Communications to the Editor

ISOLATION OF NEW ANTHRACYCLINE ANTIBIOTICS, A447 C AND D

Sir:

During the course of our screening program for new anthracycline antibiotics, four β-rhodomycinone glycosides, A447 A, B, C and D were found in the acetone extract of cultured mycelia of a strain designated as A447. Based on NMR spectral analysis and chemical degradation, A447 A and B were identified as cosmomycins D and C[1,2], respectively, while A447 C and D were found to be new congeners. A447 C and D inhibited the growth of P388 murine leukemia cells at the IC_{50} values of 2.9 ng/ml and 1.4 ng/ml, respectively.

Strain A447 was isolated from a soil sample collected at Hachijyo-island, Tokyo, Japan, and the taxonomic studies identified that the strain belongs to *Streptomyces cyaneus*. A detailed description of the classification will be reported in due course.

The organism was cultivated at 27°C for 48 hours in a 50-liter jar fermentor containing 30 liters of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4% (pH 7.0).

The mycelial cake obtained by filtration was extracted with acetone and the extract was concentrated. The aqueous residue, after being adjusted to pH 8.5, was extracted with ethyl acetate. The organic solvent layer was evaporated *in vacuo* and the dried material was dissolved in a small volume of acetic acid and diluted with 10 volumes of water. This solution was washed with chloroform and then adjusted to pH 8.5 with 5 N NaOH followed by extraction with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and concentrated to dryness to give an anthracycline mixture containing A447 A, B, C and D. This material was chromatographed on preparative silica gel TLC plates with $CHCl_3$ - MeOH - 28% NH_4OH (200: 20: 1). Each separated band corresponding to A447 A, B, C and D was scraped off and eluted with $CHCl_3$ - MeOH (10: 1) followed by evaporation to dryness *in vacuo*. These four fractions were further purified by preparative silica gel TLC developed with $CHCl_3$ - MeOH - AcOH - H_2O (40: 8: 1: 1) and Sephadex LH-20 column chromatography developed with $CHCl_3$ - MeOH (1 : 1). The eluate was concentrated and the residue was dissolved in chloroform. Then hexane was added to the solution to give precipitates. These solid materials were evaporated to dryness to give reddish powders of A447 A (40 mg), B (37 mg), C (80 mg) and D (45 mg) in pure forms. The Rf values of A447 A, B, C and D are shown in Table 1.

The physico-chemical properties of A447 C and D are as follows.

A447 C: MP 192～195°C (dec); UV λ_{max} nm ($E^{1\%}_{1cm}$) 236 (239), 294 (47), 496 (82) in MeOH, 242 (245), 296 (46), 566 (82) in alkaline MeOH; IR (KBr) cm^{-1} 3400, 1630, 1600; secondary ion mass spectra (SI-MS) *m/z* 1,157 (MH^+, corresponding to the molecular formula $C_{60}H_{88}O_{20}N_2$).

A447 D: MP 175～180°C (dec); UV λ_{max} nm ($E^{1\%}_{1cm}$) 236 (206), 254 (116), 293 (41), 496 (71) in MeOH, 241 (208), 298 (38), 566 (69) in alkaline MeOH; IR (KBr) cm^{-1} 3400, 1740, 1640 and 1600; SI-MS *m/z* 1,171 (MH^+, corresponding to the molecular formula $C_{60}H_{88}O_{21}N_2$).

The ^1H NMR spectra of A447 C and D (Figs. 1 and 2, respectively, and Table 2) as well as those of A447 A and B showed the peaks due to 1 mol of β-rhodomycinone, 2 mol of rhodosamine and 4 mol of hexoses.

Acid hydrolysis of A447 A, B, C and D gave β-rhodomycinone[3]. The component sugars present in the aqueous phase of each hydrolysate were identified by silica gel TLC developed with

Table 1. Rf values of A447 A, B, C and D.

Compound	Solvent systems (1)	(2)	(3)
A447 A	0.42	0.26	0.10
A447 B	0.46	0.31	0.18
A447 C	0.54	0.36	0.40
A447 D	0.61	0.40	0.33

Solvent systems: (1) $CHCl_3$ - MeOH - aq NH_4OH, 200: 20: 1. (2) $CHCl_3$ - MeOH - AcOH - H_2O, 40: 8: 1: 1. (3) $CHCl_3$ - $(CH_3CH_2)_3N$, 10 : 1. On Kieselgel $60F_{254}$ 0.25 mm thickness (Merck).

Fig. 1. 500 MHz ^{1}H NMR spectrum of A447 C.

