NEW ANTHRACYCLIC ANTIBIOTICS ROSEORUBICINS A AND B

Sir:

In the course of the study on antitumor anthracyclic antibiotics, roseorubicins A and B, new members of the rhodomycin group, were isolated from the cultured broth of *Actinomyces roseoviolaceus* A 529 (IFO 13081).

Roseorubicins are produced as orange-red pigments by the shaking culture of strain A 529 at 28°C for 5 days in a medium containing 4% sucrose, 2.5% soybean meal, 0.1% yeast extract, 0.25% NaCl, 0.32% $CaCO_3$, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.0005% $MnCl_2 \cdot 4H_2O$ and 0.0005% $ZnSO_4 \cdot 7H_2O$, pH 7.4, and can be extracted from the mycelium and the culture filtrate with acetone or chloroform, and separated into two major components with Rf 0.2 and 0.1 by silica gel thin-layer (60 F_{254} Merck Co.) chromatography using chloroform - methanol - acetic acid (80: 20: 4). Roseorubicins are also produced by *Actinomyces violascens* A517 (IFO 12920), *Act. violarus* A530 (IFO 13104), *Act. violaceochromogenes* A549 (IFO 13100) and *Streptomyces purpurascens* A519 (IFO 13077) as well as *Act. roseoviolaceus*.

In an example, 24 liters of the cultured broth (pH 7.4) were filtered, and roseorubicins in the mycelium were extracted with acetone followed by concentration to a quarter volume under reduced pressure and re-extracted with chloroform. Orange-red pigments in the culture filtrate were extracted with chloroform. The chloroform extracts were combined and concentrated *in vacuo*, yielding 30 g of an oily mixture of orange-red pigments. It was dissolved in 50 ml of chloroform-methanol mixture (1: 2) and subjected to Sephadex LH-20 gel filtration. Roseorubicin fractions were eluted with the same solvent mixture. By evaporating the eluates, the crude roseorubicins were obtained as a red powder. The crude red powder thus obtained was dissolved in 30 ml of 0.1 M acetate buffer, pH 3.0, and the insoluble residue which contained aglycones was extracted by addition of 30 ml of toluene.

Roseorubicin A which was contained in the acetate-buffer extract was re-extracted with chloroform after adjusting the pH of the aqueous layer to 7.0. The chloroform extract was dried over Na_2SO_4 and concentrated to a small volume, and pure roseorubicin A was precipitated by addition of ten times volume of *n*-hexane, as a red powder (263 mg), mp. 143~147°C, Anal. Calcd. for $C_{54}H_{78}N_2O_{18}$: C, 62.17; H, 7.54; N, 2.69; O, 27.61%; Found: C, 61.87; H, 7.63; N, 2.56%. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 237 (354), 256 (300), 295 (80), 470s (118), 482s (132), 495 (152), 515s (119), 530 (117), 580 (26).

Roseorubicin B was extracted with *n*-butanol from the acetate buffer extract at pH 7.0. The *n*-butanol extract was concentrated to a red residue. A small amount of the salt in the residue was removed by Sephadex LH-20 gel filtration using methanol as an eluation solvent. The eluate containing roseorubicin B was concentrated to a small volume and precipitated by addition of *n*-hexane to yield 37 mg of pure roseorubicin B as a red powder, mp. 122~124°C, Anal. Calcd. for $C_{36}H_{48}N_2O_{11}$: C, 63.14; H, 7.07; N, 4.09; O, 25.70%; Found: C, 62.31; H, 7.18; N, 3.97%. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 237 (481), 256 (436), 295 (107), 470s (158), 482s (180), 495 (211), 515s (166), 530 (172), 575 (47).

On acid hydrolysis in 0.1 N HCl for 30 minutes at 85°C, roseorubicins A and B gave γ-rhodomycinone[1,2]; mp. 210~218°C, M^+ at *m/e* 370, and the same CD spectrum as γ-rhodomycinone indicating 9R and 10R. Thin-layer chromatography of acid hydrolysates with *n*-butanol - acetic acid - water (4: 1: 1) indicated that roseorubicin A had three kinds of hexoses, corresponding to rhodosamine, 2-deoxyfucose and rhodinose, and B had only rhodosamine. On the other hand, roseorubicins A and B gave 10-deoxy-γ-rhodomycinone[1] by hydrogenolysis on palladium catalyst in methanol at room temperature.

