ISOLATION AND IDENTIFICATION OF NEW ANTHRACYCLINE ANTIBIOTICS, RUBOMYCINS F AND H

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The cultivation of *Streptomyces coeruleorubidus* produced a complex of anthracycline antibiotics, the main component being the antitumor antibiotic daunorubicin (rubomycin C)[1,2]. The complex was isolated from the culture broth by extraction with chloroform. The fifteen antibiotics of the complex were separated by Kieselgel 60 column chromatography developed with chloroform - MeOH, the concentration of MeOH being gradually increased from 1% to 20% (v/v), and by preparative silica gel TLC developed with chloroform - benzene - MeOH (20:2:3). Seven of them were identified using NMR and HPLC methods as known anthracycline antibiotic (daunomycinone, daunorubicin, bisanhydrodaunomycinone, 7-deoxy-13-dihydrodaunomycin, baumycins A and C and 13-dihydrodaunomycinone[3,4]). Moreover, two new anthracycline antibiotics were isolated (Table 1) by activity against Gram-positive bacteria (Table 2). These antibiotics were named rubomycins F and H.

Table 1. Rf values of antibiotics in TLC*.

Compounds	Solvent system		
	(I)	(II)	(III)
Rubomycin F	0.62	0.72	0.88
Rubomycin H	0.57	0.54	0.81
Daunorubicin	0.11	0.06	0.16
Daunomycinone	0.71	0.81	0.92

* On Kieselgel 60, 0.2 mm thickness (Merck).
Solvent systems: (I) chloroform - benzene - MeOH, 20:2:3 (in volume); (II) chloroform - benzene - MeOH, 20:1:2 (in volume); (III) chloroform-MeOH, 10:1 (in volume).

Table 2. Antimicrobial activity of rubomycins F and H.

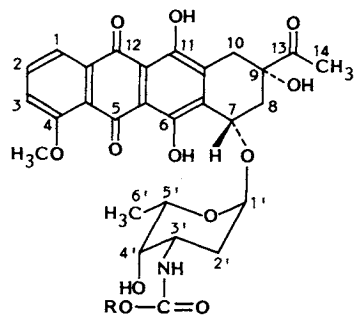
Test organism	MIC (μg/ml)	
	Rubomycin F	Rubomycin H
Bacillus subtilis ATTC 6633	8.30	4.15
Staphylococcus aureus ATCC 21027	16.00	4.20
Micrococcus luteus ATCC 9341	4.20	0.78
Escherichia coli ATCC 25922	33.03	16.60

Physico-chemical properties of rubomycins F and H are as follows: Rubomycin F: MP 145~148°C (dec); UV λ_{max}^{EtOH} nm ($E_{1cm}^{1\%}$) 482 (153), 495 (172), 534 (68); IR (KBr) cm^{-1} 3400, 1720, 1625, 1558; FD-MS *m/z* 600 $(M+H)^+$ (calcd for $C_{30}H_{34}NO_{12}$:

Table 3. ^1H NMR chemical shift data for the anthracyclines at 400 MHz and 100 MHz* in $CDCl_3$, δ in ppm.

Proton	Daunorubicin	Rubomycin F (1)	Rubomycin H (2)*
1-H	7.94 d	8.01 d	7.98 d
2-H	7.72 dd	7.78 dd	7.72 dd
3-H	7.33 d	7.41 d	7.34 d
7-H	5.20 m	5.26 m	5.22 m
8-H_2	2.30 br d	2.32 br d	2.10~2.35 m
10-H_2	3.14 d, 2.84 d	3.21 d, 2.90 d	AB-system, 3.04
14-H_3	2.40 s	2.41 s	2.44 s
15-H_3	4.04 s	4.08 s	4.07 s
1'-H	5.46 d	5.50 d	5.48
2'-H_2	1.62~1.80 m	1.72~1.90 m	1.70~1.95 m
3'-H	3.08	3.88	3.90
4'-H	3.45 br s	3.68 br s	3.66 br s
5'-H	4.08 q	4.21 q	4.20 q
6'-H_3	1.31 d	1.29 d	1.32 d
7'-H_2		4.04 q	
8'-H_3		1.19 t	
9'-H_3			3.61 s
NH		5.06 d	5.12 d

Fig. 1. Structure of rubomycins F (1) and H (2).



Rubomycin F (1) R = $\overset{7'}{C}H_2\overset{8'}{C}H_3$
Rubomycin H (2) R = $\overset{9'}{C}H_3$

600). Rubomycin H: MP 163~166°C (dec); UV λ_{max}^{EtOH} nm ($E_{1cm}^{1\%}$) 482 (162), 495 (182), 534 (72); IR (KBr) cm^{-1} 3450, 2860, 1625, 1585; FD-MS *m/z* 586 $(M+H)^+$ (calcd for $C_{29}H_{32}NO_{12}$: 586).

Acid hydrolysis (0.1 N HCl, 30 minutes at temperature 98°C) of rubomycins F and H gave daunomycinone (FD-MS) *m/z* 399 $(M+H)^+$ (calcd for $C_{21}H_{19}O_8$: 399). The ^1H NMR spectra of rubomycins F and H are presented in Table 3.

The results obtained made it possible to suggest for rubomycins F and H the structures **1** and **2**, respectively. The structure of rubomycin H was confirmed by its synthesis from daunorubicin and methyl chloroformate mixed with triethylamine and dioxane. Rf values of the semisynthetic antibiotic (3'-*N*-carbomethoxyrubomycin C) in several TLC systems identical to those of natural rubomycin H. The identical compound was prepared previously by YAMAMOTO *et al.*[5].

Thus, rubomycins F and H are new representatives of natural anthracycline antibiotics.

References

1) BRAZHNIKOVA, M. G.; N. V. KONSTANTINOVA, V. A. POMAZKOVA & B. V. ZAKHAROV: Physico-chemical properties of antibiotic rubomycin produced by *Streptomyces coeruleorubidus*. Antibiotiki 11: 763~767, 1966

2) ARCAMONE, F.; G. CASSINELLI, G. FRANCESCHI & P. OREZZI: The total absolute configuration of daunomycin. Tetrahedron Lett. 1968: 3353~3356, 1968

3) KOMIYAMA, T.; T. MATSUZAWA, T. OKI, T. INUI, Y. TAKAHASHI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Baumycins, new antitumor antibiotics related to daunomycin. J. Antibiotics 30: 619~621, 1977

4) TAKAHASHI, Y.; H. NAGANAWA, T. TAKEUCHI, H. UMEZAWA, T. KOMIYAMA, T. OKI & T. INUI: The structure of baumycins A1, A2, B1, B2, C1 and C2. J. Antibiotics 30: 622~624, 1977

5) YAMAMOTO, K.; E. M. ACTON & D. W. HENRY: Antitumor activity of some derivatives of daunorubicin at the amino and methyl ketone functions. J. Med. Chem. 15: 872~875, 1972