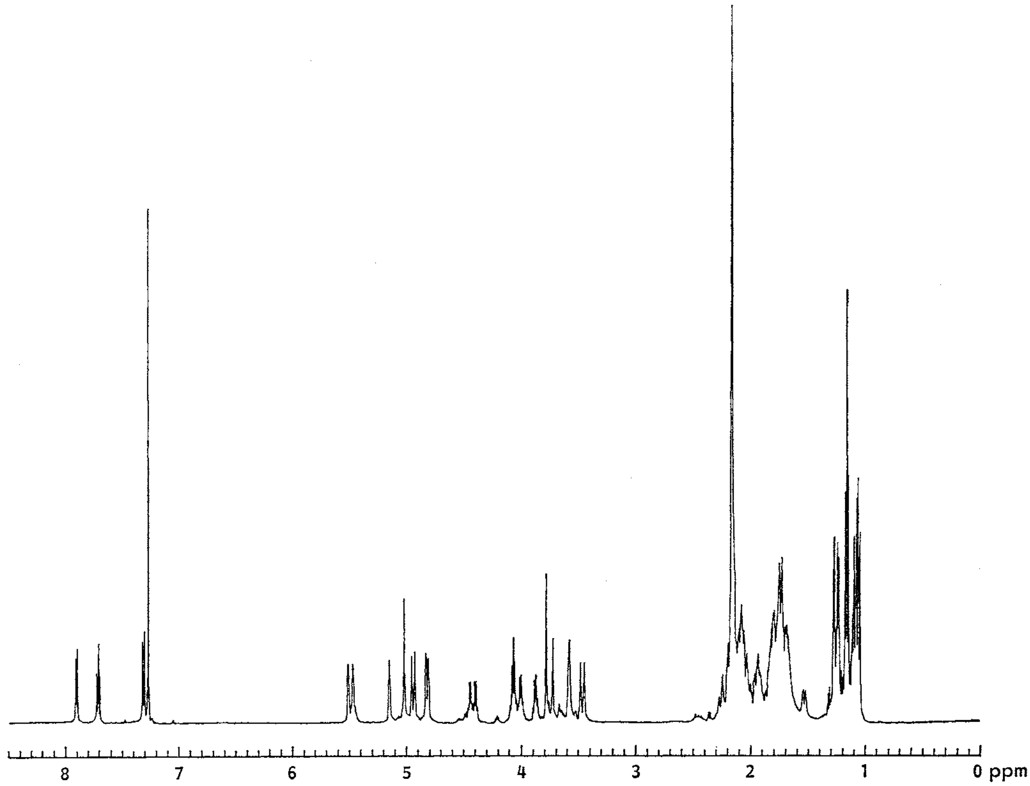


Fig. 2. 500 MHz ^{1}H NMR spectrum of A447 D.