The ^{13}C-NMR spectra (CMR, in $CDCl_3$) of roseorubicins A and B indicated the presence of 54 and 36 carbons, respectively. Off-resonance decoupled spectrum of roseorubicin A showed the presence of six C-methyls, two N-dimethyls, ten methylenes, nineteen methines, one quaternary carbon, three aromatic methines, nine substituted aromatic carbons, and two carbonyl carbons (Table 1). Thus, it was shown that roseorubicin A had eighteen carbons, corresponding to 2-deoxyfucose and two moles of rhodinose, in addition to roseorubicin B.

In order to determine the sequence of the hexoses in the sugar chain, roseorubicin A was hydrolyzed under three different conditions: (1) On mild hydrolysis with 0.05 N HCl at 23°C

Table 1. ^{13}C-Chemical shift-assignments of roseorubicins A and B*.

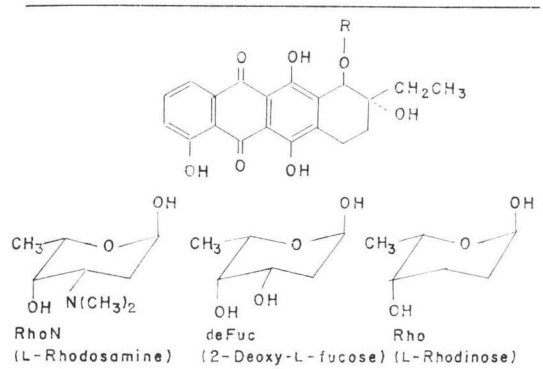
	C	A	B
γ-Rhodomycinone	5	191.0	191.0
	12	185.6	185.6
	4	162.6	162.7
	11	158.3	158.3
	6	156.3	156.3
	10a	140.2	140.2
	6a	138.3	138.2
	2	136.9	137.0
	12a	133.9	133.8
	3	124.3	124.4
	1	119.5	119.5
	4a	116.3	116.3
	5a	110.4	110.4
	11a	110.2	110.2
	10	69.8	70.7
	9	66.0	66.0
	13	29.8	29.8
	7	24.7	24.8
	8	21.0	21.0
	14	7.0	7.0
Rhodosamine	1′	91.0	91.0
	2′	26.5	26.5
	3′	59.6	59.8
	4′	77.5	77.4
	5′	66.6	66.5
	6′	16.5	16.5
	NMe_2	41.9	42.0
Rhodosamine	1″	97.8	97.9
	2″	29.7	29.5
	3″	61.4	59.5
	4″	74.4	66.3
	5″	68.1	66.5
	6″	18.0	17.2
	NMe_2	43.3	42.0
2-Deoxyfucose	1‴	100.3	
	2‴	34.3	
	3‴	65.7	
	4‴	83.6	
	5‴	66.7	
	6‴	17.0	
Rhodinose	1⁗	98.7	
	2⁗	24.0	
	3⁗	24.7	
	4⁗	75.4	
	5⁗	67.2	
	6⁗	17.1	
Rhodinose	1′′′′′	99.5	
	2′′′′′	23.7	
	3′′′′′	26.0	
	4′′′′′	67.5	
	5′′′′′	66.8	
	6′′′′′	17.1	

* In ppm (δ), obtained from $CDCl_3$ solutions containing TMS as internal reference.

for 5 ~ 30 minutes roseorubicin A gave rhodinose and two orange-red-colored products having different Rf values (0.13, 0.14) on silica gel thin-layer chromatography using chloroform - methanol - acetic acid (80: 20: 4). By further hydrolysis another rhodinose residue was liberated from the above product having Rf 0.14. Thus, it was suggested that roseorubicin A had rhodinosyl rhodinose at the terminal position. (2) On partial hydrolysis in acetone containing 0.02 N HCl for 40 minutes at room temperature, roseorubicin A was converted to roseorubicin B. Further hydrolysis of roseorubicin B obtained above in 0.1 N HCl at 50°C for 2 hours gave γ-rhodomycin I (rhodosaminyl-γ-rhodomycinone). Thus, roseorubicin B was confirmed to have 2 moles of rhodosamine at C-10 position of γ-rhodomycinone. (3) On total acid hydrolysis with 0.1 N HCl for 30 minutes at 85°C, γ-rhodomycinone, 2 moles of L-rhodosamine, one mole of 2-deoxy-L-fucose and 2 moles L-rhodinose were obtained from roseorubicin A, and γ-rhodomycinone and 2 moles of rhodosamine were obtained from roseorubicin B. Thus, the structures of roseorubicins A and B were pro-

Table 2. Structures of γ-rhodomycinone glycosides.

