STRUCTURAL STUDIES ON RHODILUNANCINS A AND B

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Antitumor antibiotics rhodilunancins A and B were isolated from a culture of *Streptomyces violaceus* var. *lunanensis* var. n. No. 1289 by acidic acetone extraction of mycelia and purified by silica gel chromatography. Rhodilunancin A is identical to cosmomycin A. Rhodilunancin B is identical to cosmomycin B (=rhodomycin Y). Both antibiotics exhibited activities against Gram-positive bacteria and inhibition on DNA synthesis of P_{388} leukemia cell *in vitro*.

In the course of our screening for new antitumor antibiotics, a *Streptomyces* strain, *Streptomyces vioaceus* var. *lunanensis* var. n. No. 1289 isolated from soil sample collected at Lunan county, Yunnan province, was found to produce two anthracycline antibiotics, rhodilunancins A (**I**) and B (**II**) (Fig. 1). Strain No. 1289 was cultured in a fermentor containing a medium of the following composition: soluble starch 1.5%, dextrin 1.5%, glucose 1.5%, soybean meal 1.2%, peptone 0.5%, yeast extract 0.5%, $(NH_4)_2SO_4$ 0.25%, NaCl 0.2%, KH_2PO_4 0.02%, $CaCO_3$ 0.3%, $MgSO_4$ 0.02%. The cultivation was carried out at 28°C for 4 days.

The antibiotics in the mycelia were extracted with acidic acetone. The extracts were concentrated *in vacuo* to remove acetone and reextracted with ethyl acetate. The basic glycosides were separated from the aglycone by extraction with aqueous acid (pH 3). The acidic solution was adjusted to pH 8

Fig. 1. Structures of **I** and **II**.

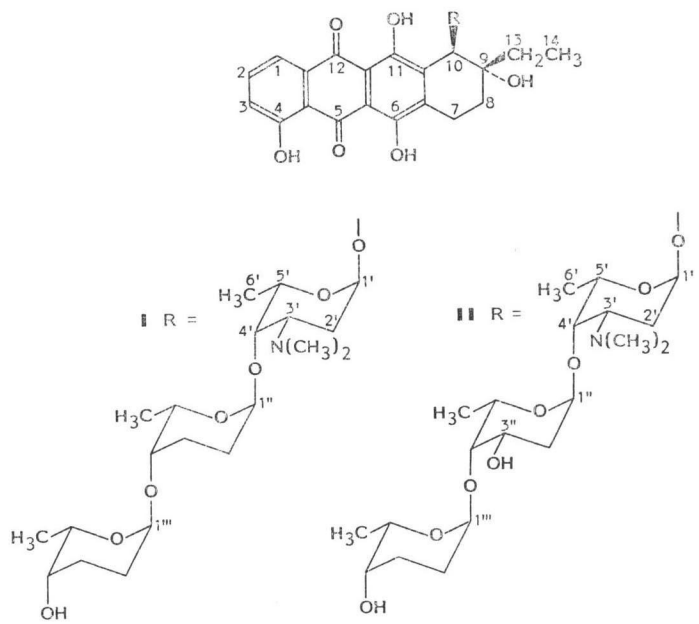
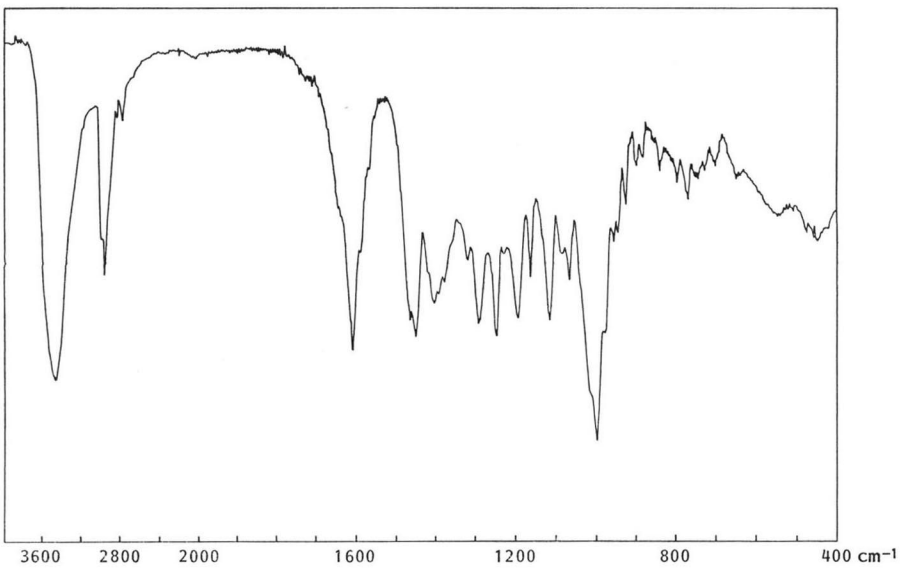