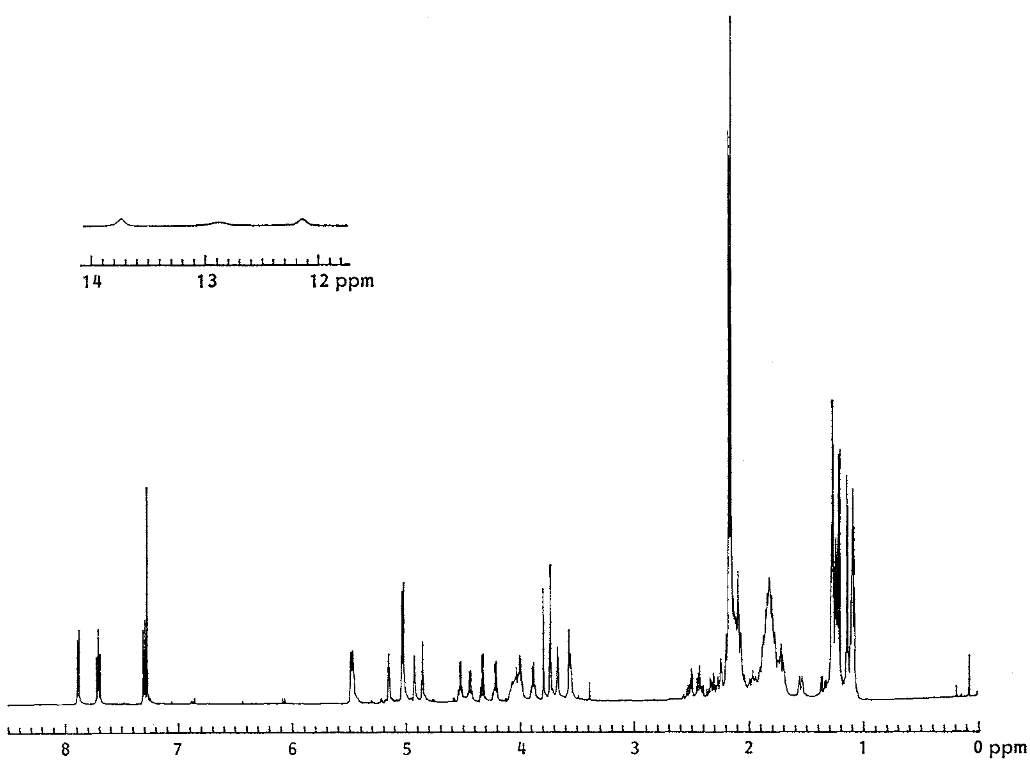


Table 2. 500 MHz ^{1}H NMR spectral data of A447 C and D in $CDCl_3$.

Proton	A447 C	A447 D
1	7.89 (d, J=7.4 Hz)	7.84 (dd, J=7.2, 1.1 Hz)
2	7.70 (dd, J=8.3, 7.4 Hz)	7.65 (dd, J=7.4, 7.2 Hz)
3	7.30 (dd, J=8.3 Hz)	7.25 (dd, J=7.2, 1.1 Hz)
7	5.14 (m)	5.15 (m)
10	5.01 (s)	5.02 (s)
14	1.10 (t, J=7.4 Hz)	1.10 (t, J=7.5 Hz)
1′	5.50 (d, J=3.6 Hz)	5.48 (d, J=3.4 Hz)
4′	3.78 (br s)	3.73 (br s)
5′	4.00 (q, J=6.4 Hz)	3.99 (q, J=6.8 Hz)
6′	1.27 (d, J=6.4 Hz)	1.28 (d, J=6.8 Hz)
3′-$N(CH_3)_2$	2.16 (s)	2.15* (s)
1″	4.95 (br s)	5.04 (br s)
4″	3.47 (br s)	4.06 (m)
5″	4.44 (q, J=6.7 Hz)	4.52 (q, J=6.3 Hz)
6″	1.07 (d, J=6.7 Hz)	1.15 (d, J=6.3 Hz)
1‴	4.82 (d, J=3.0 Hz)	4.85 (br s)
4‴	3.58 (br s)	3.66 (br s)
5‴	4.06* (q, J=6.8 Hz)	4.22 (q, J=6.7 Hz)
6‴	1.15** (d, J=6.8 Hz)	1.22 (d, J=6.7 Hz)
1⁗	5.46 (d, J=3.3 Hz)	5.46 (d, J=3.5 Hz)
4⁗	3.72 (br s)	3.73 (br s)
5⁗	3.87 (q, J=6.6 Hz)	3.88 (q, J=6.7 Hz)
6⁗	1.23 (d, J=6.6 Hz)	1.24 (d, J=6.7 Hz)
3⁗-$N(CH_3)_2$	2.16 (s)	2.17* (s)
1′′′′′	4.92 (br s)	4.92 (br s)
4′′′′′	3.44 (br s)	3.55 (br s)
5′′′′′	4.39 (q, J=6.7 Hz)	4.44 (q, J=6.3 Hz)
6′′′′′	1.06 (d, J=6.7 Hz)	1.09 (d, J=6.3 Hz)
1′′′′′′	4.80 (d, J=3.2 Hz)	5.03 (t, J=5.3 Hz)
3′′′′′′		2.51 (ddd, J=16.0, 6.7, 5.3 Hz) 2.42 (ddd, J=16.0, 9.5, 5.3 Hz)
4′′′′′′	3.58 (br s)	
5′′′′′′	4.07* (q, J=6.8 Hz)	4.33 (q, J=6.6 Hz)
6′′′′′′	1.17** (d, J=6.6 Hz)	1.27 (d, J=6.6 Hz)

Similar values marked by * and ** in the same compound may be interchangeable. Signals for H-2 and H-3 of rhodosamine and rhodinose were not assigned.

BuOH - AcOH - H_2O (4 : 1 : 1) by comparing with authentic samples. The numbers of these hexoses in each compound were revealed by mass spectrometry, ^{1}H and ^{13}C NMR as shown in Table 3.