Compounds	R
Roseorubicin A	-RhoN-RhoN-deFuc-Rho-Rho
Roseorubicin B	-RhoN-RhoN
γ-Rhodomycin I	-RhoN
γ-Rhodomycin II	$-(RhoN)_2$
γ-Rhodomycin III	$-(RhoN)_2$ -deFuc
γ-Rhodomycin IV	$-(RhoN)_2$ -deFuc -Rho
Rhodomycin Y	-RhoN-deFuc-Rho
γ-Rhodomycin analog	$-(RhoN)_2$ -Rho
γ-Rhodomycin analog	-RhoN -deFuc -Rho



posed as shown in Fig. 1. IR and PMR spectra of roseorubicins A and B are shown in Figs. 2~5.

Among known anthracyclines, γ-rhodomycin I, II, III and IV[3,4], rhodomycin Y[5], a γ-rhodomycin analogue having two moles of rhodosamine and one mole of rhodinose[6], and a γ-rhodomycin analogue having one mole of rhodosamine, 2-deoxyfucose and rhodinose[6], have the same aglycone, γ-rhodomycinone, as roseorubicins A and B. However, as summarized in Table 2, the sugar sequences of roseorubicins A and

Fig. 1. Structure of roseorubicins A and B.

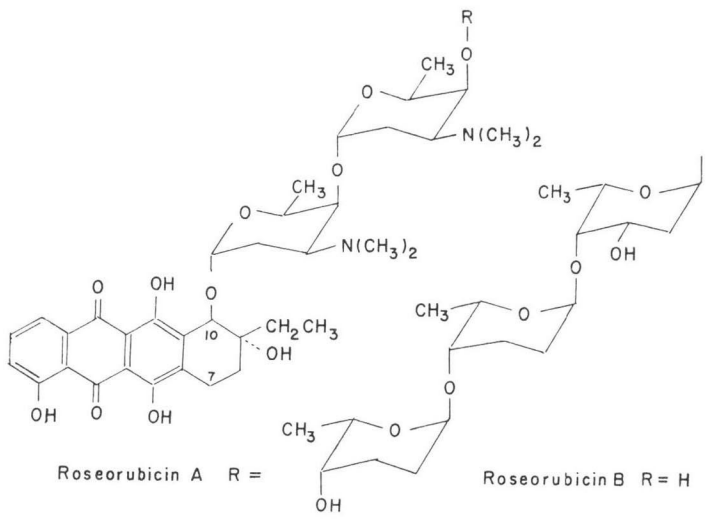
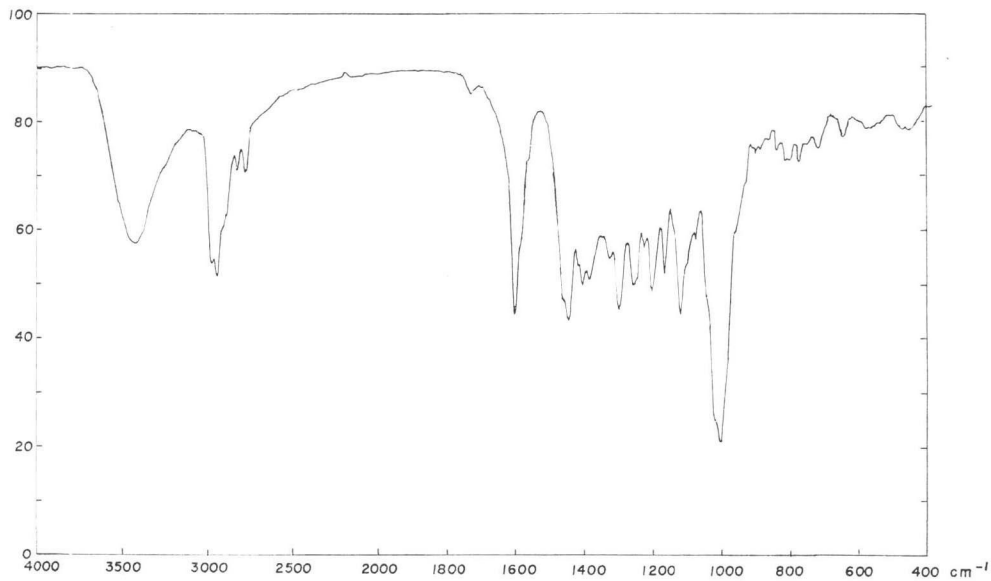


