BAUMYCINS, NEW ANTITUMOR ANTIBIOTICS RELATED TO DAUNOMYCIN

Sir:

In the course of studies on antitumor anthracycline glycosides, new members of the daunomycin group, baumycins A1, A2, B1, B2, C1 and C2 have been isolated from culture broths of *Streptomyces* sp. No. ME130-A4 which was classified as *S. coeruleorubidus*.

The strain ME130-A4 was shake-cultured on a rotary-shaking machine (230 rpm) at 28°C for 7 days in a medium containing 4% sucrose, 2.5% soybean meal, 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. Orange red pigments were extracted from the mycelium mass and the culture filtrate with acetone or chloroform and separated into 12 components by silica gel thin-layer (60 F_{254} Merck Co.) chromatography. Their Rf values developed with four solvent systems are shown in Table 1.

In an example, 10 liters of a culture broth

Fig. 1. Purification procedure of baumycin

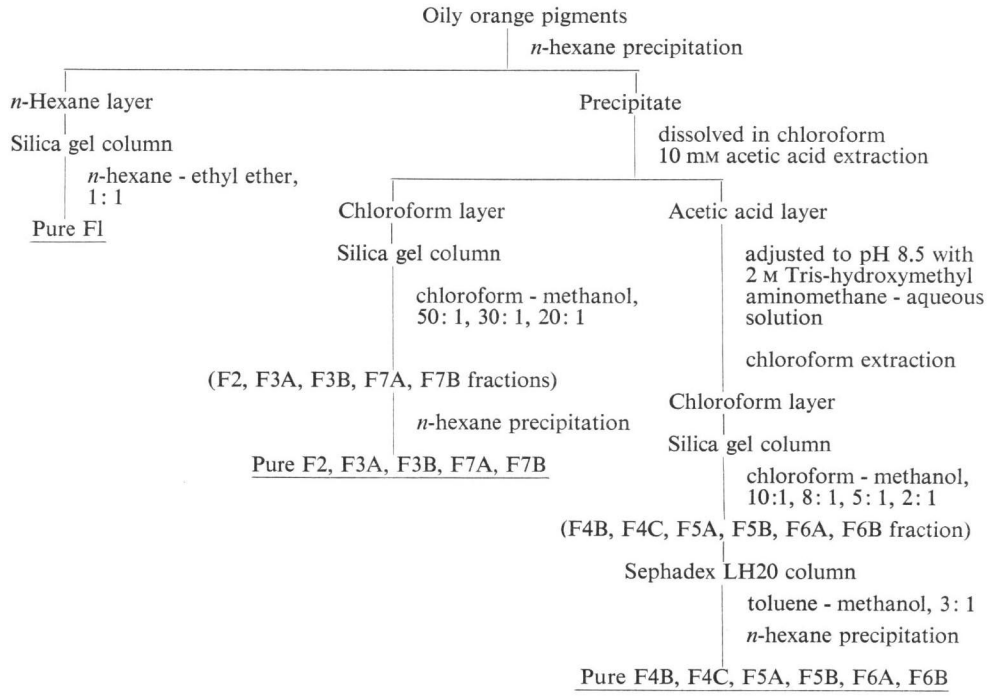

Table 1. Rf values of 12 components from the culture of *S. coeruleorubidus* ME130-A4

Solvent system	Rf value											
	F1	F2	F3A	F3B	F7A	F7B	F4B	F4C	F5A	F5B	F6A	F6B
I	0.90	0.69	0.62	0.59	0.52	0.46	0.25	0.24	0.08	0.07	0.03	0.01
II	0.89	0.64	0.56	0.54	0.48	0.44	0.26	0.30	0.17	0.18	0.07	0.10
III	0.95	0.92	0.87	0.87	0.88	0.85	0.74	0.75	0.64	0.64	0.41	0.30
IV	0.85	0.74	0.71	0.70	0.71	0.69	0.39	0.56	0.28	0.39	0.07	0.14

TLC plate: Silica gel 60 F_{254} (Merck Co.), 23°C.
Solvent system I : Chloroform - methanol = 10: 1
II : Chloroform - methanol - formic acid = 90: 10: 1
III : Chloroform - methanol - acetic acid = 80: 20: 4
IV : Chloroform - methanol - benzene = 7: 3: 3

