CHEMICAL MODIFICATION OF ANTHRACYCLINE ANTIBIOTICS. I

DEMETHOXYCARBONYLATION, 10-EPIMERIZATION AND 4-O-METHYLATION OF ACLACINOMYCIN A

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

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

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Demethoxycarbonyl derivatives of aclacinomycin A and of its 7-epimer, 10-*epi*-aclacinomycin A and 4-O-methylaclacinomycin A were chemically derived from aclacinomycin A. The cytotoxicity and inhibitory effects on RNA and DNA synthesis in cultured L1210 leukemia cells of the 4-O-methyl derivative approximated that of aclacinomycin A, while the demethoxycarbonyl derivatives and 10-*epi*-aclacinomycin A exhibited decreased activities in comparison with the parent compound.

Aclacinomycin A is a new anthracycline antibiotic which has a potent antitumor activity against various experimental tumors. With low cardiac toxicity, its clinical efficacy has been evaluated[1~3]. As a result of previous studies on the structure-activity relationship of a number of aclacinomycin analogues isolated from *Streptomyces galilaeus* culture[4~7], it was expected that the chemical modification of the antibiotic would give more potent derivatives.

The present paper deals with demethoxycarbonylation, 10-epimerization and 4-O-methylation of aclacinomycin A and the effect of chemical derivation on biological activities.

Chemistry

Demethoxycarbonylation of Aclacinomycin A

Demethoxycarbonylation of nogalamycin and ε-pyrromycinone-based anthracyclines has been recently studied[8~10], but demethoxycarbonylation of aklavinone-based anthracyclines accompanied with epimerization has not been reported yet. Demethoxycarbonylation of aclacinomycin A (**1**) under as the reaction conditions of WILEY *et al.* afforded eight demethoxycarbonyl products.

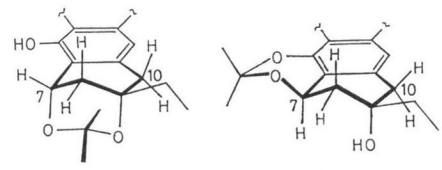
Treatment of **1** with aqueous potassium hydroxide at room temperature for several hours followed by acidification with hydrochloric acid gave the free acid products. The products, without purification, were decarboxylated with dimethylformamide at room temperature. Removal of the solvent *in vacuo* gave a gummy residue which was separated by chromatography to yield demethoxycarbonylaclacinomycin A (**2**), 7-*epi*-demethoxycarbonylaclacinomycin A (**3**), demethoxycarbonyl MA144 G1 (*cf.* MA144 G1[6]=5‴-epimer of **1**) (**4**) and 7-*epi*-demethoxycarbonyl MA144 G1 (**5**). When alkaline treatment of **1** was kept overnight at room temperature, it was converted to demethoxycarbonyl MA144 S1 (*cf.* MA144 S1[6]=decinerulosyl aclacinomycin A) (**6**) and 7-*epi*-demethoxycarbonyl MA144 S1 (**7**), and a small amount of demethoxycarbonylaklavinone (**8**) and 7-*epi*-demethoxycarbonylaklavinone (**9**). The formation of these compounds is due to the racemization at C-7 and/or C-5‴ and the glycosidic bond

cleavage of **1** during the alkaline treatment. Compound **8** was also derived from **2**, **4** and **6**, and **9** from **3**, **5** and **7** by methanolysis, respectively.

^{1}H-NMR, ^{13}C-NMR, IR spectrometries and elemental analysis of the eight compounds indicated the absence of the methoxycarbonyl group at their C-10 position. The absence of this functional group in **8** and **9** was also confirmed by mass spectra, which showed *m/z* 354 as the molecular ion peak. In ^{1}H-NMR spectrum of **2**, the signals emanated from two protons at the C-10 were observed at δ 2.83 and 3.08, which were coupled with each other (J=19 Hz). In the ^{13}C-NMR spectrum, the C-10 carbon signal of **2** appeared at δ 43.0, and shifted to the upper field by 14.2 ppm than that of **1**.

