New Anthracycline Antibiotics 10-*epi*-Oxaunomycin and 10-*epi*-11-Deoxyoxaunomycin

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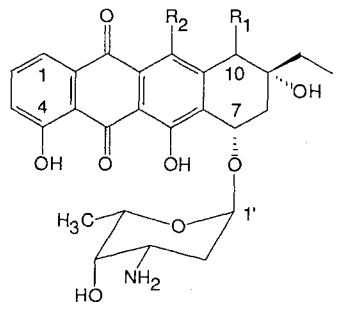
Two new anthracycline antibiotics, 10-*epi*-oxaunomycin and 10-*epi*-11-deoxyoxaunomycin, were photochemically obtained from anthracycline metabolites D788-1 (10-carboxy-13-deoxocarminomycin) and D788-3 (10-carboxy-11-deoxy-13-deoxocarminomycin) and were examined for their growth inhibitory activities on cultured L1210 leukemic cells. Effects of the S configuration of C-10 and a hydroxyl group at C-11 on the bioactivity are discussed in comparison with oxaunomycin and 11-deoxyoxaunomycin.

In the course of a screening program to obtain new anthracycline antitumor antibiotics, we have discovered oxaunomycin (**1**) (Fig. 1), a potent antibiotic of the rhodomycin-group as a minor component in the culture broth of a daunorubicin-blocked mutant[1]. Afterward, we found that it was effectively formed by photochemical conversion of the anthracycline metabolite D788-1 (**2**)[2], which is an abundantly accumulated precursor in the culture of a daunorubicin-blocked mutant[3]. **1** showed very strong cytotoxicity against murine leukemia L1210 cells and a significant antitumor effect in some experimental tumors[1]. However, it was too toxic for further development. Derivatization of **1** is one way to modify the toxicity without loss of its good antitumor effects, and several derivatives have been synthesized[4~8]. Desubstitution and configuration change in **1** itself also seem to be another interesting approach.

Fig. 1. Structures of **1**~**6**.



	R_1		R_2
1	OH	(R)	OH
2	COOH	(R)	OH
3	COOH	(R)	H
4	OH	(S)	H
5	OH	(R)	H
6	OH	(S)	OH

In this paper, we describe the photochemical preparation of 11-deoxy- and 10-*epi*-analogs (**4**~**6**) from the precursor anthracyclines D788-1 (**2**) and D788-3 (**3**)[9] and their antitumor activities *in vitro*. 11-Deoxyoxaunomycin has already been disclosed in 1987 as a product of *Actinomadura roseoviolacea* Ru7062[10], but no data were presented on their biological activities.

Results and Discussion

D788-1 (**2**) is a precursor anthracycline which is produced by the daunorubicin-blocked mutant RPM-5 of *Streptomyces* sp. D788[3]. It is a water soluble material with a carboxyl group at C-10, which is biosynthetically converted to 13-deoxocarminomycin by enzymatic decarboxylation. However, it has been found that photochemical decarboxylation of **2** is followed by hydroxylation at the same site. Thus, oxaunomycin (10-hydroxy-13-deoxocarminomycin) (**1**) occurs in the photochemical reaction. There is also the possibility that

the stereoisomeric product, 10-*epi*-oxaunomycin, is produced as a minor reaction product. Furthermore, it is certain that the photochemical treatment of D788-3 (11-deoxy D788-1) (**3**) provides 11-deoxyoxaunomycin and related isomer by analogy with **2**.

Starting materials **2** and **3** were obtained by cultureing the daunorubicin-blocked mutants RPM-5 and KL-330, respectively. Their photochemical treatments were carried out in the acidic acetone solution containing iodine according to the method described previously[2]. The reaction products were assayed in detail by HPLC after extraction with $CHCl_3$, and their time-course productions were monitored as shown in Fig. 2. **2** afforded **1** with a final conversion yield of about 80%. We found that a concomitant conversion to 10-*epi*-oxaunomycin (**6**) also occurred in this reaction although its yield was as low as 15%. No other significant product was observed.

On the other hand, the photochemical conversion of **3** gave a major 10-*epi*-11-deoxyoxaunomycin (**4**) (25% yield) and a minor 11-deoxyoxaunomycin (**5**) (<2% yield). **1** and **2** have a stereochemical configuration of 7(*S*), 9(*R*) and 10(*R*)[3,9]. The production of **4** as the main reaction product is not due to the stereochemical configuration of **3**, since it was determined to be 7(*S*), 9(*R*) and 10(*R*). However, there are significant differences in the coupling constants of 7-H and 8-CH_2 between **2** and **3**, indicating that the conformation of ring A is distorted for **3**. We believe this is a reason why **3** preferentially gave the 10-epimer in the photochemical reaction. For the structural elucidation of **4**~**6**, the photochemical reaction was conducted on a large scale using 400 mg each of **2** and **3**. The products were purified by silica gel column chromatography, reverse-phase HPLC and silica gel TLC, and pure **4**, **5**, and **6** were obtained in yields of 32 mg, 3 mg, and 15 mg, respectively.