(pH 8.3) was filtered and orange red pigments in the mycelium were extracted three times with 4 liters of acetone. The extract was concentrated to half volume under reduced pressure and re-extracted three times with 3 liters of chloroform. Pigments in the culture filtrate were extracted with 3 liters of chloroform. The chloroform extracts were combined and concentrated *in vacuo*, yielding 12 g of an oily mixture of orange red pigments. It was dissolved in 100 ml of chloroform and 400 ml of *n*-hexane was added to precipitate the pigments. The procedure of the purification of each pigment is shown in Fig. 1; yields of each pure pigment were as follows: F1, 7 mg; F2, 6.5 mg; F3A, 4 mg; F3B, 6 mg; F7A, 2.5 mg; F7B, 8.5 mg; F4B, 18 mg; F4C, 2.5 mg; F5A, 27.5 mg; F5B, 4.5 mg; F6A, 6 mg; and F6B, 4 mg. The overall yield estimated by the absorption at 530 nm was about 3~10% from the culture broth.

Partial structures of these pigments were studied by acid hydrolyses with 0.1 N HCl at 85°C for 30 minutes. F1, F2, F3A and F3B were not hydrolyzed and F1, F2 and F3A were determined to be ε-rhodomycinone[1], 7-deoxydihydrodaunomycinone[2] and dihydrodaunomycinone[2], respectively, by comparison with their authentic samples. Mass spectroscopy of F3B gave the same M^+ value as an authentic sample of dihydrodaunomycinone but F3B was differentiated from the latter by thin-layer chromatography. F3B was suggested by PMR and CD spectrum[3] to be the 7-epimer of dihydrodaunomycinone. Acid hydrolyses of F7A, F7B, F4B, F5A, F5B, F6A and F6B yielded the aglycone identical with daunomycinone[4] by the IR, ultraviolet and visible light absorption spectra, Rf value on silica gel thin-layer chromatography, the fragment ion peaks of the mass spectra and PMR, CMR and CD spectra. Thinlayer chromatography of the acid hydrolysates indicated that F4B, F5A, F5B, F6A and F6B contained daunosamine[5]. An unknown amino sugar was detected in the acid hydrolysates of F7B and F4C. On mild hydrolyses in 1% H_2SO_4 at 32°C for 30 minutes, F4B, F5A, F5B, F6A and F6B gave a spot corresponding to daunomycin, while F7A, F7B, and F4C were resistant to this hydrolysis. F6A and F7A were found to be identical with daunomycin and N-acetyldaunomycin[6], respectively, by the direct comparison with their authentic samples. F4B, F4C, F5A, F5B, F6B and F7B are new daunomycin analogues and have the following properties:

F4B: m.p. 182~185°C; $[\alpha]_D^{23}+150°$ (*c* 0.1, $CHCl_3$): anal. calcd. for $C_{34}H_{43}NO_{13}$: C 60.61, H 6.43, N 2.08, O 30.88; found: C 59.85, H 6.65, N 2.04, O 29.83. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 234.5 (452), 252.5 (327), 289 (120), 478 (127), 497 (128), 532sh (76). $\lambda_{max}^{MeOH-0.1NHCl}$ nm ($E_{1cm}^{1\%}$): 234.5 (459), 252.5 (326), 289 (121), 479 (133), 497 (133), 532sh (78). $\lambda_{max}^{MeOH-0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$): 250.5 (416), 350 (83), 558 (164), 597 (152).

F5A: m.p. 185~189°C; $[\alpha]_D^{23}+135°$ (*c* 0.1, $CHCl_3$): anal. calcd. for $C_{34}H_{43}NO_{13}$: C 60.61, H 6.43, N 2.08, O 30.88; found C 60.27, H 6.72, N 2.31, O 29.52. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 234.5 (427), 252.5 (311), 289 (108), 478 (125), 497 (128), 532sh (84).

F5B: Red needle crystals. m.p. 181~185°C; $[\alpha]_D^{23}+170°$ (*c* 0.1, $CHCl_3$-MeOH, 1:1): anal. calcd. for $C_{34}H_{41}NO_{14}\cdot 3/2\ H_2O$: C 57.14, H 6.20, N 1.96, O 34.68; found: C 57.32, H 6.45, N 2.01. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 234.5 (552), 253 (385), 290 (132), 476 (179), 495 (181), 530 (101). $\lambda_{max}^{MeOH-0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$): 251 (453), 350 (65), 556 (206), 594 (195).