It was found that the aglycone moiety of the eight compounds can be classified into two types by the C-7 configuration. For the determination of the C-7 configuration of the eight compounds, **8** and **9** were subjected to acetonation. The treatment of **8** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid afforded 7,9-O-isopropylidenedemethoxycarbonylaklavinone (**10**). Whereas, **9** gave 6,7-O-isopropylidenedemethoxycarbonylaklavinone (**11**) under the same conditions. In the ^{1}H-NMR of **10**, C-7 H, C-8 H_A and C-8 H_B were observed at δ 5.47, 1.74 and 2.56, respectively, and the coupling constant ($J_{7,8A}+J_{7,8B}$) was 6 Hz. This small coupling constant indicates that **10** has *S*-configuration at the C-7, as shown in Fig. 1-a. On the other hand, the signals of C-7 H, C-8 H_A and C-8 H_B of **11** appeared at δ 5.20, 1.65 and 2.27, respectively, and the coupling constant ($J_{7,8A}+J_{7,8B}$) was 16 Hz. This large coupling constant suggests that the C-7 of **11** is *R*-configuration (Fig. 1-b).

Fig. 1.



a b

Accordingly, **8** and **9** were decided to have the *S*- and *R*-configuration at their C-7 position, respectively. Configuration at the C-7 position of other demethoxycarbonyl derivatives was determined by ^{1}H-NMR analysis in the same way. Table 1 shows the ^{1}H-NMR spectral data concerning the C-7, 8 and 10 protons of the resulting compounds. These results coincided with following data: (i) aklavinone

Table 1. ^{1}H-NMR spectral data of 10-demethoxycarbonyl derivatives.

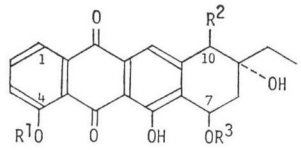
Compound	C-7 H	$J_{7,8A}+J_{7,8B}$	C-10 H_A	C-10 H_B	$J_{10A,B}$
1	δ 5.27 (bs)	6 Hz	δ 4.10 (1H, s)		—
2	5.20 (bs)	6 Hz	2.83 (d)	3.08 (d)	19 Hz
3	5.13 (t)	15 Hz	2.79	3.06	17 Hz
4	5.21 (bs)	6 Hz	2.83	3.09	19 Hz
5	5.13 (t)	15 Hz	2.79	3.06	17 Hz
6	5.19 (bs)	6 Hz	2.82	3.08	18 Hz
7	5.10 (t)	15 Hz	2.79	3.04	18 Hz
8	5.16 (bs)	7 Hz[a]	2.82	3.02	18 Hz
9	5.20 (dt)	15 Hz[b]	2.76	2.97	18 Hz
10	5.47 (t)	6 Hz[c]	2.83	3.09	18 Hz
11	5.20 (dd)	16 Hz[d]	2.98 (2H, bs)		—

Measurement at 100 MHz in $CDCl_3$, in ppm (δ) from $SiMe_4$, a; C-8 H_A: δ 1.80 (dd), $J_{7,8A}$=4.5 Hz, C-8 H_B: 2.18 (bd), $J_{7,8B}$=2.5 Hz, $J_{8A,B}$=14 Hz, C-7 OH: 4.45 (d), $J_{7,OH}$=5.5 Hz, b; C-8 H_A: 1.88 (dd), $J_{7,8A}$=8 Hz, C-8 H_B: 2.16 (ddd), $J_{7,8B}$=7 Hz, $J_{8A,B}$=14 Hz, C-7 OH: 4.12 (d), $J_{7,OH}$=3 Hz, c; C-8 H_A: 1.74 (dd), $J_{7,8A}$=3 Hz, C-8 H_B: 2.56 (ddd), $J_{7,8B}$=3 Hz, $J_{8A,B}$=14 Hz, d; C-8 H_A: 1.65 (dd), $J_{7,8A}$=11 Hz, C-8 H_B: 2.27 (ddd), $J_{7,8B}$=5 Hz, $J_{8A,B}$=13 Hz.

(7*S*, $J_{7X,8A}+J_{7X,8B}=7$ Hz) and aklavinone II (=7-*epi*-aklavinone, 7*R*, $J_{7X,8A}+J_{7X,8B}=16$ Hz) reported by TRESSELT *et al.*[11], (ii) β-rhodomycinone and 7,9-O-isopropylidene-β-rhodomycinone (7*S*, $J_{7e,8a}+J_{7e,8e}=6$ Hz) reported by BROCKMANN *et al.*[12].

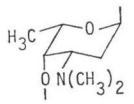
Sugar moieties of the eight compounds were identified by comparing with those of aclacinomycin A and its analogues, and are showed in Table 2.

Table 2. Structures of aclacinomycin derivatives.

