DESAMINOANTHRACYCLINES FROM THE ANTIBIOTIC COMPLEX CICLAMYCIN

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Ciclamycin, an antibiotic complex produced by *Streptomyces capoamus*[1], showed highly inhibitory activity against experimental tumors[2]. Pharmacological studies[3] and recent clinical results[4] proved its value for treatment of human neoplasias. By early investigations on chemical composition[5,6] η- and ε-pyrromycinone[7] were identified as aglycone constituents. Further work on the glycosidic part of the complex[8] showed that three major components, ciclamycins 1, 5 and 6 are identical with cinerubine B[9], cinerubine A[10] and pyrromycin[11], respectively, and that one of the minor components, ciclamycin 2, is 5‴-*epi*-cinerubine B (Fig. 1). This report describes the structure elucidation of two more components of the ciclamycin complex.

On column chromatography of the crude complex, a glycosidic compound, named ciclamycin 0, was eluted before ciclamycin 1 (=cinerubine B) in 1.7% yield and crystallized from MeOH as red needles having mp of 165~167°C.

Acidic hydrolysis of this product produced ε-pyrromycinone[7], identified by comparison with an authentic sample, and two different deoxysugars, 2-deoxyfucose and cinerulose A, as evidenced by TLC analysis, but no aminosugar was observed. Comparison of the ^1H and ^{13}C NMR spectra of ciclamycin 0 with those of ciclamycins 1, 2 and 5[8] confirmed the absence of a dimethylamino group. On the other hand, these spectra indicated clearly the presence of three molecules of deoxysugar. Detailed analysis of the 400 MHz ^1H NMR spectrum by multiple decoupling experiments revealed a composition of two molecules of 2-deoxyfucose and one of cinerulose A. Finally, fast atom bombardment mass spectra (FAB-MS) confirmed these results by strong $(M+H)^+$ and $(M+Na)^+$ peaks at *m/z* 801 and 823, respectively. The presence of fragmentation ions corresponding to the loss of one molecule of cinerulose A, $(M+H-112)^+$ at *m/z* 689, proved the terminal position of this sugar. Based on these chemical and spectroscopic findings, ciclamycin 0 should have the structure as proposed in Fig. 2.

Ciclamycin 4, the compound eluted before ciclamycin 5 (=cinerubine A), was obtained in 2.2% yield and crystallized from EtOH - EtOAc as red amorphous powder, mp at 143~145°C. On acidic hydrolysis, it produced the same aglycone as the other ciclamycins. In the aqueous layer of the hydrolysate 2-deoxyfucose and rhodinose were detected by TLC, but no aminosugar was found. High field ^1H NMR spectra (Table 1) showed clearly the presence of two molecules of rhodinose and one of deoxyfucose. FAB-MS produced strong $(M+H)^+$ and $(M+Na)^+$ peaks at *m/z* 787 and 809, respectively, and fragment ions corresponding to the loss of one and of two molecules of rhodinose, proving the terminal position of these molecules in the trisaccharide unit. Thus, the structure of ciclamycin 4 can be deduced as shown in Fig. 2.

Most natural anthracyclines are glycosidated by at least one aminosugar. The first natural nitrogen-free analogues were the monosaccharidic steffimycins[12]. Somewhat later, OKI and co-workers isolated from a mutant strain of *Streptomyces galilaeus* five aklavinone glycosides which bear no aminosugar[13,14]. Two of them, called U5 and U6, have the same trisaccharide chains as ciclamycins 0 and 4 described here, their only difference being a hydroxyl group less in position 1 of the aglycone.

Fig. 1. Structures of ciclamycins 1, 2, 5 and 6.