Hydrogenolysis of A447 C and D with 5% Pd-$BaSO_4$ in MeOH at room temp for 30 minutes gave trisaccharides of γ-rhodomycinone[4], *i.e.* A447 C′ and D′ respectively. The SI-MS of A447 C′ and D′ showed the molecular ion peaks of 756 and 754 $(M+H)^+$, respectively. Acid hydrolysis of A447 C′ gave γ-rhodomycinone, rhodosamine (Rhn) and rhodinose (Rho), and that of A447 D′ gave γ-rhodomycinone, Rhn, Rho and cinerulose A (Cin A), thereby showing that the sugar components of A447 C′ and D′ were the same as those contained in cosmomycin A[5] and ditrisarubicin C′[6], respectively (Table 4). These results were supported by direct comparison on silica gel TLC with authentic samples. Mild acid hydrolysis of A447 C (0.1 N HCl, 100 minutes, at room temp) gave A447 C″ with the molecular ion peak of 701 (MH^+, corresponding to the molecular formula $C_{36}H_{48}O_{12}N_2$). This result indicated that A447 C lost four Rho from its two sugar chains at C-7 and C-10 of the aglycone and that Rhn attached to C-7 and C-10 of the aglycone.

The ^{13}C NMR spectral data of A447 D indicated that the chemical shifts due to its sugar moieties were very similar to those of the sugar moieties contained in cosmomycin D[1]. The ^{13}C NMR shift assignments of A447 C and D are shown in Table 5.

The structure of a hydrogenolysis product of A447 D, rhodinosyl-2-deoxyfucosylrhodosamine,

Table 3. Hexose components of A447 A, B, C and D.

Hexose	Molar ratio			
	A	B	C	D
Rhodosamine	2	2	2	2
2-Deoxyfucose	2	1	0	1
Rhodinose	2	3	4	2
Cinerulose A	0	0	0	1

Obtained by acid hydrolysis in 0.1 N hydrochloric acid for 10 minutes at 100°C. The hexoses were identified by direct comparison on silica gel TLC with authentic samples. Molar ratios were determined by ^{1}H NMR, ^{13}C NMR and mass spectrometry.

Table 4. Hexose components and molecular weights of degradation products of A447 A, B, C and D.

Compound	MW m/z $(MH)^+$	Hexose components
A447 A'*	771	Rhn, deFuc, Rho
A447 B'*	756	Rhn, Rho, Rho
A447 C'*	756	Rhn, Rho, Rho
A447 D'*	754	Rhn, Rho, Cin A
A447 C''**	701	Rhn, Rhn
Cosmomycin A	756	Rhn, Rho, Rho
Ditrisarubicin C'	754	Rhn, Rho, Cin A

Rhn = Rhodosamine, deFuc = 2-deoxyfucose, Rho = rhodinose, Cin A = cinerulose A.

* Obtained by hydrogenolysis with 5% Pd-$BaSO_4$ in MeOH at room temp for 30 minutes.

** Obtained by mild acid hydrolysis (0.1 N HCl for 100 minutes at room temp) of A447 C.

Hexose components of A447 A' to D' and A447 C'' were identified by acid hydrolysis in 0.1 N HCl for 10 minutes at 100°C followed by sugar analysis of the reaction mixture after removal of the aglycone with chloroform.

Table 5. ^{13}C NMR chemical shift assignments of A447 C and D.

Carbon	A447 C	A447 D	Cosmo.
1	119.6	119.6	119.7
2	136.9	137.0	137.1
3	124.6	124.7	124.7
4	162.3	162.6	162.5
4a	115.7	116.1	115.9
5	190.3	190.8	190.6
5a	111.7	112.0	111.9
6	157.0	157.1	157.1
6a	136.4	136.5	136.4
7	70.8	70.9	71.0
8	32.9	33.0	33.0
9	71.6	71.8	71.7
10	70.3	70.5	70.3
10a	138.2	138.3	138.1
11	157.5	157.6	157.5
11a	111.5	111.7	111.7
12	185.6	186.0	185.8
12a	133.2	133.5	133.4
13	30.6	30.7	30.7
14	6.6	6.6	6.7

Carbon[a]	A447 C	A447 D	Cosmo.
	Rhn	Rhn	Rhn
1'	101.8	101.9	101.9
2'	29.1	29.3	29.3
3'	61.3*	61.4	61.4
4'	73.7	74.0	74.1
5'	68.4	68.3	68.1
6'	17.8	17.8	17.8
3'-$N(CH_3)_2$			
	43.0	43.2*	43.2
	Rho	deFuc	deFuc
1''	98.5	99.4	99.4
2''	24.4**	34.4	34.3
3''	24.7**	65.6	65.5
4''	75.2	83.7	83.5
5''	66.8	66.9	66.9
6''	16.9	16.9	17.0
	Rho	Rho	Rho
1'''	99.4	100.3	100.3
2'''	23.6	23.9	24.0
3'''	25.9	25.5	25.5
4'''	67.3	67.2	67.1
5'''	66.6	68.1	68.1
6'''	17.0	17.0	17.0