Fig. 2. IR spectrum of roseorubicin A (KBr).



B, that is, rhodinosyl-rhodinosyl-2-deoxyfucosyl-rhodosaminyl-rhodosaminyl and rhodosaminyl-rhodosaminyl, are unique. Five hexoses chain in anthracycline has been first found in roseorubicin A. It is possible that roseorubicin B may be identical to γ-rhodomycin II which was reported to contain 2 moles of rhodosamine with γ-rhodomycinone. But the physicochemical properties of the latter antibiotic has not been reported yet.

In addition to their antimicrobial activities (Table 3) against Gram-positive bacteria, roseorubicins A and B inhibited the growth of cultured L1210 cells (IC_{50} at 0.04 mcg/ml of A, and at 0.06 mcg/ml of B on day 2). Fifty percent inhibition concentrations for RNA synthesis

Fig. 3. IR spectrum of roseorubicin B (KBr).

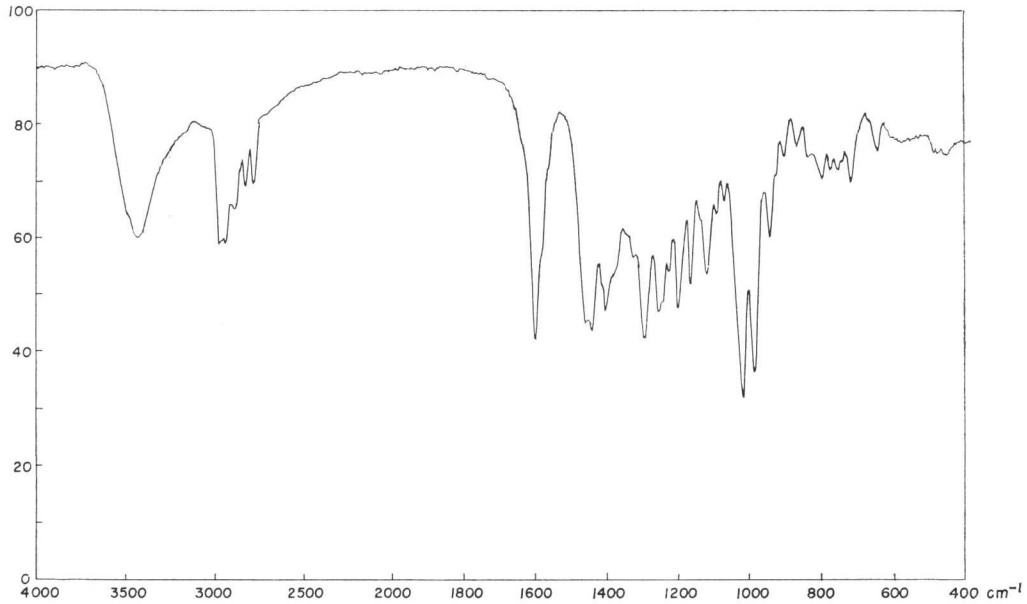
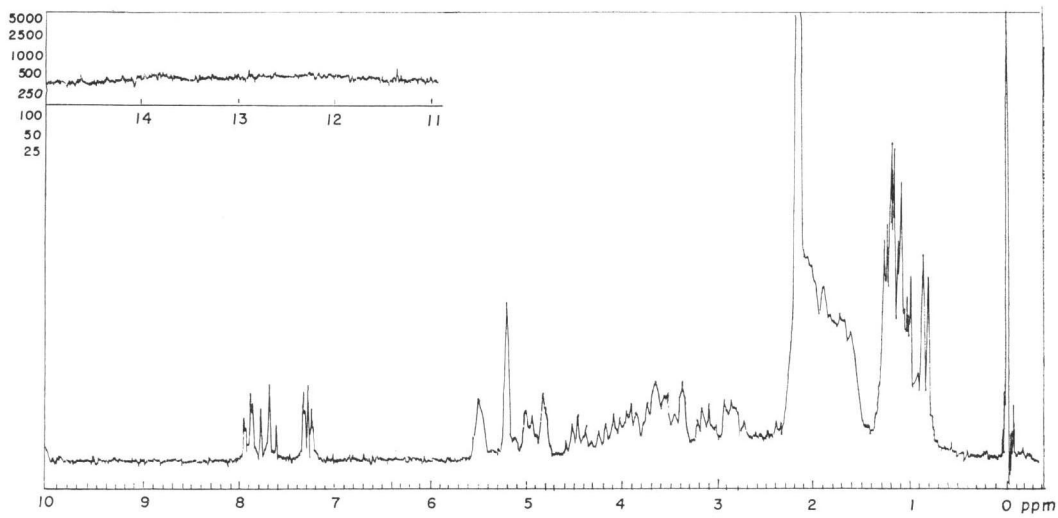