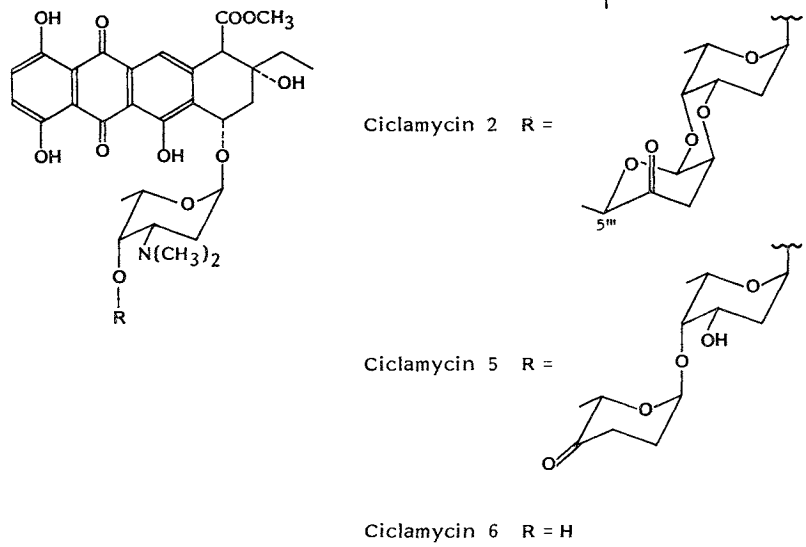
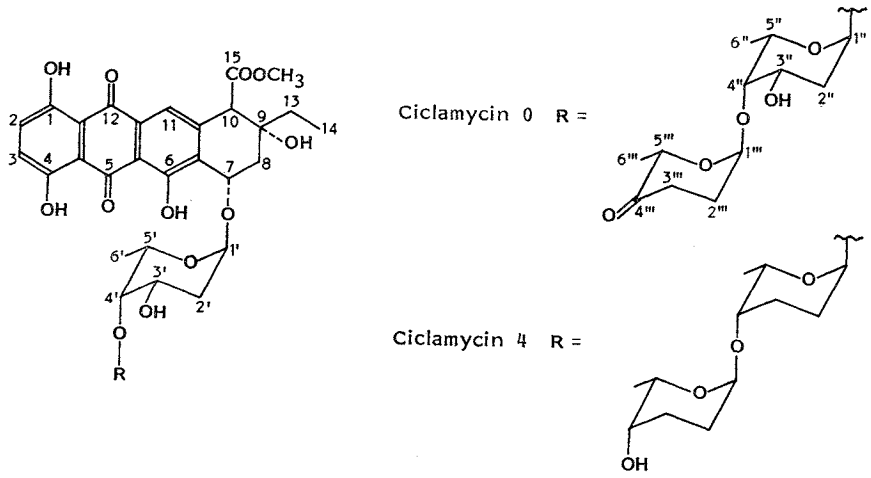


Fig. 2. Structures of ciclamycins 0 and 4.



Some of these compounds were also obtained by microbial glycosidation of aklavinone using blocked mutants[15].

In conclusion, ciclamycins 0 and 4 represent the first analogues of desaminoanthracyclines of the pyrromycinone group. Preliminary toxicologic evaluation indicates a much lower acute toxicity in comparison with traditional anthracyclines (unpublished results). Present work in our laboratories aims to increase the production of these components in order to evaluate their pharmacological properties, especially their antitumor activity.

Table 1. ^{1}H NMR chemical shift assignments of ciclamycins 0 and 4 (after H/D exchange).