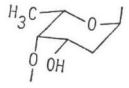


Compound	R¹	R²	R³	Configuration C-7	Configuration C-10
1	H	$COOCH_3$	(L)-Rho-(L)-dFu-(L)-Cin A	*S*	*R*
2	H	H	(L)-Rho-(L)-dFu-(L)-Cin A	*S*	—
3	H	H	(L)-Rho-(L)-dFu-(L)-Cin A	*R*	—
4	H	H	(L)-Rho-(L)-dFu-(D)-Cin A	*S*	—
5	H	H	(L)-Rho-(L)-dFu-(D)-Cin A	*R*	—
6	H	H	(L)-Rho-(L)-dFu	*S*	—
7	H	H	(L)-Rho-(L)-dFu	*R*	—
8	H	H	H	*S*	—
9	H	H	H	*R*	—
12	H	$COOCH_3$	(L)-Rho-(L)-dFu-(L)-Cin A	*S*	*S*
13	CH_3	$COOCH_3$	(L)-Rho-(L)-dFu-(L)-Cin A	*S*	*R*

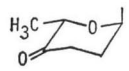
(L)-Rho: (L)-rhodosamine



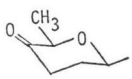
(L)-dFu: (L)-2-deoxyfucose



(L)-Cin A: (L)-cinerulose A



(D)-Cin A: (D)-cinerulose A



10-Epimerization of Aclacinomycin A

10-*epi*-Aclacinomycin A (**12**) was prepared by the treatment of **1** with bases such as sodium azide, triethylamine and pyridine in dimethylformamide (DMF). Treatment of **1** with 1.5 molar equivalent of sodium azide in DMF at room temperature for 1 hour gave a mixture of **1** and **12** in about 1 : 1.2 ratio which was chromatographycally separated to afford **12** in 36% yield. Compound **12** also gave a mixture of **1** and **12** in the same procedure described above. On the other hand, treatment of **1** with triethylamine or pyridine for 20 hours gave a mixture of **12** and **1** in 1 : 1.7 and 1 : 4 ratios, respectively.

The IR spectrum of **12** is very similar to that of **1**. In ^{1}H-NMR spectrum of **12**, the methyl resonance of the methoxycarbonyl group was observed at δ 3.84, which was lower than that of **1** by 0.14 ppm, but the signal of C-10 H appeared at δ 3.96, which was shifted by 0.15 ppm to the upper field than the corresponding signal of **1**. Furthermore, the ^{1}H-NMR and ^{13}C-NMR spectra of **12** indicated that the stereochemical structure is kept in the natural conformation except for the C-10 position.

Preparation of 4-O-Methylaclacinomycin A

Methylation of the hydroxy group at the C-4 of the anthracyclines which have a trisaccharide such as aclacinomycin A has not been reported as yet. It was already known that the methylation of the peri-hydroxy group of naphthoquinones is performed with methyl iodide using silver oxide in chloroform[13].

By direct methylation of the phenolic functions of **1** with excess methyl iodide and silver oxide in chloroform, 4-O-methylaclacinomycin A (**13**) was obtained in 37% yield after column chromatography, however the 6-O-methyl derivative could not be obtained.

Table 3. ^{13}C-NMR spectral data of aclacinomycin A derivatives.

Compound	5	12	15[a]	7	10	16[b]	4-OMe	1′	NMe_2	4′	1″	4″	1‴	4‴	5‴
1[c]	192.7	181.3	171.3	70.6	57.2	52.5	—	101.6	43.3	74.1	100.2	83.0	99.4	210.0	71.8
MA144 G1[c]	193.1	181.6	171.6	70.8	57.3	52.5	—	101.9	43.3	74.2	99.7	83.4	101.8	207.7	76.6
MA144 S1[c]	192.8	181.3	171.3	70.7	57.2	52.5	—	101.6	43.2	74.3	99.1	71.6	—	—	—
2	192.5	181.4	—	70.0	43.0	—	—	101.2	43.3	74.2	100.1	83.0	99.4	210.0	71.8
3	192.7	181.7	—	70.9	42.2	—	—	99.9	43.3	74.4	100.1	83.0	99.4	210.0	71.8
4	192.5	191.5	—	70.1	43.0	—	—	101.2	43.2	74.1	99.5	83.2	101.8	207.3	76.4
5	192.7	181.6	—	70.9	42.1	—	—	99.9	43.3	74.4	99.5	83.2	101.8	207.3	76.4
6	192.4	181.3	—	70.1	42.9	—	—	101.1	43.2	74.2	99.1	71.6	—	—	—
7	192.7	181.3	—	70.9	42.0	—	—	99.9	43.3	74.5	99.2	71.5	—	—	—
8	193.9	181.8	—	62.6	43.1	—	—	—	—	—	—	—	—	—	—
9	193.9	181.8	—	64.4	43.1	—	—	—	—	—	—	—	—	—	—
12	192.6	181.2	171.4	69.2	56.0	52.4	—	100.6	43.2	74.1	100.1	82.9	99.4	210.0	71.8
13	188.8	182.3	171.6	71.0	57.0	52.4	56.7	101.7	43.2	74.2	100.1	83.0	99.5	210.0	71.8

