REDUCTION AND HYDROLYSIS OF 2-HYDROXYACLACINOMYCIN A

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(Received for publication July 17, 1981)

In studies on the microbial modification of anthracycline antibiotics[1~4], we have produced a new potent antitumor anthracycline 2-hydroxyaclacinomycin A by the glycosidation of 2-hydroxyaklavinone fed to the growing culture of an aclacinomycin-negative mutant strain KE303 obtained from *Streptomyces galilaeus* MA144-M1. The present paper describes four derivatives; 2-hydroxyaclacinomycins M, N, S, and T derived by the reduction and hydrolysis of 2-hydroxyaclacinomycin A.

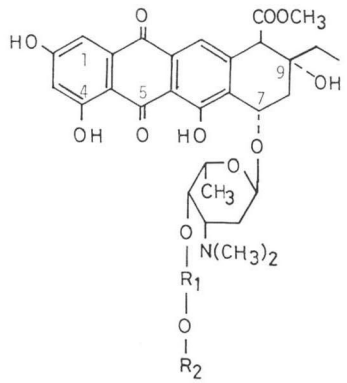
The reduction of the carbonyl group of L-cinerulose of 2-hydroxyaclacinomycin A (**I**) with 40 mg of sodium borohydride gave 2-hydroxyaclacinomycins M(**II**) and N(**III**), each of which had L-amicetose or L-rhodinose as a terminal sugar. To a solution of 400 mg of **I** in 120 ml of chloroform and 18 ml of ethanol was added a solution of 40 mg of sodium borohydride in 5 ml of ethanol, and the mixture was stirred for 45 minutes at room temperature and thereafter shaken with 100 ml of chloroform and 150 ml of distilled water. The chloroform layer was washed with 10^{-2} M EDTA and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue (400 mg) was dissolved in a small amount of chloroform and subjected to preparative layer chromatography of silica gel (PF_{254}, E. Merck Co.) using chloroform - methanol (100 : 15, v/v) mixture. The major bands corresponding to **II** and **III** showing Rf 0.29 and 0.21, respectively, were separately scraped off and extracted with chloroform - methanol - aqueous ammonia (100: 15: 0.2, v/v/v) mixture, and the extracts were concentrated under reduced pressure. To the concentrate was added excess *n*-hexane to form dark yellow precipitate, and 230 mg of pure **II** and 76 mg of pure **III** were obtained by filtration and drying of the precipitate *in vacuo*.

The physicochemical properties of **II** and **III** are as follows: **II**: m.p. 168~170°C; $[\alpha]_D^{23}$ +123° (*c* 0.04, MeOH); IR (KBr): 1730, 1670, 1620, 1250, 1010, 1000 cm^{-1}; $\lambda_{max}^{90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 222 (390), 256 (235), 294 (210), 440 (116), 525s (38); *Anal.* Calcd. for $C_{42}N_{55}NO_{16}$ (m.w. 829.9): C 60.79, H 6.68, N 1.69; Found: C 60.34, H 6.87, N 1.63 %. **III**: m.p. 167~169°C; $[\alpha]_D^{23}$ +103° (*c* 0.04, MeOH); IR (KBr): 1735, 1675, 1620, 1250, 1000 cm^{-1}; $\lambda_{max}^{90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 223 (381), 256 (227), 294 (204), 440 (113), 520s (37); *Anal.* Calcd. for $C_{42}H_{55}NO_{16}$ (m.w. 829.9): C 60.79, H 6.68, N 1.69; Found: C 60.30, H 6.85, N 1.71 %.

