Notes

NEW ANTHRACYCLINE ANTIBIOTICS: SERIRUBICIN AND 1-HYDROXYSERIRUBICIN†

TAKESHI UCHIDA, MASAYA IMOTO, TŌRU MASUDA, HIROSHI YOSHIMOTO††, KANJI IMAMURA††, TSUTOMU SAWA, HIROSHI NAGANAWA, TOMIO TAKEUCHI and HAMAO UMEZAWA

Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
††Applied Bioscience Laboratory, Kirin Brewery Co. Ltd., 1-2-2 Souja, Maebashi, Gunma 371, Japan

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New anthracycline antibiotics, serirubicin (**1**) and 1-hydroxyserirubicin (**2**) have been found in the culture filtrate of *Streptomyces cyaneus* MG344-hF49 (FERM BP-314), which had been previously reported to produce ditrisarubicins A, B (**3**) and C[1]. In the present paper, the production, isolation and characterization of anthracyclines **1** and **2** are reported.

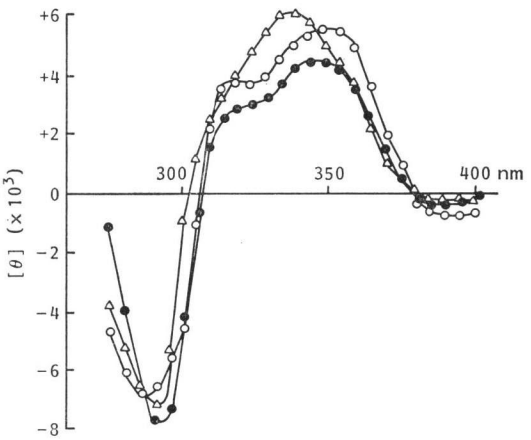
The strain was cultured at 27°C for 4 days in a jar fermentor with aeration and agitation in a medium consisting of 3% dextrin, 1.5% soybean meal ("Toast-soya" Nisshin-seiyu Co.), 0.6% glucose, 0.2% $CaCl_2$ and 0.012% $CoCl_2 \cdot 6H_2O$. Red pigments in the filtrate (100 liters) were extracted with butyl acetate (100 liters) at pH 8.0, followed by concentration *in vacuo*. The resulting residue was dissolved in a small amount of $CHCl_3$ and placed on a silicic acid column (ϕ 5×20 cm). After washing with a mixture of $CHCl_3$ - MeOH (100: 1), the fractions containing compounds **1**, **2** and **3** and ditrisarubicin C were eluted with a mixture of $CHCl_3$ - MeOH (100: 2), combined and evaporated to dryness. The red powder (125 mg) thus obtained was chromatographed on silicic acid thin-layer plates (Kieselgel $60F_{254}$, Merck Co.), using $CHCl_3$ - MeOH - NH_4OH (100: 5: 0.02) system. The first band (Rf 0.56) was ditrisarubicin B (**3**), whereas the second band (Rf 0.47) was a mixture of serirubicin (**1**), 1-hydroxyserirubicin (**2**) and ditrisarubicin C. This band was scraped off and eluted with a mixture of $CHCl_3$ - MeOH (10: 1). Subsequently the mixture was separated into three components by silicic acid thin-layer chromatography (TLC) developed with EtOAc - MeOH - NH_4OH (20: 1: 0.01). The bands corresponding to **1** (Rf 0.50) and **2** (Rf 0.43) were scraped off and extracted with $CHCl_3$ - MeOH (10: 1). After drying of the extracts, each pigment was further purified by chromatography on a Sephadex LH-20 column with MeOH. Thus, 15.2 mg of **1** and 10.4 mg of **2** were obtained.

The physico-chemical properties of **1** and **2** are as follows:

1: MP 179~183°C (dec); $[\alpha]_D^{25}$ −76° (*c* 0.1, $CHCl_3$); UV $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 229 (347), 255 (250), 290 (81), 419 (106), 436 (106); UV $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 239 (411), 280 (116), 500 (115); IR (KBr) cm^{-1} 3450, 1735, 1625, 1460, 1220, 1120, 1010; *Anal* Calcd for $C_{60}H_{80}N_2O_{21}$: C 61.84, H 6.92, N 2.40; Found: C 60.71, H 7.15, N 2.40; FD-MS *m/z* 1,165 $(M+H)^+$.

Fig. 1. CD curves of α-citromycinone (**4**), α_2-rhodomycinone (**6**) and β-rhodomycinone (**7**) (in acetonitrile).

△ **4** 1.35×10^{-4} M, ● **6** 2.00×10^{-4} M, ○ **7** 2.01×10^{-4} M.