Measurement at 25.2 MHz in $CDCl_3$, in ppm (δ) from $SiMe_4$, a: carbonyl of methoxycarbonyl group, b: methyl of methoxycarbonyl group, c: ref. 6).

The IR spectrum of **13** showed the absorption bands of the aromatic ether (1250 cm^{-1}), hydrogen-bonded (1630) and nonhydrogen-bonded (1675) quinone carbonyl groups. Visible spectrum of **13** indicated an absorption maximum at 419 nm, which is shifted 16 nm toward shorter wavelengths, compared with that of **1**. In ^{1}H-NMR of **13**, the signal of the 4-O-methyl protons was observed at δ 4.07, and the aromatic proton signals of C-1, C-2 and C-3 were shifted to the lower field than those of **1**. The signal indicated at δ 56.7 in ^{13}C-NMR showed the presence of the methoxy group at the C-4.

Table 3 shows the ^{13}C-NMR spectral data of aclacinomycin derivatives.

Biological Activity

The cytotoxicity against L1210 leukemia cells and the inhibitory effect on macromolecular synthesis measured by the incorporation of radioactive precursors into the acid-insoluble fraction of L1210 cells in culture were expressed as the concentration of the compounds required for 50% inhibition (IC_{50} value). The results are shown in Table 4. Composition of **2** with **1** indicates that demethoxycarbonylation of aclacinomycin A resulted in a 2- to 8-fold loss in inhibitory effect on RNA and DNA synthesis and on cell growth of L1210 cells.

Epimers at the C-7 position (**3**, **5**, **7**) have a 1.5- to 2-fold loss in cytotoxicity in comparison with demethoxycarbonylaclacinomycin A (**2**), -MA144 G1 (**4**) and -MA144 S1 (**6**) as well as a decrease in inhibitory effect on RNA and DNA synthesis.

Table 4. *In vitro* antitumor activity of aclacinomycin A derivatives against cultured L1210 leukemia cells.

Compound	IC_{50} (μg/ml)		
	Cytotoxicity*	DNA synth.	RNA synth.
1	0.03	0.65	0.085
2	0.1	1.2	0.68
3	0.23	1.65	1.1
4	0.2	2.4	1.8
5	0.35	2.5	2.25
6	0.18	0.95	0.35
7	0.37	1.7	0.95
12	0.04	5.5	1.9
13	0.04	0.48	0.05

* day 2

The IC_{50} value for cell growth of aclacinomycin A (0.03 μg/ml) and its 10-epimer (0.04 μg/ml) are approximately equivalent, but for RNA and DNA synthesis the IC_{50} value of the 10-epimer is nearly 10- to 20-fold higher than that of aclacinomycin A. In their works on nogalamycin, marcellomycin and rudolfomycin, LI *et al.*[14] and DUVERNAY *et al.*[10] have shown that removal of the methoxycarbonyl function results in a 5- to 10-fold loss in potency.

The IC_{50} values for cell growth and macromolecular synthesis of 4-O-methylaclacinomycin A approximate that of aclacinomycin A.

Experimental

General:

The isolation and physicochemical properties of aclacinomycin A (**1**), starting material, has been described in detail elsewhere[1,4~6]. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. IR and UV spectra were recorded on a Hitachi 260–30 spectrophotometer and a Hitachi 200–20 spectrophotometer, respectively. Optical rotation was measured using a JASCO DIP-181 polarimeter. ^{1}H-NMR and ^{13}C-NMR data were obtained with a Varian XL-100 spectrometer. Chemical shifts are expressed in parts per million down field from internal tetramethylsilane. Abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and bs=broad singlet. Mass spectrum was taken on a Hitachi RMU-6 mass spectrometer. Silica gel thin-layer chromatography (TLC) was carried out using precoated plates of Silica Gel 60 F_{254} (E. Merck, Darmstadt). Column chromatography was performed on Silica Gel 60 (70~230 mesh, E. Merck, Darmstadt).