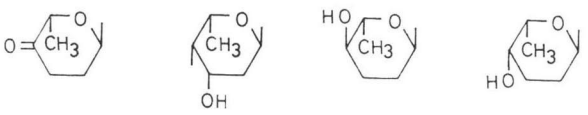
2-Hydroxyaclacinomycin M (**II**) was partially hydrolyzed in diluted acid to yield 2-hydroxyaclacinomycins S (**IV**) and T (**V**). One hundred seventy milligrams of **II** were dissolved in 200 ml of 0.05 N HCl, allowed to stand for 5 hours at room temperature, neutralized with 0.1 N NaOH at pH 7.5, and extracted four times with 200 ml of chloroform. The chloroform extract was dried over anhydrous sodium sulfate and concentrated to dryness. The residue (160 mg) was dissolved in a small amount of chloroform and subjected to preparative layer chromatography of silica gel using chloroform - methanol (100: 15, v/v) mixture. Bands corresponding to **IV** showing Rf 0.09 and **V** showing Rf 0.05 were scraped off, and extracted separately with chloroform - methanol - aqueous ammonia (100: 15: 0.2, v/v/v). After concentrated the extract to a small volume, the pure pigments were obtained as dark yellow powders by precipitating with *n*-hexane. These yielded as 46 mg of **IV** and 7 mg of **V**, and their physicochemical properties are as follows: **IV**: m.p. 170~172°C; $[\alpha]_D^{23}$ +140° (*c* 0.04, MeOH); IR (KBr): 1735, 1675, 1620, 1255, 1010, 995 cm^{-1}; $\lambda_{max}^{90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 222 (423), 256 (270), 295 (233), 450 (125), 510s (65); *Anal.* Calcd. for $C_{36}H_{45}NO_{14}$ (m.w. 715.7): C 60.41, H 6.34, N 1.96; Found: C 60.28, H 6.51, N 1.93 %. **V**: m.p. 169~171°C; $[\alpha]_D^{23}$ +299° (*c* 0.01, MeOH); IR (KBr): 1730, 1670, 1620, 1250, 1010, 980 cm^{-1}; $\lambda_{max}^{90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 222 (515), 256 (330), 297 (278), 455 (148), 510s (90); *Anal.* Calcd. for $C_{30}H_{35}NO_{11}$ (m.w. 585.6): C 61.53, H 6.02, N 2.39; Found C 61.14, H 6.41, N 2.32 %.

Total hydrolysis of four derivatives; **II**, **III**, **IV** and **V** in 0.1 N hydrochloric acid at 85°C for 30 minutes gave the same aglycone, which was identified to be 2-hydroxyaklavinone[5] by direct comparison with PMR, IR and mass spectra and its melting point of the authentic sample, and sugar moieties, which were identified to be com-

Fig. 1. Structure of 2-hydroxyaclacinomycin derivatives.



	R_1	R_2
I: 2-Hydroxyaclacinomycin A	2-Deoxyfucose	Cinerulose A
II: 2-Hydroxyaclacinomycin M	2-Deoxyfucose	Amicetose
III: 2-Hydroxyaclacinomycin N	2-Deoxyfucose	Rhodinose
IV: 2-Hydroxyaclacinomycin S	2-Deoxyfucose	H
V: 2-Hydroxyaclacinomycin T	H	-



L-Cinerulose A 2-Deoxy-L-fucose L-Amicetose L-Rhodinose

posed of rhodosamine, 2-deoxyfucose, amicetose and rhodinose on silica gel thin-layer as detected in acid hydrolysate of aclacinomycins[6]. The structure of **II** is similar to 2-hydroxyaclacinomycin A[4] consisting of 2-hydroxyaklavinone and trisaccharide. **II** yielded rhodosaminyl-2-hydroxyaklavinone (**V**) and methyl glycoside on partial methanolysis. The spectral properties and behavior on TLC of the methyl glycoside obtained from **II** completely agreed with those of methyl-L-amicetosyl-2-deoxy-L-fucoside from MA144 M1[6]. **III** gave **IV** and rhodinose by mild hydrolysis in 0.5 % HCl for 10 minutes at room temperature, and **IV** gave rhodosaminyl-2-hydroxyaklavinone (**V**) and methy 2-deoxy-fucoside by methanolysis. Thus, the structures of these derivatives of 2-hydroxyaclacinomycin A are as shown in Fig. 1.

