PREPARATION OF 4- AND 6-*O*-METHYLACLACINOMYCIN DERIVATIVES AND THEIR ANTITUMOR ACTIVITIES*

HIROSHI TANAKA, TAKEO YOSHIOKA, YASUTAKA SHIMAUCHI, YOSHIYUKI MATSUSHITA, YASUE MATSUZAWA, TOSHIKAZU OKI and TAIJI INUI

Central Research Laboratories, Sanraku-Ocean Co., Ltd., Johnan, Fujisawa, Kanagawa, Japan

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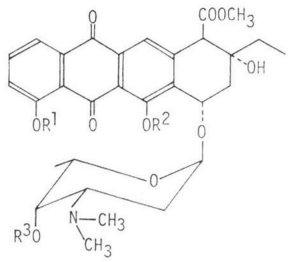
In a preceding paper, we have reported the preparation of 4-*O*-methylaclacinomycin A (**1**)[1]. Since **1** showed a remarkable antitumor activity against L1210 leukemia in mice, we prepared various 4- or 6-*O*-methylaclacinomycin derivatives (Table 1) for investigation of their structure-activity relationships.

In addition to **1**, 6-*O*-methylaclacinomycin A (**2**) was obtained as a minor component by the methylation of aclacinomycin A (ACM)[2,3] with methyl iodide - silver oxide in chloroform. A chloroform solution of methyl iodide (about 40-fold excess for ACM) was added over a 3-hour period at 15°C to a suspension of ACM and silver oxide in chloroform and the resulting mixture stirred for 1 hour. After work-up, chromatographic purification (Silica Gel 60, E. Merck: $CHCl_3$ - MeOH, 60 : 1) gave **1** and **2** in 26.2 and 6.5% yields. Properties of **2** are: mp. 144~

Table 1. Structure of 4- and 6-*O*-methylaclacinomycin derivatives.



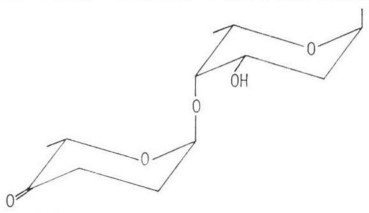
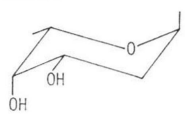
Compound	R^1	R^2	R^3
ACM	H	H	
1	CH_3	H	
2	H	CH_3	
3	CH_3	H	R^4 = OH, R^5 = H
4	CH_3	H	R^4 = H R^5 = OH
5	CH_3	H	
6	CH_3	H	H

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Table 2. Selected ^{1}H-NMR spectral data of the derivatives.

Compound	C-1H	C-2H	C-3H	C-11H	Aromatic OCH_3	Aromatic OH	$N(CH_3)_2$	C-1'H	C-1''H	C-1'''H
ACM	7.79 dd	7.65 t	7.25 dd	7.65 s	—	11.9 b 12.6 b	2.17 s	5.52 bs	5.03 bs	5.07 t
1	7.97 dd	7.72 t	7.35 dd	7.62 s	4.08 s	13.68 s	2.18 s	5.53 bs	5.03 overlap	
2	7.80 dd	7.65 t	7.31 dd	8.02 s	4.02 s	12.88 s	2.15 s	5.46 bs	5.03 overlap	
3	7.93 dd	7.71 t	7.34 dd	7.60 s	4.05 s	13.68 s	2.16 s	5.51 bs	5.01 bs	4.75 bs
4	7.98 dd	7.75 t	7.36 dd	7.64 s	4.06 s	13.65 s	2.18 s	5.51 bs	5.02 bs	4.85 bs
5	7.98 dd	7.75 t	7.37 dd	7.53 s	4.06 s	13.66 bs	2.20 s	5.52 bs	5.00 bs	—
6	7.97 dd	7.73 t	7.36 dd	7.62 s	4.06 s	13.67 bs	2.25 s	5.52 bs	—	—

Measurement at 90 MHz in $CDCl_3$. In ppm (δ) from $SiMe_4$. Abbreviations: s=singlet, d=doublet, t=triplet, b=broad.