Table 1. Physico-chemical properties of **I** and **II**.

		I	II
Nature		Red powder	Red powder
MP (°C)		140~142	154~156
$[\alpha]_D^{22}$ (c 0.01, $CHCl_3$)		+157.5°	+132.5°
MW (FD-MS)		755	771
UV/VIS	MeOH	235 (33,000), 253 (30,000),	235 (33,000), 253 (30,000),
λ_{max} nm (ε)		294 (7,700), 464 (11,000),	294 (76,000), 465 (12,000),
		480 (13,000), 493 (15,000),	480 (13,000), 493 (15,000),
		513 (12,000), 527 (12,000)	513 (11,000), 528 (11,000)
	0.1 N HCl - MeOH	234 (35,000), 253 (29,000),	234 (36,000), 253 (29,000),
		293 (8,000), 464 (12,000),	294 (7,700), 465 (12,000),
		480 (13,000), 492 (15,000),	480 (13,000), 493 (15,000),
		513 (12,000), 526 (11,000)	513 (11,000), 528 (11,000)
	0.1 N NaOH - MeOH	240 (36,000), 296 (7,500),	240 (38,000), 295 (7,500),
		493 (9,300), 540 (13,000),	495 (9,100), 543 (13,000),
		584 (9,300)	548 (9,600)
IR (KBr) ν cm^{-1}		3450, 2980, 2940, 1602,	3450, 2980, 2950, 1603,
		1000	1000

Fig. 2. IR spectrum of **I**.



and extracted with ethyl acetate. The extracts were concentrated to dryness and chromatographed on a column of silica gel (Qing Dao Guei Qiu 80~120 mesh) and developed with chloroform - methanol (20:1, 15:1 and 10:1) successively to yield crude powders of **I** and **II**. Further purification of the two compounds was carried out by centrifugal and preparative thin-layer chromatography using ethyl acetate - methanol (10:1) and benzene - ethyl acetate - methanol (5:5:1) as developing solvents respectively to obtain red powders of **I** and **II** in pure form. Their physico-chemical properties are shown in Table 1 (Figs. 2, 3).

Acid hydrolysis (0.1 N HCl, 90°C, 30 minutes) of **I** and **II** gave the same aglycone (**III**). UV/VIS spectrum (λ_{max} 234, 252, 298, 462, 482, 492, 527 nm), IR spectrum (ν 1600 cm^{-1}) (hydrogen-bonded

Fig. 3. IR spectrum of **II**.

