NEW ANTHRACYCLINE ANTIBIOTICS, RHODIRUBINS

Sir:

We have previously reported on aclacinomycins A and B[1] and will report on their nineteen analogues[2] produced by a strain No. MA144-M1, which was classified as *Streptomyces galilaeus*. In a continuing search for other new anthracycline antibiotics, we have found rhodirubins in the culture broths of a strain designated as ME505-HE1 belonging to *Streptomyces* sp. In this communication, the isolation of rhodirubins and their properties are reported.

The strain ME505-HE1 was cultivated at 28°C for 5 days on a rotary shaker in a medium consisting of 2% potato starch, 2% glucose, 2% soybean meal, 0.5% yeast extract, 0.25% NaCl, 0.3% $CaCO_3$, 0.0003% $CuSO_4 \cdot 5H_2O$, 0.0003% $MnCl_2 \cdot 4H_2O$ and 0.0003% $ZnSO_4 \cdot 7H_2O$, pH 7.4. Red pigments in the mycelial cake were extracted with acetone, evaporated under reduced pressure and re-extracted with chloroform. After concentration, the dried residue was dissolved in a small amount of ethyl acetate and crude rhodirubin was precipitated by addition of 10 times volume of *n*-hexane. The crude dark red powder thus obtained was dissolved in a small amount of toluene and subjected to a silicic acid column chromatography. After eluting the red non-carbohydrate containing pigments with toluene - methanol in 100: 2 volume ratio, rhodirubins C, G, B and A were successively eluted with a 100: 3 mixture of the same solvents. Rhodirubins D and E were eluted with a 10: 1 mixture. Each rhodirubin fraction described above was subjected to preparative silicic acid thin-layer chromatography using chloroform - methanol (20: 1 or 10: 1). Thus, 33 mg of rhodirubin A, 87.6 mg of rhodirubin B and 105 mg of rhodirubin C were obtained in pure red amorphous states from 2.4 g of the crude powder described above. The minor components D, E and G were obtained in quantities sufficient only for determination of TLC Rf values and for analysis of sugar moieties by acid hydrolysis. Their Rf values on a silicic acid thin-layer chromatography (Kieselgel $60F_{254}$, Merck Co.) with several solvent systems are shown in Table 1.

On acid hydrolysis in 0.1 N HCl for 30 minutes at 85°C, all rhodirubins gave ε-pyrromycinone[3,4]. Thin-layer chromatography of acid hydrolysates with *n*-butanol - acetic acid - water (4: 1: 1) indicated that rhodirubin A contained three hexoses corresponding to rhodosamine, 2-deoxyfucose and rhodinose, B contained rhodosamine and two moles of rhodinose, C contained rhodosamine, 2-deoxyfucose and cinerulose, E contained rhodosamine and 2 moles of 2-deoxyfucose, and G contained rhodosamine, 2-deoxyfucose and D-cinerulose, while D had two hexoses corresponding to rhodosamine and 2-deoxyfucose, as shown in Table 2. For identification of the hexoses, authentic samples of aclacinomycin A[1], MA144 G1[2] and streptolydigin[5] were hydrolyzed and the hexoses thus obtained were compared with those obtained from hydrolysis of rhodirubins. Rhodirubin C was found to be identical with cinerubin A[6] by the mixed melting point determination, IR, PMR and ^{13}C-NMR spectra, and the physicochemical properties of the methyl disaccharide obtained by their methanolysis in methanol containing 0.01 N HCl for 30 minutes.

Physicochemical properties of rhodirubins A and B are as follows: Rhodirubin A: m.p. 141~143°C; $[\alpha]_D^{20} + 120°$ (*c* 1.0, $CHCl_3$);

Anal. calcd. for $C_{42}H_{55}NO_{16}$: C 60.77, H 6.67, O 30.81, N 1.69.

Found: C 60.39, H 6.63, O 30.72, N 1.71.

The ultraviolet and visible absorption spectra in methanol show the following maxima ($E_{1cm}^{1\%}$): 234 (527), 258 (280), 292 (120), 412sh (53), 465sh (133), 480 (153), 490 (160), 510sh (127), 524sh (107), 570 nm (20). The IR spectrum in KBr shows peaks at the following wavelengths in cm^{-1}: 3430, 2950, 2930, 2810, 2750, 1735, 1640, 1600, 1450, 1320, 1300, 1220, 1160, 1120, 1040, 1000, 970, 960, 920, 800 and 760. The PMR spectrum of rhodirubin A in $CDCl_3$ (100 MHz)

Table 1. Rf values of rhodirubins

Components	Solvent systems		
	(1)	(2)	(3)
Rhodirubin A	0.52	0.48	0.26
Rhodirubin B	0.52	0.56	0.29
Rhodirubin C	0.88	0.70	0.53
Rhodirubin D	0.28	0.26	0.13
Rhodirubin E	0.28	0.17	0.11
Rhodirubin G	0.85	0.66	0.49

Solvent systems:

(1) chloroform - methanol (10: 1)

(2) chloroform - methanol - formic acid (9: 1: 0.5)

(3) benzene - ethyl acetate - methanol (5: 5: 1)

Table 2. Rf values of hexoses obtained by hydrolysis of rhodirubins

Rhodirubins	Rhodosamine Rf=0.14	2-Deoxyfucose 0.55	Rhodinose 0.70	Cinerulose 0.81
A	+	+	+	−
B	+	−	++	−
C	+	+	−	+
D	+	+	−	−
E	+	++	−	−
G	+	+	−	+

Rf: Silica gel thin-layer chromatography with *n*-butanol - acetic acid - water (4: 1: 1)