146°C; $[\alpha]_D^{22}$ −58° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 414 nm (106); ν_{max}^{KBr} 1735, 1675, 1635 cm^{-1}; *Anal.* calcd. for $C_{43}H_{55}NO_{15}$: C 62.53, H 6.71, N 1.70; found: C 62.01, H 6.89, N 1.52. The compound **2** was produced in the beginning 3 ~ 4 hours of the reaction and degraded thereafter. The methylation for 11 hours did not give **2**, and produced **1** (in 37% yield at the best) and minor by-products: 4-*O*-methylbisanhydroaklavinone (mp. 192 ~ 196°C) and 6-*O*-methylbisanhydroaklavinone (mp. 199 ~ 201°C). The presence of the methoxy group at the C-6 position in **2** was confirmed by the singlet at δ 4.08 attributed to aromatic methoxy group and by the down-field shift (0.37 ppm) of the C-11 proton[4] in comparison with that of ACM, as shown in Table 2.

The reduction of the C-4''' carbonyl function of **1** with $NaBH_4$ or $NaBH_3CN$ afforded a mixture of 4-*O*-methyl MA144 M1 (**3**) and -N1 (**4**). For example, the reduction (20°C, 10 minutes) of **1** with 0.25 molar equivalent of $NaBH_4$ in benzene containing a small amount of MeOH followed by column chromatography ($CHCl_3$ - MeOH, 20 : 1) gave **3** and **4** in 33 and 17% yields, respectively, **3**: mp. 160 ~ 162°C; $[\alpha]_D^{22}$ −2° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$); 420 nm (122); ν_{max}^{KBr} 1735, 1675, 1630 cm^{-1}; *Anal.* calcd. for $C_{43}H_{57}NO_{15}\cdot H_2O$: C 61.05, H 7.03, N 1.66; found: C 60.47, H 6.76, N 2.02; and **4**: mp. 156 ~ 157°C; $[\alpha]_D^{22}$ −8° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 420 nm (120); ν_{max}^{KBr} 1735, 1675, 1630 cm^{-1}; *Anal.* calcd. for $C_{43}H_{57}NO_{15}$: C 62.38, H 6.94, N 1.69; found: C 62.01, H 7.04, N 1.67. In both compounds, the absence of the carbonyl function at the C-4''' was proved by the diminished absorption of IR at 1735 cm^{-1}.

4-*O*-Methyl MA144 S1 (**5**) and 4-*O*-methylaklavin (**6**) were obtained by hydrolysis of **3** with 0.1 N HCl at 22°C for 1.5 hours in 47 and 33% yields, after preparative layer chromatographic purification (Silica Gel 60 F_{254}, E. Merck; $CHCl_3$ - MeOH, 5 : 1), **5**: mp. 159 ~ 161°C; $[\alpha]_D^{22}$ +48° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 420 nm (147); ν_{max}^{KBr} 1735, 1675, 1630 cm^{-1}; *Anal.* calcd. for $C_{37}H_{47}NO_{13}\cdot H_2O$: C 60.79, H 6.76, N 1.92; found: C 60.55, H 6.57, N 2.08; and **6**: mp. 131 ~ 135°C; $[\alpha]_D^{22}$ +156° (*c* 0.05, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ ($E_{1cm}^{1\%}$) 420 nm (173); ν_{max}^{KBr} 1735, 1675, 1630 cm^{-1}; *Anal.* calcd. for $C_{31}H_{37}NO_{10}\cdot H_2O$; C 61.95, H 6.54, N 2.33; found: C 61.77, H 6.28, N 2.32.