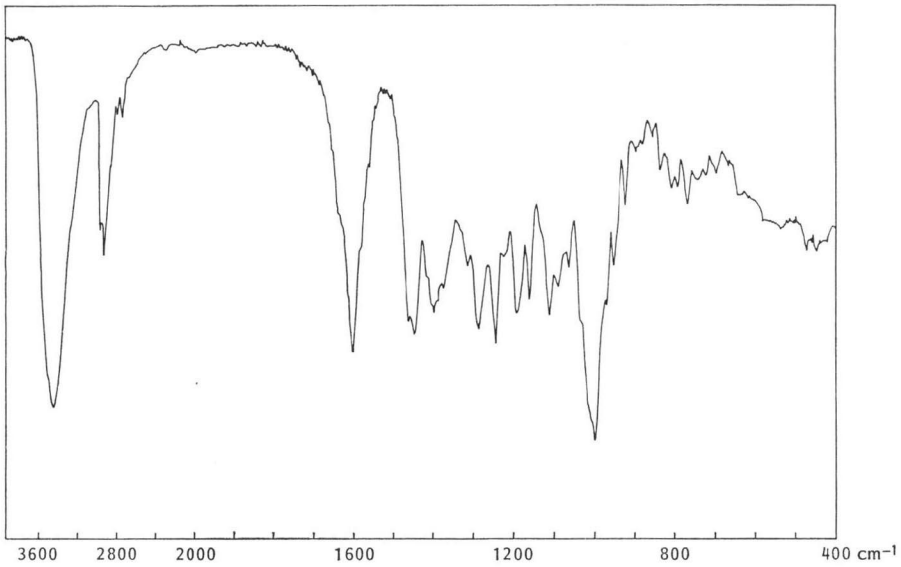


Fig. 4. Structure of **III**.

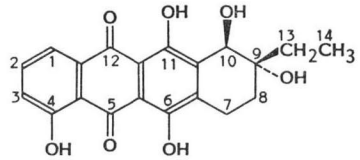


Table 2. TLC comparison of Rf values and color development of sugars of **I** and **II** with authentic samples.

Sugar	Rf value*									Color**
	I	II	3082 A	3082 B	Acl[a]	MA144-M_1	MA144-N_1	Red	Ref 8)	
Rhodosamine	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.12	Sky blue
2-Deoxyfucose		0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.56	Grayish blue
Rhodinose	0.64	0.64					0.64	0.64	0.71	Yellowish brown***
Amicetose						0.67		0.67	0.74	Yellowish brown
Cinerulose A			0.75		0.75				0.82	Greenish blue
Cinerulose B				0.71					0.80	Greenish blue

[a] Acl: Aclarubicin A, Red: reduction products of 3082 A.

* Silica gel thin-layer (60 F_{254} Merck Co.) BuOH - AcOH - H_2O (4: 1: 1).

** Visualization was carried out with *p*-anisaldehyde.

*** In our experiment the spot corresponding to rhodinose gave yellow brown color, which is different from that reported.[8]

quinone C=O and C=C) of **III** demonstrated that it has a three-α-hydroxyl anthraquinone chromophores.[1] A ^{13}C NMR spectrum of **I** in $CDCl_3$ showed the signals assigned to two carbonyl groups hydrogen-bonded to one (δ 186.5 ppm) and two (δ 190.7 ppm) peri-hydroxyl groups, respectively.[2] Further, by direct comparison of melting points, TLC Rf values, MS, ^1H NMR and ^{13}C NMR spectra with that of the authentic sample, **III** was identified as γ-rhodomycinone.[3~5] The same CD curves

Fig. 5. ^{1}H NMR spectrum of **I** (acetone-d_6).

