmomycin D, a New Anthracycline Antibiotic

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In the course of studies on antitumor anthracycline antibiotics, we have isolated cosmomycin D, a new member of the β -rhodomycin group, from the mycelium extract of *Streptomyces cosmosus* TMF518. From this strain three other anthracycline compounds were purified. They are cosmomycons A and B,¹⁾ belonging to the γ -rhodomycin group of antibiotics, and cosmomycin C, identified as β -rhodomycin S-2.²⁾ We report here

on the elucidation of the structures of cosmomycons C and D.

A chloroform-methanol extract of the mycelia (2.6 kg, wet) of *S. cosmosus* was applied to silica gel column chromatography and eluted with chloroform-methanol (1:1). The concentrated residue of the active fraction was separated by silica gel thin-layer chromatography (developing solvent, chloroform-triethylamine (10:1)) to give pure cosmomycons C (5.8 mg) and D (22 mg). The solvent systems used were, 1, chloroform-methanol (9:1) and, 2, chloroform-triethylamine (10:1). *R_f* values in these systems were 0.24 and 0.15 for cosmomycin C and 0.25 and 0.10 for cosmomycin D, respectively. Details of fermentation and isolation methods will be given in another paper.³⁾

The physicochemical properties of cosmomycons C and D were as follows:

Cosmomycin C: mp 180~183°C; UV $\lambda_{\text{max}}^{\text{MeOH}}/\text{nm}$ ($E_1^{1\%}$) 234 (305), 253 (181), 292 (58), 466 sh (90), 483 sh (102), 495 (110), 515 sh (86), 529 (74); IR (neat)/cm⁻¹ 3460 (br), 2970, 2930 (s), 2820, 2770, 1605 (s), 1580, 1460, 1440,

Compounds	R ₁	R ₂	MW
Cosmomycin D	-O-RhN-deFuc-Rho-H	-O-RhN-deFuc-Rho-H	1188
Cosmomycin C	-O-RhN-Rho-Rho-H	-O-RhN-deFuc-Rho-H	1172
Cosmomycin B	-O-RhN-deFuc-Rho-H	-H	771
Cosmomycin A	-O-RhN-Rho-Rho-H	-H	755
Product I ^a	-O-RhN-deFuc-H	-O-RhN-deFuc-H	960
Product II ^a	-O-RhN-H	-O-RhN-deFuc-H	830
Product III ^a	-O-RhN-H	-O-RhN-H	700
β -Rhodomycinone	-OH	-OH	386

^a Partial hydrolysis products.

FIG 1. Structures of Cosmomycons and Their Analogs.

1405, 1380, 1315, 1295, 1240, 1225, 1200, 1165, 1120, 1080, 1070, 1000 (s), 975, 925, 885, 810; $[\alpha]_D^{25} + 181^\circ$ ($c=0.1$, CHCl_3); *Anal.* Found: C, 61.32; H, 7.66; N, 2.53; O, 28.49, Calcd for $\text{C}_{60}\text{H}_{88}\text{N}_2\text{O}_{21}$: C, 61.42; H, 7.56; N, 2.39; O, 28.63%; FD-MS m/z 1173 ($\text{M}+\text{H}$)⁺.

Cosmomycin D: mp 188~191°C; UV $\lambda_{\text{max}}^{\text{MeOH}}/\text{nm}$ ($E_{1\text{cm}}^{1\%}$) 235 (329), 254 (194), 293 (62), 470 sh (97), 485 sh (111), 496 (120), 515 sh (93), 530 (79); IR (neat)/ cm^{-1} 3430 (br), 2980, 2940 (s), 2820, 2770, 1605 (s), 1460, 1440, 1405, 1380, 1315, 1295, 1240, 1225, 1200, 1190, 1170, 1120, 1100, 1085, 1000 (s), 975, 925, 885, 810; $[\alpha]_D^{25} + 190^\circ$ ($c=0.1$, CHCl_3); *Anal.* Found: C, 60.36; H, 7.59; N, 2.24; O, 29.81, Calcd for $\text{C}_{60}\text{H}_{88}\text{N}_2\text{O}_{22}$: C, 60.59; H, 7.46; N, 2.36; O, 29.59%; FD-MS m/z 1189 ($\text{M}+\text{H}$)⁺.

On acid hydrolysis with 0.1 N HCl at 85°C for 30 min, cosmomycons C and D gave β -rhodomycinone, which was identified by EI-MS (m/z 386 (M^+)),⁴⁾ $^1\text{H-NMR}$ ⁵⁾ and CD⁶⁾ spectra. The sugar components in the aqueous phase of the hydrolysate were purified by silica gel thin-layer chromatography and developed with *n*-butanol–acetic acid–water (4:1:1). These sugars were identified by comparison with authentic hexoses prepared from the reduced aclacinomycin A1.⁷⁾ The identified sugar components of cosmomycons C and D were as follows:

Cosmomycin C: Rhodosamine (2), 2-Deoxyfucose (1), Rhodnose (3).

Cosmomycin D: Rhodosamine (2), 2-Deoxyfucose (2), Rhodnose (2).

The numbers of these hexoses in each cosmomycin were deduced from its molecular weight measurement by field desorption mass spectrometry (FD-MS).