† Taken in part from the Ph. D. Thesis of T. UCHIDA, the University of Tokyo, 1984

Table 1. ^{13}C NMR chemical shifts* of serirubicin (**1**), 1-hydroxyserirubicin (**2**) and ditrisarubicin B (**3**) (100 MHz).

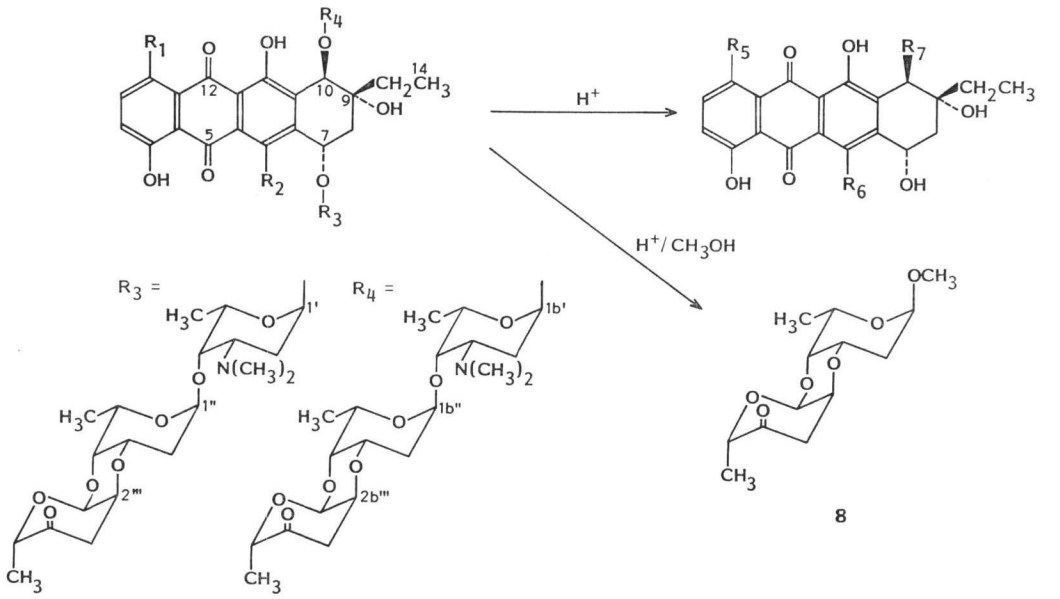
Carbon	1	2	3	Remarks
1	119.5	(158.4)	119.8	Aglycone
2	136.9	(130.0)	137.1	
3	125.0	(129.8)	124.7	
4	162.9	(157.9)	162.7	
4a	116.1	(112.7)	116.2	
5	(187.70)	186.0	190.9	
5a	132.5	(132.8)	(112.1)	
6	118.9	119.0	(157.1)	
6a	146.9	147.3	136.4	
7	74.0	73.9	71.0	
8	36.7	36.7	33.1	
9	72.2	72.2	71.8	
10	70.9	70.4	70.6	
10a	132.2	(131.9)	138.3	
11	162.2	162.0	(157.7)	
11a	115.1	115.1	(111.9)	
12	(187.66)	190.7	186.1	
12a	133.3	112.5	133.6	
13	31.1	31.1	30.7	
14	6.7	6.7	6.6	
1′, 1b′	(96.6), (100.2)	(96.3), (100.2)	(97.4), (101.9)	Rhodosamine
2′, 2b′	29.6, 29.6	29.6, 29.6	(29.4), (29.8)	
3′, 3b′	(61.4), (61.6)	(61.4), (61.5)	61.4, 61.4	
4′, 4b′	(74.4), (74.6)	(74.3), (74.5)	(74.3), (74.6)	
5′, 5b′	(68.3), (68.5)	(68.3), (68.4)	(68.3), (68.5)	
6′, 6b′	(17.9), (18.1)	(18.0), (18.1)	(17.9), (18.1)	
$NMe_2\times2$	(43.28), (43.31)	(43.29), (43.33)	(43.2), (43.3)	
1″, 1b″	99.2, 99.2	99.1, 99.1	99.1, 99.1	2-Deoxyfucose
2″, 2b″	(26.9), (27.0)	(26.9), (27.0)	(26.9), (27.0)	
3″, 3b″	(67.28), (67.35)	(67.2), (67.3)	(67.3), (67.4)	
4″, 4b″	67.0, 67.0	66.9, 66.9	67.0, 67.0	
5″, 5b″	(65.3), (65.4)	(65.2), (65.3)	(65.3), (65.4)	
6″, 6b″	16.0, 16.0	(15.96), (16.00)	16.0, 16.0	
1‴, 1b‴	91.6, 91.6	91.6, 91.6	91.6, 91.6	Cinerulose B
2‴, 2b‴	(63.0), (63.1)	(62.97), (63.04)	(63.0), (63.1)	
3‴, 3b‴	(39.7), (39.8)	(39.70), (39.75)	(39.7), (39.8)	
4‴, 4b‴	208.1, 208.1	208.1, 208.1	208.1, 208.1	
5‴, 5b‴	(77.88), (77.94)	(77.88), (77.94)	(77.88), (77.94)	
6‴, 6b‴	(16.1), (16.2)	(16.16), (16.20)	(16.1), (16.2)	