Fig. 4. PMR spectrum of roseorubicin A (100 MHz, in $CDCl_3$).



in L1210 cultured cells were 0.33 mcg/ml of A and 0.4 mcg/ml of B and those for DNA synthesis were 2.8 mcg/ml of A and 2.15 mcg/ml of B.

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Fig. 5. PMR spectrum of roseorubicin B (100 MHz, in $CDCl_3$).

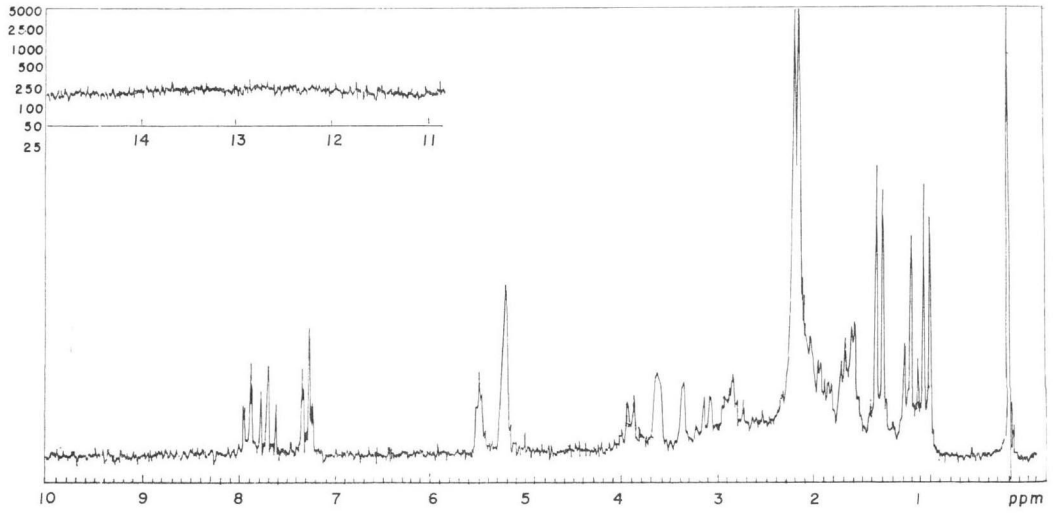

Table 3. Antimicrobial spectrum of roseorubicins A and B*.

Organisms	MIC (mcg/ml)	
	A	B
Staphylococcus aureus FDA209P	0.4	3.1
Bacillus subtilis ATCC6633	0.2	3.1
Bacillus cereus ATCC9634	0.4	6.2
Bacillus megaterium NRRL B-938	<0.1	6.2
Sarcina lutea ATCC9341	0.4	12.5
Micrococcus flavus	0.2	12.5
Corynebacterium bovis	<0.1	6.2
Pseudomonas fluorescens NIHJ B-254	>50	>50
Mycobacterium smegmatis ATCC607	0.8	25
Candida albicans IAM 4905	>50	>50

* Broth dilution method.

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