Base treatment of **1** gave only the desired disaccharide (**5**) with satisfactory yield. Namely, treatment of **1** with 2 molar equivalent of NaOMe in dry 5% methanolic tetrahydrofuran at 20°C for 1 hour followed by column chromatography ($CHCl_3$ - MeOH, 20 : 1) afforded **5** in 65% yield. It seems that the C-4''' carbonyl function of **1** relates to the C-1''' glycosidic bond cleavage by the base. Also, compound **6** was obtained by acidic hydrolysis of **1** in 87% yield.

The selected ^{1}H-NMR spectral data of the derivatives are summarized in Table 2. Methylation of the aromatic hydroxy groups caused down-field shifts of the C-11H resonance[4] in **2** and the C-1H, C-2H and C-3H resonances in others (**1** & **3** ~ **6**) in comparison with the corresponding position in ACM. The phenolic

Table 3. Antitumor activities of 4- or 6-*O*-methylaclacinomycin derivatives against L1210 leukemia.

Compound	*In vivo* ILS (%), Dose (mg/kg/day)							*In vitro* IC_{50} (μg/ml)		
	45	30	20	15	10	5	2.5	Cytotoxicity	DNA synth.	RNA synth.
ACM					Tox.	46	75	0.01	0.65	0.09
1	12	93	67	67	39	14	8	0.04	0.48	0.05
2	—	—	26	80	32	14	9	0.03	1.42	0.09
3	—	26	50	—	56	22	4	0.01	0.41	0.04
5	—	20	20	—	52	22	4	0.01	0.31	0.05

In vivo antitumor activity: CDF_1 mice transplanted intraperitoneally by 10^5 L1210 leukemia cells were treated by intraperitoneal administration of the compound daily for 10 days starting 24 hours after implantation. Death or survival of the test and the control mice was recorded daily for 30 days and the antitumor activity was evaluated in terms of the percentage increase in life span (ILS) over the control.

Cytotoxicity: L1210 cells (4×10^4 cell/ml) were cultured in RPMI 1,640 medium containing 20 % calf serum with test compounds (0.01~0.5 μg/ml) at 37°C under 5 % CO_2-95 % air atomsphere. The cell growth was periodically determined using a hemocytometer by counting viable cells stained with trypan blue (0.17 %). Cytotoxicity was expressed as IC_{50} of the control growth on day 2.

Nucleic acid biosynthesis: After preincubation of L1210 cell suspension (5×10^5 cells/ml) with test compound (0.01~2.5 μg/ml) at 37°C for 15 minutes, 2-^{14}C-TdR and -UR were added with 0.05 μCi/ml, respectively, and incubated at 37°C for 60 minutes. The reaction was terminated by rapid chilling and adding 1 ml of cold 10 % TCA to 1 ml of reaction 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 BRAY's scintillator.

proton was indicated by the resonance at δ 12.9 in 6-*O*-methyl derivative and at δ 13.6 in others. Spectral patterns of the sugar moieties in each derivatives are very similar to those of the corresponding aclacinomycin analogs: ACM, MA144 M1, -N1 and -S1[3] and aklavin[5].

The antitumor activity of 4- and 6-*O*-methylaclacinomycin derivatives against L1210 leukemia was tested in comparison with that of ACM, and the results are shown in Table 3. In this test, **1** produced a fairly good ILS ranging from 39 to 93 in doses 10~30 mg/kg once daily for 1~10 days. This activity was somewhat better than that of ACM and **2**, while the potency of **1** as well as other 4- and 6-*O*-methyl derivatives was much lower (1/10) than that of the parent compound (ACM). Compound **3** and **5** showed a slightly lower activity than others. It is interesting that methylation at the C-4 or -6 position of ACM decreases the toxicity more markedly than antitumor activity.

4- And 6-*O*-methylaclacinomycin derivatives showed a marked cytotoxicity against cultured L1210 leukemia cells and inhibited preferentially RNA synthesis, as shown in Table 3. The IC_{50} values for RNA synthesis of ACM and **2** was about twice those of 4-*O*-methyl derivatives, and **2** showed about 3-times higher IC_{50} value for DNA synthesis than the other compounds.

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