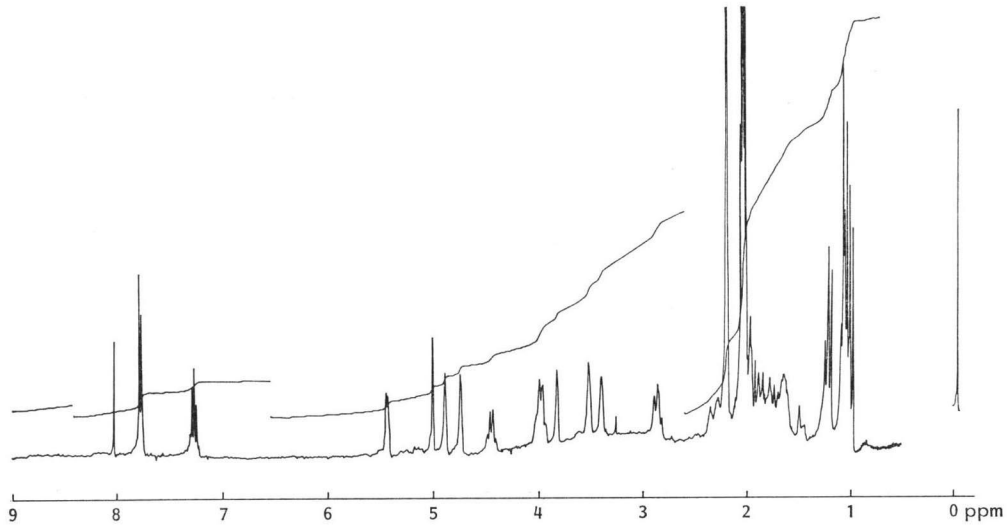
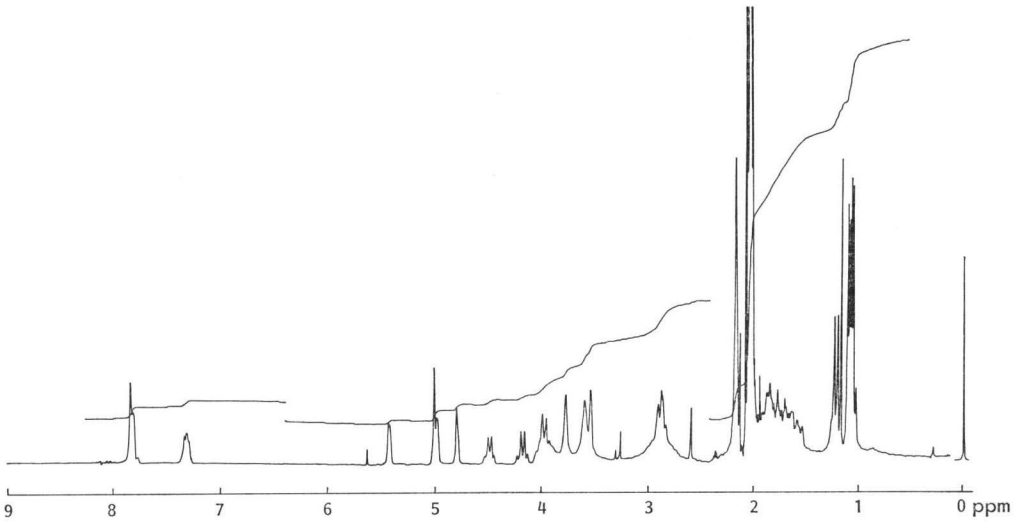


Fig. 6. ^{1}H NMR spectrum of **II** (acetone-d_6).



of **III** and γ-rhodomycinone indicated the same configuration at the chiral centers (9*R*,10*R*)[6] (Fig. 4).

Thin-layer chromatography of two acid hydrolysates indicated that the sugar moieties of **I** and **II** are composed of rhodosamine and rhodinose; rhodosamine, 2-deoxyfucose and rhodinose, respectively by direct comparison with authentic samples from 3082 A*, 3082 B**, aclarubicin A, MA144-M_1, MA144-N_1 and the reductive products of 3082 A.[7,8] The results are shown in Table 2.

The anomeric proton signals in ^{1}H NMR spectra of **I** (δ 5.43, 4.98, 4.75 ppm) and **II** (δ 5.43, 4.98, 4.80 ppm) indicated that both **I** and **II** have trisaccharide moieties (Figs. 5, 6). The small coupling constants (J=3 Hz or $W_{\frac{1}{2}}$=6 Hz) indicated that the glycosidic bond of every sugar has the α-configuration. The sequence of sugar moieties of **I** and **II** was found to be rhodinosyl-rhodinosyl-rhodo-

* 3082 A: Aclarubicin A. ** 3082 B: Aclarubicin B.

Table 3. Products analysis for partial acid hydrolysis of **I** and **II**.