Demethoxycarbonylaclacinomycin A (**2**), 7-*epi*-demethoxycarbonylaclacinomycin A (**3**), demethoxycarbonyl MA144 G1 (**4**) and 7-*epi*-demethoxycarbonyl MA144 G1 (**5**)

A solution of **1** (1.0 g) in 0.5 N KOH (11 ml) was stirred at room temperature for 4.5 hours followed by neutralization with 2 N HCl to form a yellow precipitate. The resulting precipitate was dissolved in dry DMF (10 ml), and stirred at room temperature for 1 hour. Evaporation of the solvent *in vacuo* gave a gummy residue. The residue was chromatographed on silica gel column using $CHCl_3$ - MeOH (30: 1). The first eluate was evaporated, and the resulting residue was further separated into **2** (78 mg, Rf: 0.55) and **3** (64 mg, Rf: 0.50) by preparative TLC using $CHCl_3$ - benzene - MeOH (6: 2: 1). The successive eluate also contained two components which were purified by the same way to give **4** (18 mg, Rf: 0.45) and **5** (19 mg, Rf: 0.42).

2: Yellow powder, mp. 139~141°C, $[\alpha]_D^{21}$ −44.7° (*c* 1, $CHCl_3$), UV ($CHCl_3$) λ_{max} 262 nm ($\varepsilon \times 10^3$ 24.3), 282 (11.2), 293 (11.6), 436 (12.7), IR (KBr) 1730, 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.18 (6H, s, NMe_2), 2.83 (1H, d, C-10 H_A), 3.08 (1H, d, C-10 H_B), 5.04 (2H, m, C-1″ & 1‴ H), 5.20 (1H, bs, C-7 H), 5.50 (1H, bs, C-1′ H), 7.20~7.85 (4H, m, aromatic).

Anal. Calcd. for $C_{40}H_{51}NO_{13}\cdot\frac{1}{2}H_2O$: C 62.98; H 6.87; N 1.84.
Found: C 62.94; H 6.55; N 2.03.

3: Yellow powder, mp. 142~144°C, $[\alpha]_D^{21}$ −43.0° (*c* 0.8, $CHCl_3$), UV ($CHCl_3$) λ_{max} 262 nm ($\varepsilon \times 10^3$ 24.3), 283 (10.6), 294 (10.6), 437 (12.2), IR (KBr) 1735, 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.22 (6H, s, NMe_2), 2.79 (1H, d, C-10 H_A), 3.06 (1H, d, C-10 H_B), 5.04 (2H, m, C-1″ & 1‴ H), 5.13 (1H, t, C-7 H), 5.43 (1H, bs, C-1′ H), 7.20~7.85 (4H, m, aromatic).

Anal. Calcd. for $C_{40}H_{51}NO_{13}\cdot\frac{1}{2}H_2O$: C 62.98; H 6.87; N 1.84.
Found: C 63.18; H 6.81; N 2.16.

4: Yellow powder, mp. 141~143°C, $[\alpha]_D^{21}$ −12.0° (*c* 1, $CHCl_3$), UV ($CHCl_3$) λ_{max} 263 nm ($\varepsilon \times 10^3$ 23.4), 282 (11.0), 293 (10.3), 436 (12.3), IR (KBr) 1730, 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.18 (6H, s, NMe_2), 2.83 (1H, d, C-10 H_A), 3.09 (1H, d, C-10 H_B), 4.93 (1H, t, C-1‴ H), 5.03 (1H, bs, C-1″ H), 5.21 (1H, bs, C-7 H), 5.50 (1H, bs, C-1′ H), 7.20~7.85 (4H, m, aromatic).

Anal. Calcd. for $C_{40}H_{51}NO_{13}$: C 63.73; H 6.82; N 1.86.
Found: C 63.44; H 6.63; N 1.89.

5: Yellow powder, mp. 136~139°C, $[\alpha]_D^{21}$ +1.25° (*c* 0.4, $CHCl_3$), UV ($CHCl_3$) λ_{max} 263 nm ($\varepsilon \times$

10^3 25.0), 283 (10.9), 293 (10.7), 437 (12.6), IR (KBr) 1730, 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.23 (6H, s, NMe_2), 2.79 (1H, d, C-10 H_A), 3.06 (1H, d, C-10 H_B), 4.92 (1H, t, C-1‴ H), 5.04 (1H, bs, C-1″ H), 5.13 (1H, t, C-7 H), 5.43 (1H, bs, C-1′ H), 7.20~7.85 (4H, m, aromatic).