Ciclamycin 0			Ciclamycin 4		
Position	δ (ppm)	J (Hz)	Position	δ (ppm)	J (Hz)
14	1.08 t	7.5	14	1.07 t	7.2
6″	1.24 d	6.8	6′	1.16 d	6.5
6′	1.30 d	6.8	6″	1.19 d	6.5
6‴	1.33 d	6.8	6‴	1.21 d	6.5
13a	1.51 m		13a	1.52 m	
13b	1.75 m		2′$_{ax}$	1.55 d	13.5
2′$_{ax}$	1.79 td	13.0/4.0	2″$_{ax}$		
2″$_{ax}$	1.92 td	13.0/4.0	2‴$_{ax,eq}$	1.65～1.85 m	
2′$_{eq}$	1.95 dd	13.0/5.0	13b		
2″$_{eq}$	2.08 dd	13.0/5.0	3″$_{ax,eq}$		
2‴$_{ax}$	2.16 m		2′$_{eq}$		
8$_{ax}$	2.30 d	15.0	2″$_{eq}$	1.90～2.10 m	
2‴$_{eq}$	2.41～2.50 m		3‴$_{ax,eq}$		
3‴$_{ax,eq}$			8$_{ax}$	2.34 d	15.0
8$_{eq}$	2.53 dd	15.0/4.2	8$_{eq}$	2.50 dd	15.0/4.0
4′	3.59 s		4‴	3.48 s	
OCH_3	3.70 s		4′	3.57 s	
4″	3.74 s		4″	3.65 s	
3′	3.78 m		OCH_3	3.68 s	
10	4.11 s		3′	3.98 m	
3″	4.14 m		5′	3.99 q	6.5
5′	4.15 q	6.8	5‴	4.08 q	6.5
5″	4.23 q	6.8	10	4.10 s	
5‴	4.49 q	6.8	5″	4.19 q	6.5
1″	4.98 d	3.5	1″	4.84 s	
1‴	5.09 t	6.0	1‴	4.93 d	3.0
7	5.24 dd	4.2/1.5	7	5.27 d	2.3
1′	5.49 d	4.0	1′	5.40 d	2.0
2	7.26 d	9.5	2	7.25 d	9.5
3	7.29 d	9.5	3	7.28 d	9.5
11	7.69 s		11	7.68 s	

Table 2. ^{13}C NMR chemical shift assignments of ciclamycins 0 and 4.

Position	Ciclamycin 0	Ciclamycin 4	Position	Ciclamycin 0	Ciclamycin 4
1	157.79	157.70	15	171.23	171.19
2	129.68	129.62	OCH_3	52.51	52.42
3	130.06	129.94	1′	102.13	101.21
4	158.39	158.28	2′	34.34	34.32
4a	112.33	112.30	3′	65.48	65.54
5	190.52	190.54	4′	82.87	82.97
5a	114.79	114.71	5′	67.86	68.10
6	162.20	162.13	6′	17.03	17.06
6a	132.82	132.69	1″	101.07	100.22
7	71.08	70.52	2″	34.15	25.42
8	33.82	33.64	3″	64.92	24.28
9	71.58	71.57	4″	82.01	77.73
10	57.10	57.13	5″	67.43	67.28
10a	142.40	142.40	6″	16.99	17.00
11	120.40	120.31	1‴	100.17	100.18
11a	131.19	131.48	2‴	27.52	24.28
12	185.68	185.72	3‴	33.40	23.91
12a	112.51	112.47	4‴	209.83	76.44
13	32.07	32.08	5‴	71.86	66.92
14	6.67	6.60	6‴	14.75	16.83

Experimental

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 400 spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. FAB-MS were obtained by bombarding glycerol solutions of ciclamycins with 4~6 keV xenon atoms. Acidic hydrolysis and TLC analysis of the sugars were carried out according to OKI and co-workers[16].

Ciclamycin 0: 10 g of crude ciclamycin complex were fractionated by column chromatography (300 g of Silica gel 60). After elution with $CHCl_3$ containing 0.5% of MeOH, 165 mg (1.7%) of ciclamycin 0 were crystallized from MeOH: MP 165~167°C; FAB-MS *m/z* 823 (M+Na), 801 (M+H), 711 (M+Na−112), 689 (M+H−112).

Ciclamycin 4: After elution of the column with $CHCl_3$ containing 1.5% of MeOH, 217 mg (2.2%) of ciclamycin 4 were crystallized from EtOH - EtOAc: MP 144~145°C; FAB-MS *m/z* 809 (M+Na), 787 (M+H), 679 (M+Na−130), 657 (M+H−130), 527 (M+H−260).

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