Table 3. Antimicrobial spectrum of rhodirubins A and B

Organisms	MIC, μg/ml	
	Rhodirubin A	Rhodirubin B
Bacillus subtilis ATCC6633	0.78	1.56
Bacillus cereus ATCC9634	0.2	0.4
Bacillus megaterium NRRL B-938	0.78	1.56
Staphylococcus aureus FDA209P	1.56	1.56
Staphylococcus aureus Smith	0.4	0.78
Sarcina lutea ATCC9341	0.4	0.78
Micrococcus flavus	<0.2	<0.2
Corynebacterium bovis 1810	0.2	0.4
Pseudomonas fluorescens NIHJ B254	100	100
Proteus morganii	>100	>100
Mycobacterium smegmatis ATCC607	6.25	3.1
Candida albicans IAM4905	>100	>100
Candida tropicalis IAM4942	>100	>100

shows the following chemical shifts (ppm): 7.6, s; 7.24, s; 5.50, m; 5.62, m; 5.02, m; 4.84, m; 4.52, q; 4.7 ~ 3.90, overlapping m; 3.72, s; 3.60 ~0.09, overlapping m and 2.18 s. Rhodirubin B: m.p. 135~137°C; $[\alpha]_D^{20}+190°$ (*c* 0.1, $CHCl_3$);

Anal. calcd. for $C_{42}H_{55}NO_{15}$: C 61.99, H 6.77, O 29.52, N 1.72.
Found: C 61.23, H 6.80, O 28.77, N 1.94.

The ultraviolet and visible absorption spectra in methanol show the following maxima ($E_{1cm}^{1\%}$): 235 (600), 257 (310), 290 (120), 410sh (53), 464sh (153), 476sh (173), 491 (187), 510sh (147), 522sh (113) and 570 nm (19). The infrared spectrum in KBr shows peaks at the following wavelengths in cm^{-1}: 3470, 2960, 2940, 2820, 2790, 1740, 1640, 1600, 1450, 1300, 1220, 1160, 1120, 1040,

Fig. 1. Structure of rhodirubins A and B

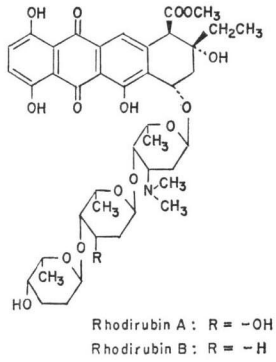
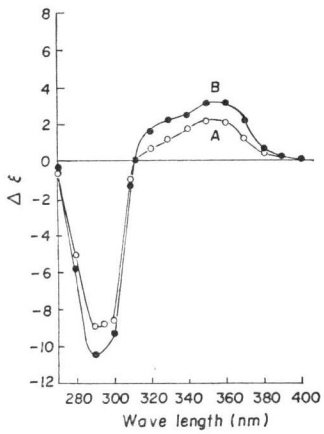


Fig. 2. CD curves of rhodirubins A and B in chloroform - methanol (1: 1)



1000, 980, 960, 920, 800, 790 and 760. The PMR spectrum of rhodirubin B in $CDCl_3$ (100 MHz) shows the following chemical shifts (ppm): 7.6, s; 7.24, s; 5.50, m; 5.02, m; 4.84, m; 4.52, q; 4.7~ 3.90, overlapping m; 3.72, s; 3.6 ~ 0.09 overlapping m and 2.18, s.

In order to determine the sequence of the hexoses in the sugar chain, rhodirubins A and B were hydrolyzed under three different conditions. On mild hydrolysis with 0.5% HCl at 20°C for 10 minutes both rhodirubins A and B liberated L-rhodinose. On methanolysis in methanol containing 0.01 N HCl for 30 minutes at room temperature pyrromycin[3] (rhodosaminyl-ε-pyrromycinone) was obtained from both A and B. On total acid hydrolysis (0.1 N HCl for 60 minutes at 85°C) ε-pyrromycinone, L-rhodosamine, 2-deoxy-L-fucose and L-rhodinose were obtained from rhodirubin A and L-rhodinose was obtained from rhodirubin B in place of 2-

deoxy-L-fucose from rhodirubin A. Thus, the structures shown in Fig. 1 are proposed for rhodirubins A and B. Detailed structural studies will be reported elsewhere.

Among the known anthracycline antibiotics, rhodomycins X and Y[7], violamycins[10] and the compound MA144-N1 have the same sugar moiety (rhodinosyl-2-deoxyfucosyl-rhodosaminyl) as rhodirubin A, but their aglycones were different: rhodomycinones in rhodomycins X and Y and violamycin; aklavinone in MA144-N1; ε-pyrromycinone in rhodirubins. The sugar moiety (rhodinosyl-rhodinosyl-rhodosaminyl) of rhodirubin B has not been found in anthracycline antibiotics.

On the stereochemistry of aglycones of anthracycline antibiotics, BROCKMANN *et al.*[8] have reported the absolute configuration of ε-pyrromycinone, and ECKARDT *et al.*[9] have proposed two stereoisomers having the aklavinone structure. The CD curves of rhodirubins A and B are shown in Fig. 2. The same absolute configuration (7S, 9R and 10R) as ε-pyrromycinone proposed by BROCKMANN *et al.* has been confirmed by the PMR analysis.

The antimicrobial activity of rhodirubins A and B was tested by the broth dilution method with the results shown in Table 3. These antibiotics are strongly active against Gram-positive bacteria. Rhodirubins A and B (2.5~5 mg/kg/day) prolonged the survival period of CDF_1 mice inoculated with L-1210 leukemia. Their LD_{50} values in mice were 7.5~10 mg/kg and 10~12.5 mg/kg, i.p., respectively.

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