Compound	Acid hydrolysis	Liberated sugars			Remainder	
		Rf*	Color	Sugar	Rf**	Compound
I	0.5% HCl, 25°C, 10 minutes	0.70	Yellowish brown	Rod	0.52	**III** - Roa - Rod
	0.1 N HCl, 25°C, 60 minutes	0.70	Yellowish brown	Rod	0.23	**III** - Roa
	0.1 N HCl, 90°C, 30 minutes	0.70	Yellowish brown	Rod	0.95	**III**
		0.14	Sky blue	Roa		
III - Roa	0.1 N HCl, 90°C, 30 minutes	0.14	Sky blue	Roa	0.95	**III**
II	0.5% HCl, 21°C, 15 minutes	0.70	Yellowish brown	Rod	0.44	**III** - Roa - dF
	0.5% HCl, 21°C, 75 minutes	0.70	Yellowish brown	Rod	0.23	**III** - Roa
		0.57	Grayish blue	dF		
		0.14	Sky blue	Roa		
	0.1 N HCl, 90°C, 30 minutes	0.70	Yellowish brown	Rod	0.95	**III**
		0.57	Grayish blue	dF		
III - Roa	0.1 N HCl, 50°C, 3 hours	0.14	Sky blue	Roa	0.95	**III**

* Silica gel thin-layer (60 F_{254} Merck Co.) BuOH - AcOH - H_2O (4: 1: 1).
** Silica gel thin-layer (Qin Dao Guei Gao H) benzene - EtOAc - MeOH (5: 5: 1).
III: γ-Rhodomycinone, Roa: rhodosamine, Rod: rhodinose, dF: 2-deoxyfucose.

Fig. 7. ^{13}C NMR spectrum of **I** (acetone-d_6).