A solution of cosmomycin (2.0 mg) in 1.0 ml of methanol was hydrogenated over 5% Pd/BaSO₄ (5 mg) at room temperature and at atmospheric pressure for 2 hr. Hydrogenolysis of cosmomycons C and D gave single main products, identified as cosmomycons A and B (Fig. 1), respectively, by silica gel thin-layer chromatography developed with two solvent systems. The identification was further supported by $^1\text{H-NMR}$ spectra and FD-mass

spectra in which a molecular ion peak was observed for the reduced product of cosmomycin C (m/z 756 ($\text{M}+\text{H}$)⁺), identical with cosmomycin A, or cosmomycin D (m/z 772 ($\text{M}+\text{H}$)⁺), identical with cosmomycin B. Hydrogenolysis occurs at the C-7 position of β -rhodomycinone. From these results, we concluded that cosmomycons C and D possess two sugar chains consisting of three hexoses. One sugar chain that links to the C-10 position of the aglycone is rhodinosyl-rhodinosyl-rhodosamine for cosmomycin C and rhodinosyl-2-deoxyfucosyl-rhodosamine for cosmomycin D. The other glycoside chain that attaches to the C-7 position of the aglycone consists of rhodosamine, 2-deoxyfucose and rhodnose for both cosmomycons C and D. The bonding positions of the sugar chains were deduced from the ^{13}C -chemical shifts of C-7 (δ_c 71.0 ppm) and C-10 (δ_c 70.4 or 70.3 ppm) (Table I).

In order to determine the sequence of the hexoses in the sugar chain attached to the C-7 position, cosmomycin C and D (0.1 mg) were partially hydrolyzed in 0.1 N HCl (0.1 ml) at 25°C for 30 min. After the reaction was stopped by addition of a saturated aqueous solution of sodium bicarbonate, the hydrolysate was extracted with chloroform, and concentrated. The residue was subjected to FD-MS. The molecular ions ($\text{M}+\text{H}$)⁺ of the partial hydrolysates were as follows:

Cosmomycin C: m/z 1059, 945, 831, 701.

Cosmomycin D: m/z 1075, 961, 831, 701.

The ions at m/z 831 and m/z 701 for cosmomycin C suggest that cosmomycin C gave a partial hydrolysis product II (MW 830) and III (MW 700). For cosmomycin D, partial hydrolysis products I (MW 960) and III (MW 700) were detected as ions at m/z 961 and m/z 701. The structures of Products I, II and III shown in Fig. 1 were deduced from the molecular weights and sugar components.

The $^{13}\text{C-NMR}$ assignments of cosmomycons C and D shown in Table I were achieved by comparison of their $^{13}\text{C-NMR}$ spectra with MA-144 N1,⁷⁾ sulfurmycin F,⁸⁾ roseorubicin A⁹⁾ and cosmomycin A and B.¹⁾ All rhodi-

TABLE I. ^{13}C -CHEMICAL SHIFT ASSIGNMENTS OF COSMOMYCINS C AND D (ppm)

Carbons	Cosmomycins		Carbons ^a	Cosmomycins		Carbons ^b	Cosmomycins	
	C	D		C	D		C	D
1	119.6	119.7	1'	102.0	101.9	1'	97.4	97.1
2	137.0	137.1	2'	29.3	29.1	2'	29.8	29.6
3	124.7	124.7	3'	61.4	61.4	3'	61.4	61.4
4	162.6	162.5	4'	74.1	74.1	4'	74.1	74.2
4a	116.1	115.9	5'	68.3	68.1	5'	68.7	68.1
5	190.7	190.6	6'	18.0	17.8	6'	18.1	18.0
5a	112.0	111.9	N(CH ₃) ₂	43.2	43.2	N(CH ₃) ₂	43.2	43.2
6	157.1	157.1	1''	99.5	99.4	1''	98.6	99.4
6a	136.5	136.4	2''	34.4	34.3	2''	24.7	34.3
7	71.0	71.0	3''	65.6	65.5	3''	24.7	65.5
8	33.0	33.0	4''	83.7	83.5	4''	75.4	83.5
9	71.8	71.7	5''	66.8	66.9	5''	66.8	66.9
10	70.4	70.3	6''	17.1	17.0	6''	17.1	17.0
10a	138.3	138.1	1'''	100.3	100.3	1'''	99.5	100.3
11	157.7	157.5	2'''	24.0	24.0	2'''	23.6	24.0
11a	111.7	111.7	3'''	25.5	25.5	3'''	26.0	25.5
12	186.0	185.8	4'''	67.2	67.1	4'''	66.8	67.1
12a	133.5	133.4	5'''	68.2	68.1	5'''	67.5	68.1
13	30.7	30.7	6'''	17.1	17.0	6'''	17.1	17.0
14	6.7	6.7						

^a The sugar chain at the C-7 position of aglycon.^b The sugar chain at the C-10 position of aglycon.

noses bonded to 2-deoxyfucose in cosmomycins C and D prove to be attached at the C-4 position of 2-deoxyfucose from a low-field shift by about 12 ppm of C-4 (δ_c 83.5 ppm) of all the 2-deoxyfucose moieties, since the chemical shift of the C-4 position with a free hydroxy group is typically δ_c 71.5 ppm.^{7,8)} In the ^1H -NMR spectra of cosmomycins C and D, the anomeric proton of each sugar component appeared as a broad singlet. The small coupling constant (1 or 2 Hz) indicated the α -configurations at all anomeric carbons.

Due to the paucity of the samples, the configurations of the sugar components were not determined. However, all sugar components are presumed to have the same configuration (L-) as that of the hexoses of the known anthracycline antibiotics.^{7,8)}

Among the known anthracycline antibiotics, the β -rhodomycin analogs having six hexoses are ditrisarubicins A, B and C¹⁰⁾ and β -rhodomycin S-2.²⁾ Cosmomycin C seems to be identical with β -rhodomycin S-2, the

stereochemistry of which has not been described. Cosmomycin D, however, is different from the known β -rhodomycin analogs. Therefore, cosmomycin D is considered to be a new anthracycline antibiotic.

During investigation of the biological activities of these compounds, we found that cosmomycins C and D caused differentiation of Friend leukemia cells. Details of their biological properties will be reported elsewhere.

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