Anal. Calcd. for $C_{40}H_{51}NO_{13}$: C 63.73; H 6.82; N 1.86.
Found: C 63.41; H 6.60; N 2.21.

Demethoxycarbonyl MA144 S1 (**6**), 7-*epi*-demethoxycarbonyl MA144 S1 (**7**), demethoxycarbonylaklavinone (**8**) and 7-*epi*-demethoxycarbonylaklavinone (**9**)

A solution of **1** (800 mg) in 0.5 N KOH (8.8 ml) was stirred at room temperature for 17 hours. Acidification of the solution with 2 N HCl (pH 6) gave a yellow precipitate which was filtered and dried. The resulting solid was dissolved in dry DMF (10 ml) and stirred at room temperature for 1 hour. Removal of the solvent *in vacuo* gave a gummy residue. The residue was chromatographed on silica gel column. The elution with $CHCl_3$ - MeOH (20: 1) gave two components which were further separated into **8** (8.2 mg, Rf: 0.42) and **9** (5.1 mg, Rf: 0.37) by preparative TLC using $CHCl_3$ - MeOH (20 :1). The elution with $CHCl_3$ - MeOH (10: 1) afforded **6** (97 mg, Rf: 0.13*) and **7** (13 mg, Rf: 0.09*).

6: Yellow powder, mp. 154~155°C, $[\alpha]_D^{21}$ +20.8° (*c* 0.25, $CHCl_3$), UV ($CHCl_3$) λ_{max} 263 nm ($\varepsilon \times 10^3$ 22.6), 283 (10.2), 293 (10.1), 436 (11.5), IR (KBr) 1680, 1630 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.22 (6H, s, NMe_2), 2.82 (1H, d, C-10 H_A), 3.08 (1H, d, C-10 H_B), 5.06 (1H, bs, C-1″ H), 5.19 (1H, bs, C-7 H), 5.50 (1H, bs, C-1′ H), 7.19~7.84 (4H, m, aromatic).

Anal. Calcd. for $C_{34}H_{43}NO_{11}\cdot\frac{1}{2}H_2O$: C 62.76; H 6.82; N 2.15.
Found: C 62.47; H 6.46; N 2.14.

7: Yellow powder, mp. 155~157°C, $[\alpha]_D^{21}$ +41.0° (*c* 0.2, $CHCl_3$), UV ($CHCl_3$) λ_{max} 262 nm ($\varepsilon \times 10^3$ 22.8), 282 (9.9), 293 (9.7), 437 (11.5), IR (KBr) 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.24 (6H, s, NMe_2), 2.79 (1H, d, C-10 H_A), 3.04 (1H, d, C-10 H_B), 5.02 (1H, bs, C-1″ H), 5.10 (1H, t, C-7 H), 5.42 (1H, bs, C-1′ H), 7.18~7.84 (4H, m, aromatic).

Anal. Calcd. for $C_{34}H_{43}NO_{11}\cdot\frac{1}{3}H_2O$: C 63.05; H 6.79; N 2.16.
Found: C 63.18; H 6.94; N 2.03.

8: Yellow powder, mp. 185~188°C, $[\alpha]_D^{21}$ +133.0° (*c* 0.1, $CHCl_3$ - MeOH, 2: 1 v/v), UV ($CHCl_3$) λ_{max} 262 nm ($\varepsilon \times 10^3$ 26.5), 292 (11.3), 433 (12.8), IR (KBr) 1675, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 1.00 (3H, t, $-CH_2C\underline{H}_3$), 1.57 (2H, q, $-C\underline{H}_2CH_3$), 1.80 (1H, dd, C-8 H_A), 2.18 (1H, bd, C-8 H_B), 2.82 (1H, d, C-10 H_A), 3.02 (1H, d, C-10 H_B), 5.16 (1H, bs, C-7 H), 7.20~7.84 (4H, m, aromatic), MS *m/z* 354 (M^+).

Anal. Calcd. for $C_{20}H_{18}O_6$: C 67.79; H 5.12.
Found: C 67.60; H 5.11.