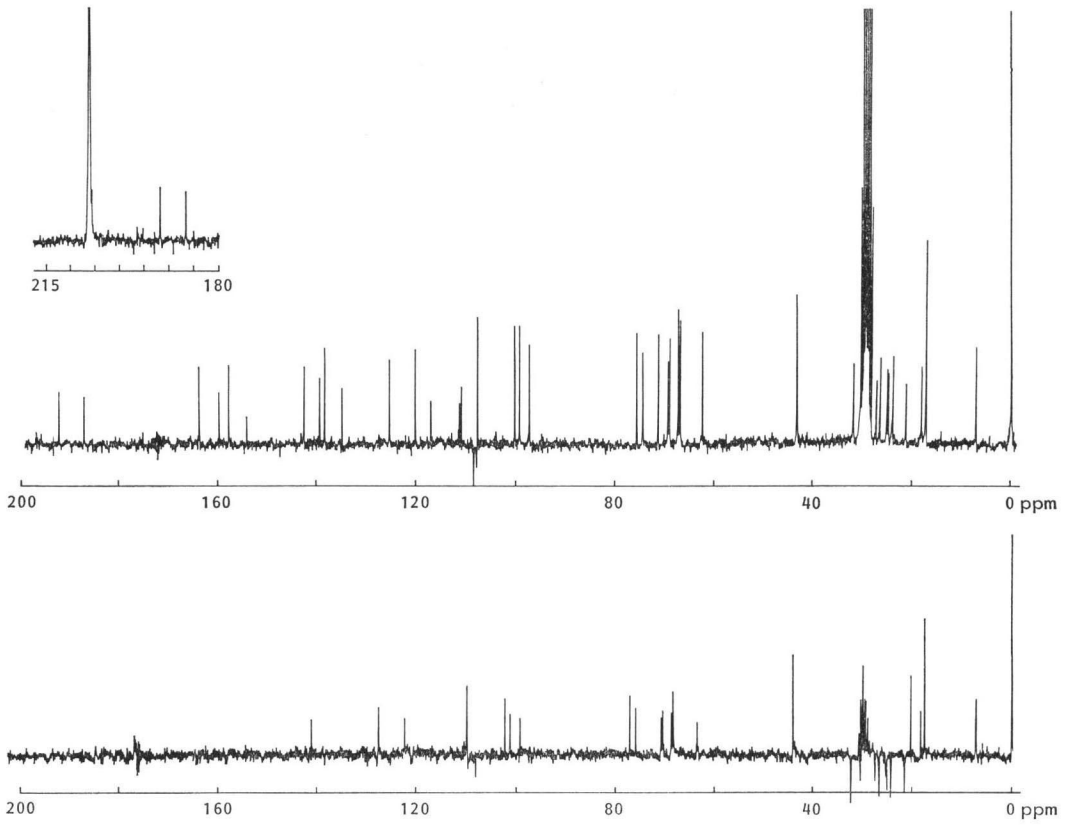


Table 4. Assignment of ^{13}C NMR spectrum of I (50 MHz, acetone-d_6).

Carbon	I δ (ppm)	Carbon	I δ (ppm)
5	191.7	1′	97.2
12	186.5	2′	30.5
4	163.3	3′	62.7
11	159.2	4′	74.7
6	157.2	5′	67.5
10a	142.1	6′	17.7
6a	139.0	$N(CH_3)_2$	43.7
2	138.1	1″	100.1
12a	134.5	2″	24.5
3	125.0	3″	25.3
1	119.9	4″	76.0
4a	116.8	5″	67.3
5a	111.2	6″	18.4
11a	110.8	1‴	99.1
10	69.8	2‴	25.5
9	71.6	3‴	27.0
13	32.3	4‴	67.8
7	27.7	5‴	69.4
8	21.8	6‴	17.7
14	7.2		

samine; rhodinosyl-2-deoxyfucosyl-rhodosamine, respectively, by mild hydrolysis under different conditions (Table 3) and was also confirmed by their FD-MS.

I FD-MS: m/z 755=M^+=(III - rhodosamine - rhodinose - rhodinose)$^+$, m/z 527=(III - rhodosamine)$^+$, m/z 370=(III)$^+$.

II FD-MS: m/z 771=M^+=(III - rhodosamine - 2-deoxyfucose - rhodinose)$^+$, m/z 658=(III - rhodosamine - 2-deoxyfucose)$^+$, m/z 527=(III - rhodosamine)$^+$, m/z 370=(III)$^+$.

The C-H decoupling resonance ^{13}C NMR spectrum and DEPT-^{13}C NMR spectrum of I showed the presence of forty carbons (Fig. 7). It was shown that I had twenty carbons corresponding to γ-rhodomycinone; twenty carbons corresponding to one mol of rhodosamine and two mol of rhodinose (Table 4). The signal assigned to C-10 is at 4.2 ppm (δ 69.8 ppm) lower field than that (δ 65.6 ppm) of γ-rhodomycinone. This demonstrated that the trisaccharide of I is linked to the C-10 position of γ-rhodomycinone.

The structures of I and II are shown in Fig. 1. The only difference between I and II is that II has one more hydroxyl group than I at the C-3″ position. This fact was also confirmed by ^1H NMR spectrum. The multiplet peak in the area of 3.84~4.10 ppm was assigned to the C-3″ proton of II by comparison with that of aclarubicin A.[9]

Among the known anthracycline antibiotics, auramycin G,[10] sulfurmycin G[10] and rhodirubin B[11] have the same sugar moieties as I, but their aglycones are different from that of I. Therefore, I is a new anthracycline antibiotic which is designated rhodilunancin A. II (rhodilunancin B) was identified as rhodomycin Y.[12] But the physico-chemical properties of the latter has not been reported.

In addition to their antimicrobial activities against Gram-positive bacteria, I and II inhibited the DNA synthesis of P_{388} leukemia cell *in vitro*. The IC_{50} value for DNA synthesis of I and II were 9.2 μg/ml and 5.47 μg/ml, respectively.

Addendum in Proof

After we finished our structural studies on rhodilunancins, we found an article on the structure of antibiotics, cosmomycins A and B in Agric. Biol. Chem. 49 in 1985 by T. ANDO *et al.*[13] Rhodilunancins A and B seem to be identical to cosmomycins A and B, respectively. The latter is also identical to rhodomycin Y found in 1972.[12]

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