NEW ANTHRACYCLINE DERIVATIVES FROM BETACLAMYCIN A

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We have been studying the preparation of new anthracyclines by fermentation, chemical synthesis or microbial glycosidation[1~4]. Recently we isolated a potent new antitumor anthracycline betaclamycin A (newly named CG7[2]) obtained by feeding β-rhodomycinone to the growing culture of an aclacinomycin-negative mutant strain KE303 derived from *Streptomyces galilaeus* MA144-M1. This compound showed excellent antitumor activity against L1210 leukemia with a T/C % of over 200. In an attempt to produce derivatives with improved therapeutic properties we chemically hydrolyzed and reduced betaclamycin A. We now report four derivatives thus obtained: betaclamycins M, N, S and T.

Reduction of the carbonyl group of L-cinerulose of betaclamycin A (**I**) with sodium borohydride gave betaclamycins M (**II**) and N (**III**), which had L-amicetose or L-rhodinose respectively as the terminal sugar. To a solution of 1 g of **I** in 300 ml of $CHCl_3$ and 45 ml of EtOH was added 100 mg of sodium borohydride and the mixture was stirred at 30°C for 15 minutes. Then 100 ml of $CHCl_3$ and 100 ml of distilled H_2O were added and the layers were separated. The $CHCl_3$ layer was washed with 10^{-2} M EDTA aq solution and then H_2O, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was subjected to preparative layer chromatography (PLC) on silica gel (PF_{254}, E. Merck Co.) using $CHCl_3$ - MeOH (10: 1) as eluant. The major bands at Rf 0.37 and 0.32 corresponded to **II** and **III** respectively. They were separately scraped off and extracted with $CHCl_3$ - MeOH - NH_4OH (100: 15: 0.2) mixture. An excess of *n*-hexane was added to the concentrated extract to form a red precipitate. In this way 220 mg of pure **II** and 40 mg of pure **III** were obtained. The physico-chemical properties of **II** and **III** are as follows:

II: mp 168~170°C; IR (KBr) 1600 cm^{-1}; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 497 (175); *Anal* Calcd for $C_{40}H_{53}NO_{15}$ (MW 787.9): C 60.98, H 6.78, N 1.78; Found: C 60.95, H 6.81, N 1.76%.

III: mp 170~172°C; IR (KBr) 1600 cm^{-1}; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 495 (187); *Anal* Calcd for $C_{40}H_{53}NO_{15}$ (MW 787.9): C 60.98, H 6.78, N 1.78; Found: C 60.94, H 6.80, N 1.77%.

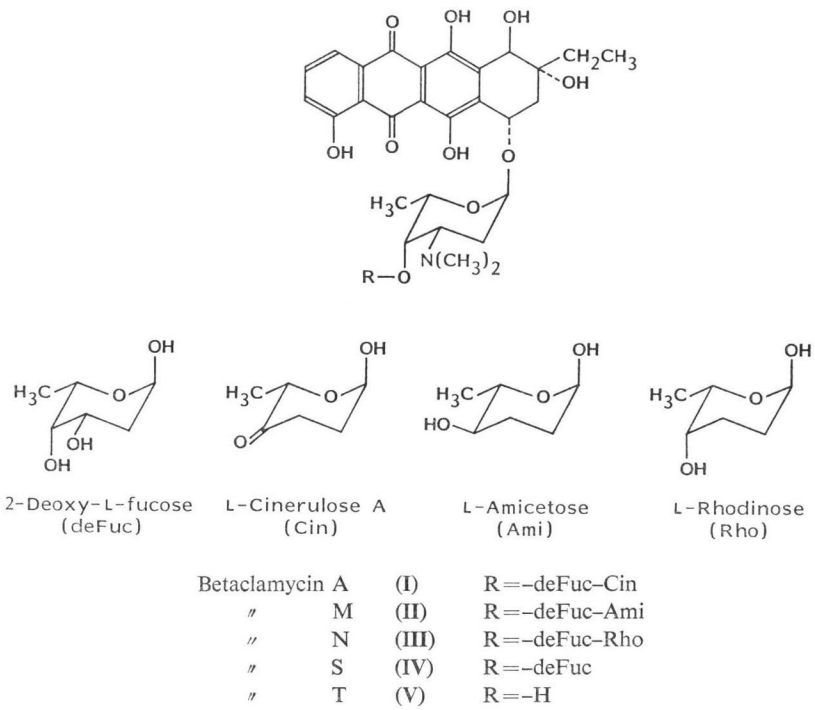
III (200 mg) was partially hydrolyzed by stirring for 2 hours at 30°C in 200 ml of 0.05 N HCl. The solution was neutralized with saturated sodium bicarbonate solution and extracted with $CHCl_3$ (4×200 ml). The combined extracts was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was subjected to preparative TLC on silica gel using $CHCl_3$ - MeOH - NH_4OH (100: 15: 0.2) as eluant. Bands corresponding to betaclamycin S (**IV**) (Rf 0.4) and betaclamycin T (**V**) (Rf 0.24) were scraped off and extracted separately with the eluting solvent. After concentration of the extract to a small volume, the pure pigments were obtained as a red powder by precipitation with *n*-hexane. The yields were 50 mg of **IV** and 27 mg of **V**. Their physico-chemical properties are as follows:

IV: mp 170~172°C; IR (KBr) 1600 cm^{-1}; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 496 (206); *Anal* Calcd for $C_{34}H_{43}NO_{13}$ (MW 673.7): C 60.62, H 6.43, N 2.08; Found: C 60.60, H 6.45, N 2.10%.

V: mp 155~158°C; IR (KBr) 1600 cm^{-1}; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 496 (211).

Acid hydrolysis of derivatives **II**, **III**, **IV** and **V** in 0.1 N HCl at 85°C for 30 minutes gave the same aglycone, which was identified as β-rhodomycinone by direct comparison with ^1H NMR, IR, MS and melting point with those of the authentic sample. The sugar moieties were found to be composed of rhodosamine, 2-deoxyfucose, amicetose and rhodinose using the silica gel thin-layer method as done with the acid hydrolysate of aclacinomycins[5]. **II** consisted of β-rhodomycinone and trisaccharide and yielded 7-*O*-rhodosaminyl-β-rhodomycinone (**V**) and a methyl glycoside on partial methanolysis. The spectral properties and behavior on TLC of the methyl glycoside obtained from **II** completely agreed

Fig. 1. Structure of betaclamycin derivatives.



Betaclamycin	A	(I)	R=-deFuc-Cin
〃	M	(II)	R=-deFuc-Ami
〃	N	(III)	R=-deFuc-Rho
〃	S	(IV)	R=-deFuc
〃	T	(V)	R=-H

Table 1. Effect of betaclamycin derivatives on growth and macromolecular biosyntheses of cultured L1210 leukemia cells.

Compound	IC_{50} (nM)			Ratio $\frac{IC_{50}\ DNA}{IC_{50}\ RNA}$
	Cytotoxicity	DNA synthesis	RNA synthesis	
Betaclamycin M	36	660	127	5.2
〃 N	20	685	114	6.0
〃 S	15	534	133	4.0
〃 T	18	386	92	4.2
〃 A	13	573	89	6.4

Cytotoxicity: L1210 cells (4×10^4 cells/ml) were cultured in RPMI1640 medium containing 20% calf serum with test compound at 37°C under 5% CO_2 - 95% air atmosphere for 2 days.

Macromolecular biosynthesis: After preincubation of L1210 cells (5×10^5 cells/ml) with test compound at 37°C for 15 minutes, 2-[^{14}C]thymidine or -uridine was added at 0.05 μCi/ml, and incubated for 60 minutes at 37°C. The radioactivity in the acid insoluble fraction was counted with a Aloka LSC-900 liquid scintillation spectrometer in BRAY's cocktail.

with those of methyl-L-amicetosyl-2-deoxy-L-fucoside obtained from MA144-M1[5]. **III** gave **IV** and rhodinose after mild hydrolysis in 0.5% HCl at 25°C for 10 minutes. **IV** gave rhodosaminyl-β-rhodomycinone (V) and a methyl-2-deoxy-L-fucoside by subsequent methanolysis. Thus, the structures of betaclamycins M, N, S and T are as shown in Fig. 1. Betaclamycin T is identical to rhodomycin B[6].

As shown in Table 1, the three new betaclamycin derivatives showed marked cytotoxicities against cultured L1210 leukemia cells and inhibited DNA and RNA syntheses to the same extent as did betaclamycin A.

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