F6B: Red needle crystals. m.p. 197~201°C: $[\alpha]_D^{23}+170°$ (*c* 0.1, $CHCl_3$ - MeOH, 1:1): anal. calcd. for $C_{34}H_{41}NO_{14}\cdot 2H_2O$: C 56.43, H 6.27, N 1.94, O 35.37; found: C 56.59, H 5.96, N 1.92. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 234 (575), 252 (414), 290 (130), 478 (176), 495 (183), 530 (120), 577 (40).

F7B: m.p. 154~157°C: $[\alpha]_D^{23}+260°$ (*c* 0.1, $CHCl_3$): anal. calcd. for $C_{28}H_{29}NO_{11}$: C 60.53, H 5.26, N 2.52, O 31.68: found: C 60.53, H 5.26, N 2.52. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 234.5 (633), 252 (480), 290 (145), 478 (195), 496 (204), 531 (137), 575 (44). $\lambda_{max}^{MeOH-0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$): 251 (570), 360 (85), 557 (254), 596 (240).

F4C: Red needle crystals. m.p. 213~215°C: $[\alpha]_D^{23}+240°$ (*c* 0.1, MeOH): anal. calcd. for $C_{28}H_{31}NO_{11}\cdot 3/2H_2O$: C 57.52, H 5.86, N 2.40, O 34.21: found: C 57.38, H 6.16, N 2.29. λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (510), 252.5 (422), 291 (125), 478 (161), 496 (172), 531 (124), 575 (44). $\lambda_{max}^{MeOH-0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$): 251.5 (502), 360 (80), 556 (217), 594 (203).

These new products were named as follows: F4B = baumycin A1; F5A = baumycin A2; F5B = baumycin B1; F6B = baumycin B2; F7B = baumycin C1; F4C = baumycin C2.

Table 2. Antimicrobial spectrum of baumycins, daunomycin and N-acetyldaunomycin

Organisms	Minimum inhibitory concentrations (mcg/ml)							
	DM	N-Acetyl DM	Baumycin					
			A1	A2	B1	B2	C1	C2
Staphylococcus aureus FDA209P	6.25	6.25	1.56	3.12	50	100	12.5	25
Staphylococcus aureus Smith	3.12	3.12	0.78	1.56	50	50	3.12	6.25
Bacillus subtilis ATCC6633	6.25	12.5	0.78	3.12	100	>100	3.12	6.25
Bacillus cereus ATCC9634	6.25	3.12	1.56	3.12	100	>100	1.56	12.5
Bacillus megaterium NRRLB-938	12.5	6.25	1.56	6.25	100	>100	3.12	25
Sarcina lutea ATCC9341	1.56	3.12	0.78	3.12	50	25	1.56	6.25
Micrococcus flavus	3.12	1.56	0.78	1.56	50	50	1.56	12.5
Corynebacterium bovis 1810	12.5	1.56	1.56	6.25	50	50	1.56	12.5
Pseudomonas fluorescens NIHJB-254	—	100	>100	100	100	>100	100	>100
Proteus morganii	—	>100	>100	>100	>100	>100	>100	>100
Mycobacterium smegmatis ATCC607	12.5	25	6.25	12.5	100	>100	100	100
Candida albicans IAM4905	—	100	100	100	100	>100	100	>100
Candida tropicalis IAM4942	—	100	100	100	100	>100	>100	>100

Broth dilution method. DM: Daunomycin

The structures of these compounds will be reported in the accompanying paper.

Antimicrobial activities of baumycins described above have been tested by the broth dilution method and the results are shown in Table 2. Baumycins A1, A2, C1 and C2 showed a strong inhibition against Gram-positive bacteria, while baumycins B1 and B2 showed only a weak activity. Baumycins A1 and A2 showed a strong inhibition against the growth and syntheses of RNA and DNA of cultured L1210 cells: RNA synthesis inhibition (%) at 0.5 μg/ml, A1, 64.7; A2, 60.6; B1, 17.6; B2, 14.3: DNA synthesis inhibition (%) at 1 μg/ml, A1, 69.6; A2, 40.4; B1, 25.0; B2, 6.7.

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