9: Yellow powder, mp. 190~193°C, $[\alpha]_D^{21}$ +121.3° (*c* 0.08, $CHCl_3$ - MeOH, 2: 1 v/v), UV ($CHCl_3$) λ_{max} 262 nm ($\varepsilon \times 10^3$ 26.3), 282 (11.2), 292 (11.3), 433 (12.6), IR (KBr) 1670, 1620 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 0.98 (3H, t, $-CH_2C\underline{H}_3$), 1.61 (2H, q, $-C\underline{H}_2CH_3$), 1.88 (1H, dd, C-8 H_A), 2.16 (1H, ddd, C-8 H_B), 2.76 (1H, d, C-10 H_A), 2.97 (1H, d C-10 H_B), 5.20 (1H, dt, C-7 H), 7.20~7.84 (4H, m, aromatic), MS *m/z* 354 (M^+).

Anal. Calcd. for $C_{20}H_{18}O_6$: C 67.79; H 5.12.
Found: C 67.55; H 5.10.

Preparation of **8**

To a solution of **2** (50 mg) in MeOH - benzene (0.1 ml: 10 ml) was added 25 mg of *p*-toluenesulfonic acid, and refluxed for 10 minutes. The mixture was washed with 1% aqueous $NaHCO_3$ and water, and evaporated. The residue was purified by preparative TLC ($CHCl_3$ - MeOH, 20: 1) to afford **8** (15 mg, Rf: 0.42).

Preparation of **9**

By the same procedure described above **3** (40 mg) was converted to **9** (12 mg, Rf: 0.37).

8 and **9** obtained by this procedure showed the same physicochemical properties as those of the previous ones.

7,9-O-Isopropylidenemethoxycarbonylaklavinone (**10**)

To a solution of **8** (31 mg) in 2,2-dimethoxypropane (4 ml) and Me_2CO (4 ml) was added 10 mg of

* $CHCl_3$ - benzene - MeOH (6: 2: 1)

p-toluenesulfonic acid, and stirred at 50°C for 6 hours. The reaction mixture was diluted with EtOAc, and washed with 1% aqueous $NaHCO_3$ and water. Evaporation of the solvent gave a yellow residue. The residue was purified by preparative TLC using $CHCl_3$ to afford **10** (17 mg, Rf: 0.75 $CHCl_3$ - MeOH, 20: 1): mp. 210~214°C, $[\alpha]_D^{22}$ +226° (*c* 0.05, $CHCl_3$), UV ($CHCl_3$) λ max 261 nm ($\varepsilon \times 10^3$ 27.1), 289 (10.4), 433 (13.2), IR (KBr) 1670, 1625 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 1.06 (6H, s & t, $-CH_2C\underline{H}_3$, $C\langle^{CH_3}_{CH_3}$), 1.52 (3H, s, $C\langle^{CH_3}_{C\underline{H}_3}$), 1.74 (1H, dd, C-8 H_A), 1.82 (2H, q, $-C\underline{H}_2CH_3$), 2.56 (1H, ddd, C-8 H_B), 2.83 (1H, d, C-10 H_A), 3.09 (1H, dd, C-10 H_B), 5.47 (1H, t, C-7 H), 7.15~7.85 (4H, m, aromatic).

Anal. Calcd. for $C_{23}H_{22}O_6$: C 70.04; H 5.62.
Found: C 70.20; H 5.51.

6,7-O-Isopropylidenedemethoxycarbonylaklavinone (**11**)

To a solution of **9** (7.4 mg) in 2,2-dimethoxypropane (1 ml) and Me_2CO (1 ml) was added 2 mg of *p*-toluenesulfonic acid, and the mixture was stirred at 50°C for 30 minutes. The reaction mixture was worked up as above, and the resulting residue was chromatographed on silica gel column using $CHCl_3$ to yield **11** (7.0 mg, Rf: 0.44 $CHCl_3$ - MeOH, 20: 1): mp. 86~88°C, $[\alpha]_D^{21}$ −78° (*c* 0.05, $CHCl_3$), UV ($CHCl_3$) λ_{max} 264 nm ($\varepsilon \times 10^3$ 26.3), 290 (10.8), 417 (10.5), IR (KBr) 1665, 1635 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 1.04 (3H, t, $-CH_2C\underline{H}_3$), 1.4~1.8 (3H, m, $-C\underline{H}_2CH_3$, C-8 H_A), 1.75 (6H, s, CMe_2), 2.27 (1H, ddd, C-8 H_B), 2.98 (2H, bs, C-10 H_2), 5.20 (1H, dd, C-7 H), 7.15~7.80 (4H, m, aromatic).