* In ppm (δ), obtained from $CDCl_3$ solutions containing TMS as internal reference at 100 MHz. Tentative assignments are given on the basis of the value of ref 6, 7. Similar values in parentheses may be interchanged.

2: MP 189~192°C (dec); $[\alpha]_D^{25}$ +1° (*c* 0.1, $CHCl_3$); UV $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 232 (374), 256 (190), 290 (68), 493 (119), 513 (sh, 89), 525 (73); UV $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 238 (373), 290 (sh, 69), 565 (145), 606 (139); IR (KBr) cm^{-1} 3450, 1740, 1605, 1480, 1220, 1120, 1020; *Anal* Calcd for $C_{60}H_{80}N_2O_{22}$: C 61.01, H 6.83, N 2.37; Found: C 60.83, H 7.12, N 2.27; FD-MS *m/z* 1,181 $(M+H)^+$.

Total acid hydrolysis of **1** in 0.1 N HCl at 85°C for 30 minutes gave α-citromycinone (**4**)[2], which was identified by comparison of its UV, IR, mass and ^1H NMR spectra with those reported[2,3]. The identification was also sup-

Fig. 2. Structures of anthracyclines **1**, **2** and **3**, and their degradation products.



Glycoside	R_1	R_2
Serirubicin (**1**)	H	H
1-Hydroxyserirubicin (**2**)	OH	H
Ditrisarubicin B (**3**)	H	OH

Aglycone	R_5	R_6	R_7
α-Citromycinone (**4**)	H	H	OH
γ-Citromycinone (**5**)	H	H	H
α_2-Rhodomycinone (**6**)	OH	H	OH
β-Rhodomycinone (**7**)	H	OH	OH

Table 2. Antitumor activity of serirubicin (**1**) and 1-hydroxyserirubicin (**2**) against L1210 leukemia.

Dose (mg/kg/day)	T/C (%)	
	1	**2**
1.0	149	114*
0.5	143	139
0.25	273	145
0.125	137	151
0.0625	113	127
0.0313	119	108

Inoculum: 1×10^5 cells/CDF_1 mouse, ip.
Treatment: Day 0~10, ip.
* Toxic.

ported by the isolation of γ-citromycinone (**5**)[2,4] after hydrogenolysis of **1** with 5% Pd/$BaSO_4$ in MeOH. On total acid hydrolysis, compound **2** liberated an orange red aglycone **6**, which was determined to be α_2-rhodomycinone[2] by comparison of its $[\alpha]_D$ and Rf values with those of an authentic sample. The absolute configurations of the aglycones **4** and **6** have been reported by BROCKMANN *et al.*[5]; their ^{1}H NMR spectra and CD curves (Fig. 1) show the β-rhodomycinone (**7**) configuration (7*S*, 9*R*, 10*R*).

The physico-chemical properties of **4**, **5** and **6** are as follows:

4: MP 197~200°C (dec); $[\alpha]_D^{25}$ +123° (*c* 0.05, $CHCl_3$); UV $\lambda_{max}^{cyclohexane}$ nm ($E_{1cm}^{1\%}$) 227 (1,213), 257 (595), 290 (225), 417 (300), 435 (298); UV $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 228 (1,143), 255 (746), 287 (265), 417 (350), 436 (350); UV $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 238 (1,350), 278 (354), 487 (382); IR (KBr) cm^{-1} 3400, 1630, 1605; FD-MS *m/z* 370 $(M)^+$; ^{1}H NMR (400 MHz, C_5D_5N) δ 1.36 (3H, t, *J*=7.4 Hz, H-14), 2.25 (2H, m, H-13), 2.48 (1H, ddd, *J*=14.4, 2.2, ~1 Hz, H-8B), 2.77 (1H, dd, *J*=14.4, 5.2 Hz, H-8A), 5.26 (1H, ddd, *J*=7.5, 5.2, 2.2 Hz, H-7), 5.59 (1H, dd, *J*=3.8, ~1 Hz, H-10), 6.36 (1H, s, OH-9), 6.78 (1H, d, *J*=7.5 Hz, OH-7), 7.36 (1H, dd, *J*=8.5, ~1 Hz, H-3), 7.59 (1H, OH-10), 7.61 (1H, dd, *J*=8.5, 7.5 Hz, H-2), 7.86 (1H, dd, *J*=7.5, ~1 Hz, H-1), 8.35 (1H, s, H-6), 13.01 (1H, s, OH), 13.62 (1H, s, OH).