Among 2-hydroxyaclacinomycin derivatives, **II** and **III** having trisaccharide moiety as well as 2-hydroxyaclacinomycin A showed a marked cytotoxicity against cultured L1210 leukemia cells and inhibited preferentially RNA synthesis, as shown in Table 1. However, **IV** and **V** having di- or monosaccharide moiety were less active. The IC_{50} values for RNA synthesis of **II** and **III** were almost equal to that of 2-hydroxyaclacinomycin A, but **IV** and **V** showed about 10 times higher IC_{50} value for DNA and RNA syntheses than trisaccharide derivatives.

Table 1. Effect of 2-hydroxyaclacinomycin derivatives on the growth and macromolecular biosynthesis of cultured L1210 leukemia cells

Compound	IC_{50}(μg/ml)			Ratio
	Cyto-toxicity	DNA synthesis	RNA synthesis	IC_{50} DNA / IC_{50} RNA
2-Hydroxy-aclacino-mycin M	0.04	1.2	0.1	12.0
〃 N	0.04	1.2	0.18	6.6
〃 S	0.24	5.0	1.3	3.8
〃 T	0.20	2.6	1.0	2.6
〃 A	0.04	0.9	0.14	6.4

Cytotoxicity: L1210 cells (4×10^4 cells/ml) were cultured in RPMI 1640 medium containing 20 % calf serum with test compound (0.01～0.5 μg/ml) at 37°C under 5 % CO_2-95 % air atmosphere. Cytotoxicity was expressed as IC_{50} of the control growth on day 2.

Macromolecular biosynthesis: After preincubation of L1210 cells (5×10^5 cells/ml) with test compound (0.01～2.5 μg/ml) at 37°C for 15 minutes, 2-^{14}C-thymidine or -uridine was added with 0.05 μCi/ml, and incubated for 60 minutes at 37°C. Uptake of precursor was terminated by rapid chilling and adding 1 ml of cold 10 % TCA to 1 ml of the incubation mixture. The precipitate was washed twice with 2 ml of cold 5 % TCA, and dissolved in 0.25 ml of 99 % formic acid. The radioactivity was counted with a Aloka LSC-653 liquid scintillation spectrometer in BRAYS' scintillator. IC_{50} values were estimated by probit analysis.

Acknowledgments

The authors wish to thank Drs. H. UMEZAWA and T. TAKEUCHI, Institute of Microbial Chemistry, Tokyo, for their valuable discussions.

References

1) YOSHIMOTO, A.; Y. MATSUZAWA, T. OKI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Microbial conversion of ε-pyrromycinone and ε-isorhodomycinone to 1-hydroxy-13-dihydrodaunomycin and N-formyl-1-hydroxy-13-dihydrodaunomycin and their bioactivities. J. Antibiotics 33: 1150~1157, 1980
2) OKI, T.; A. YOSHIMOTO, Y. MATSUZAWA, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of anthracycline antibiotics by *Streptomyces galilaeus*. I. Glycosidation of various anthracyclinones by an aclacinomycin-negative mutant and biosynthesis of aclacinomycins from aklavinone. J. Antibiotics 33: 1331~1340, 1980
3) YOSHIMOTO, A.; Y. MATSUZAWA, Y. MATSUSHITA, T. OKI, T. TAKEUCHI & H. UMEZAWA: Trisarubicinol, new antitumor anthracycline antibiotic. J. Antibiotics 34: 1492~1494, 1981
4) OKI, T.; A. YOSHIMOTO, Y. MATSUZAWA, T. TAKEUCHI & H. UMEZAWA: New anthracycline antibiotic, 2-hydroxyaclacinomycin A. J. Antibiotics 34: 916~918, 1981
5) MATSUZAWA, Y.; A. YOSHIMOTO, N. SHIBAMOTO, H. TOBE, T. OKI, H. NAGANAWA, T. TAKEUCHI & & H. UMEZAWA: New anthracycline metabolites from mutant strains of *Streptomyces galilaeus* MA144-M1. II. Structure of 2-hydroxyaklavinone and new aklavinone glycosides. J. Antibiotics 34: 959~964, 1981
6) OKI, T.; I. KITAMURA, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, A. YOSHIMOTO, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. II. Structural determination. J. Antibiotics 32: 801~819, 1979