Carbon[b]	A447 C	A447 D	Cosmo.
	Rhn	Rhn	Rhn
1''''	97.2	97.4	97.1
2''''	29.6	29.8	29.6
3''''	61.5*	61.5	61.4
4''''	73.9	74.2	74.2
5''''	68.6	68.7	68.1
6''''	17.9	18.0	18.0
3''''-$N(CH_3)_2$			
	43.0	43.3*	43.2
	Rho	Rho	deFuc
1'''''	98.5	98.6	99.4
2'''''	24.4	24.6**	34.3
3'''''	24.7	24.8**	65.5
4'''''	75.1	75.6	83.5
5'''''	66.8	66.5	66.9
6'''''	17.0	17.0	17.0
	Rho	Cin A	Rho
1''''''	99.3	98.9	100.3
2''''''	23.6	28.6	24.0
3''''''	25.9	33.6	25.5
4''''''	67.3	211.0	67.1
5''''''	66.6	71.1	68.1
6''''''	17.0	14.8	17.0

In ppm, TMS as internal references at 100 MHz in $CDCl_3$.

Similar values marked by * and ** in the same compound may be interchanged.

Rhn = Rhodosamine, Rho = rhodinose, deFuc = 2-deoxyfucose, Cin A = cinerulose A.

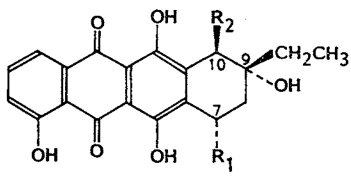
Cosmo.: Cosmomycin D.

The assignments were made by the chemical shift data of cosmomycin D[2].

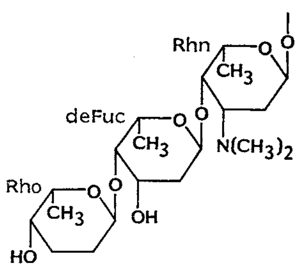
a Sugar chain at C-7 of β-rhodomycinone.

b Sugar chain at C-10 of β-rhodomycinone.

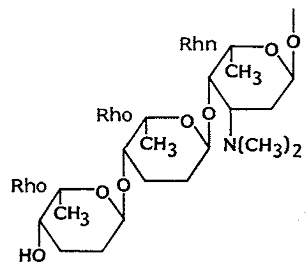
Fig. 3. Structures of A447 A, B, C, D, C′ and D′.



A447 A	R_1 = (1)	R_2 = (1)
A447 B	R_1 = (1)	R_2 = (2)
A447 C	R_1 = (2)	R_2 = (2)
A447 D	R_1 = (1)	R_2 = (3)
A447 C′	R_1 = H	R_2 = (2)
A447 D′	R_1 = H	R_2 = (3)



(1)



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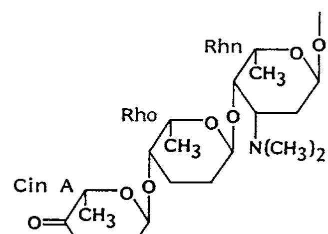

(3)

indicated that the sugar chain at C-7 of A447 D was the same as the sugar chain contained in cosmomycin D. Acid hydrolysis products of A447 D′ indicated that the sugar components at C-10 of A447 D (Rhn, Rho and Cin A) were the same as those contained in ditrisarubicin C′. The position of Cin A in the sugar chain at C-10 was fixed due to its structure and the position of Rhn was determined by ^{1}H and ^{13}C NMR spectral data. Thus, based on the NMR spectral data together with the degradation study, we led to the structure of A447 D as shown in Fig. 3.

A447 C had two sugar chains consisting of Rhn, Rho and Rho at C-7 and C-10, and its ^{13}C NMR chemical shifts indicated that two Rhn attached to C-7 and C-10 of β-rhodomycinone. A447 C″ obtained by mild acid hydrolysis of A447 C also supported that Rhn attached to C-7 and C-10 of β-rhodomycinone. Thus the structure of A447 C was determined as shown in Fig. 3.

These results clearly indicate that A447 D is a novel anthracycline, while A447 C may be identical with cytorhodin A[7]. The detailed structural studies and the biological activities of the new compounds will be reported in due course.

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