Anal. Calcd. for $C_{23}H_{22}O_6$: C 70.04; H 5.62.
Found: C 70.19; H 5.64.

10-*epi*-Aclacinomycin A (**12**)

To a solution of **1** (100 mg) in dry DMF (5 ml) was added 12 mg of NaN_3. After stirring at room temperature for 1 hour, benzene was added. The solution was washed with water, and evaporation of the solvent gave a yellow residue. Separation of the residue by silica gel column chromatography ($CHCl_3$ - MeOH, 40: 1) gave crude **1** (42 mg) and crude **12** (40 mg). Crude **12** was further purified by preparative TLC ($CHCl_3$ - MeOH, 20: 1) to yield pure **12** (36 mg, Rf: 0.36): yellow powder, mp. 139~142°C, $[\alpha]_D^{21}$ −118° (*c* 0.15, $CHCl_3$), UV ($CHCl_3$) λ_{max} 261 nm ($\varepsilon \times 10^3$ 23.2), 280 (10.1), 292 (10.2), 435 (12.4), IR (KBr) 1735, 1675, 1625 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.21 (6H, s, NMe_2), 3.84 (3H, s, COOMe), 3.96 (1H, s, C-10 H), 5.05 (2H, m, C-1'' & 1''' H), 5.23 (1H, bs, C-7 H), 5.48 (1H, bs, C-1' H), 7.22~7.86 (4H, m, aromatic).

Anal. Calcd. for $C_{42}H_{53}NO_{15}$: C 62.14; H 6.58; N 1.72.
Found: C 62.03; H 6.61; N 1.70.

4-O-Methylaclacinomycin A (**13**)

A solution of MeI (0.8 ml) in $CHCl_3$ (10 ml) was added to a stirring mixture of **1** (300 mg) and Ag_2O (600 mg) in $CHCl_3$ (30 ml) over a 3-hour period at room temperature. The mixture was then stirred for 8 hours. The solid material was filtered off and the filtrate was evaporated to give a residue. Purification of the residue by silica gel column chromatography ($CHCl_3$ - MeOH, 70: 1) afforded **13** (114 mg, Rf: 0.47 $CHCl_3$ - benzene - MeOH, 3: 1: 1): yellow powder, mp. 145~148°, $[\alpha]_D^{21}$ −38.0° (*c* 0.2, $CHCl_3$), UV ($CHCl_3$) λ max 258 nm ($\varepsilon \times 10^3$ 23.3), 288 (11.4), 419 (10.9), IR (KBr) 1735, 1675, 1630 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ 2.17 (6H, s, NMe_2), 3.69 (3H, s, COOMe), 4.07 (3H, s, C-4 OMe), 5.07 (2H, m, C-1'' & 1''' H), 5.28 (1H, bs, C-7 H), 5.53 (1H, bs, C-1' H), 7.20~8.0 (4H, m, aromatic).

Anal. Calcd. for $C_{43}H_{56}NO_{15}$: C 62.53; H 6.71; N 1.70.
Found: C 62.14; H 6.74; N 1.67.

L1210 cell growth in culture

RPMI (Rosewell Park Memorial Institute) medium 1640 was used for growing L1210 leukemia cells. Calf serum (20%), sodium bicarbonate (0.1%) and penicillin G (0.1 mg/ml) were added as the supplements. For the growth study, 0.1-ml aliquots of diluent or drug were pipetted into each culture tube. The experiment was then initiated by the addition of 0.9 ml of cells (approximately 5.5×10^4 cells/ml), and the tubes were incubated at 37°C for 2 days in a CO_2-incubater. Cells were counted by a hemocytometer after trypan-blue staining.

Macromolecular synthesis:

L1210 cells in the logarithmic phase of growth were used in the incorporation studies. Cells/ml (5×10^5) in RPMI medium 1640 containing 10% calf serum were incubated with gentle shaking at 37°C with the compound at a given concentration for 15 minutes, and then 0.05 μCi/ml of a labeled precursor (^{14}C-thymidine or ^{14}C-uridine) was added. After incubation for 60 minutes, 10% trichloroacetic acid (TCA) was added to the reaction mixture, and the acid-insoluble materials were precipitated. The precipitate was washed three times with 5% TCA, and dissolved in a small amount of formic acid, and then the radioactivity was measured by an Aloka model LSC653 scintillation spectrometer using 10 ml of BRAY's scintillator.

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