5: MP 219~224°C (dec), UV $\lambda_{max}^{cyclohexane}$ nm ($E_{1cm}^{1\%}$) 418 (337), 437 (337); EI-MS *m/z* 354

$(M)^+$, 336 $(M-H_2O)^+$, 318, 282, 280, 279; ^1H NMR (250 MHz, $CDCl_3$) δ 1.10 (3H, t, J=7.4 Hz, H-14), 1.77 (2H, m, H-13), 1.88 (1H, s, OH-9), 1.93 (1H, ddd, J=14.5, 5, 1.3 Hz, H-8B), 2.36 (1H, ddd, J=14.5, 2, 2 Hz, H-8A), 2.67 (1H, d, J=19 Hz, H-10B), 3.16 (1H, dd, J=19, 2 Hz, H-10A), 3.93 (1H, d, J=10 Hz, OH-7), 4.87 (1H, ddd, J=10, 5, 2 Hz, H-7), 7.33 (1H, dd, J=8.5, ~1 Hz, H-3), 7.69 (1H, dd, J=8.5, 7.5 Hz, H-2), 7.85 (1H, dd, J=7.5, ~1 Hz, H-1), 8.00 (1H, s, H-6), 12.73 (1H, s, OH), 13.17 (1H, s, OH).

6: MP 214~217°C (dec); $[\alpha]_D^{25}$ +210° (*c* 0.03, $CHCl_3$); UV $\lambda_{max}^{0.1N\ HCl-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 232 (1,192), 256 (614), 288 (219), 489 (383), 590 (sh, 295), 523 (sh, 236); UV $\lambda_{max}^{0.1N\ NaOH-90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 241 (1,200), 285 (sh, 246), 553 (432); IR (KBr) cm^{-1} 3400, 1595, 1450, 1280; EI-MS *m/z* 386 $(M)^+$, 368 $(M-H_2O)^+$, 350 $(M-2H_2O)^+$, 334, 311, 296; ^1H NMR (100 MHz, C_5D_5N) δ 1.36 (3H, t, H-14), 2.24 (2H, m, H-13), 2.45 (1H, ddd, J=14, 2, 2 Hz, H-8B), 2.74 (1H, dd, J=14, 5 Hz, H-8A), 5.24 (1H, m, H-7), 5.55 (1H, br s, H-10), 7.37 (2H, s, H-2 and H-3), 8.38 (1H, s, H-6).

The sugar moieties in **1** and **2** were found to consist of rhodosamine, 2-deoxyfucose and cinerulose B as in ditrisarubicin B (**3**) by a silicic acid TLC analysis of their acid hydrolysates. Their ^{13}C NMR spectra (Table 1) suggested that **1** and **2** consisted of two trisaccharides similar to **3**. Methanolysis of **1** and **2** in 0.046 N methanolic hydrogen chloride at room temperature for 3 hours gave a methyl disaccharide **8**, which was identified as methyl 2′,3-anhydro-2,6-dideoxy-4-*O*-(3,6-dideoxy-α-L-*erythro*-hexopyrano-4-ulose-1-yl)-α-L-*lyxo*-hexopyranoside by comparison of $[\alpha]_D$ and ^1H NMR spectrum with reported data[6]. These results indicated that both sugar substituents of compounds **1** and **2** are composed of two trisaccharides, whose sequences are identical with that of **3**. Furthermore the ^{13}C NMR spectra indicated that the hydroxyl groups at C-7 and C-10 of the aglycone linked to the trisaccharides in both **1** and **2**. Thus, we propose the structures shown in Fig. 2 for serirubicin (**1**) and 1-hydroxyserirubicin (**2**).

The cytotoxic activity of **1** and **2** was compared with that of **3**; the IC_{50} values against P388 leukemia cells were 0.0098 μg/ml, 0.012 μg/ml and 0.004 μg/ml, respectively. The antitumor activity of **1** and **2** was also tested against L1210 leukemia cells in CDF_1 mice and the results are shown in Table 2. The median lethal doses (LD_{50}) by a single intraperitoneal injection of **1** and **2** in mice were 10~20 mg/kg and 5~10 mg/kg, respectively.

Though cosmocarcin A[8], which was identical with serirubicin[9], was recently reported, this study was performed independently.

Acknowledgments

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