^1H and ^{13}C NMR data for **4**~**6** are shown in Tables 1 and 2. Chemical-shift assignments were mainly carried out by ^1H-^1H and ^1H-^{13}C COSY, and HMBC. The chemical shifts of C-10 for **4**~**6** were quite different from those of their parent compounds (δ 58.68 for **3**[9] and δ 53.2 for **2**[3]) and the molecular ion peaks of the products appeared at 28 mass units lower than the corresponding

Fig. 2. Photochemical reaction of **2** (A) and **3** (B).

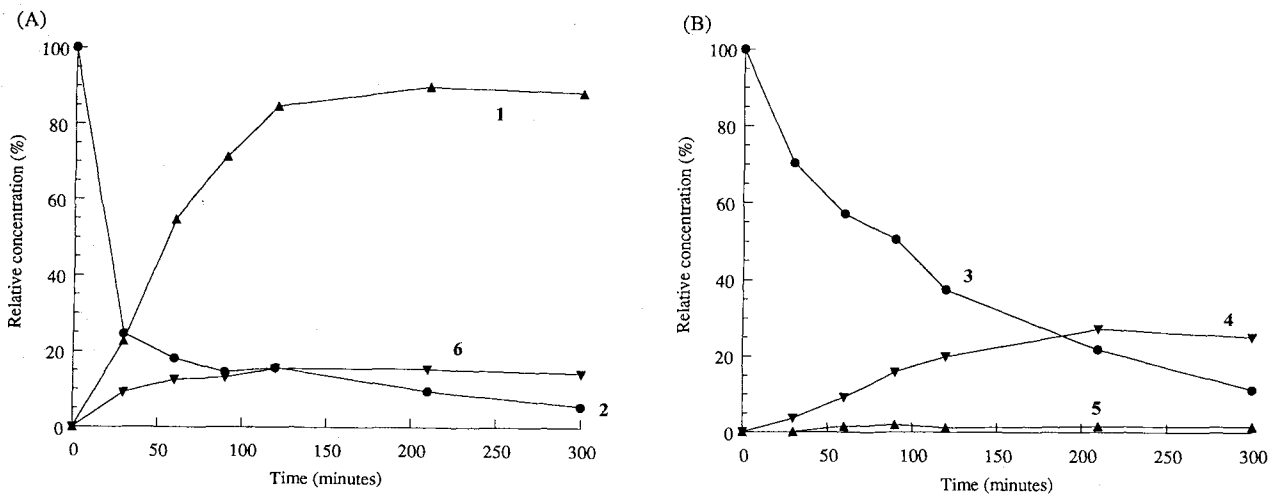


Table 1. ^1H NMR data for **4**~**6** and **1**.

Proton	4	5	6	1[a]
7-H	5.11 br t (4.4, 2.9)	4.93	4.93 t (5.9)	5.15 dd (4.0, 2.0)
8-Ha	2.33 dd (14.7, 5.1)	2.27 dd (14.7, 5.1)	2.36 dd (13.9, 5.9)	2.23 dd (15.0, 2.0)
8-Hb	2.18 dd (14.7, 2.9)	2.18 d (13.9)	2.08 dd (13.9, 5.9)	2.13 dd (15.0, 4.0)
10-H	4.52 s	4.94 s	4.74 s	4.87 s
11-H	7.84 s	7.81 s	-	-
Solvent	$CDCl_3$-CD_3OD (10:1)	$CDCl_3$-CD_3OD (20:1)	$CDCl_3$-CD_3OD (10:1)	$CDCl_3$-CD_3OD (10:1)

[a] Data cited from ref 1.

Table 2. ^{13}C NMR data for **4**~**6** and **1**.

Carbon	4	5	6	1
6a	133.54 [a]	134.08 [a]	136.89	134.77
7	71.01	73.47	71.57	70.75
8	33.50	33.71	35.69	32.48
9	73.08	72.33	72.19	71.94
10	72.82	66.76	67.91	66.09
10a	147.73	142.99	138.89	138.56
11	121.76	120.48	157.61 [a]	156.98 [a]
11a	130.65	132.06	112.08	111.49 [a]

[a] May be interchanged with similar one of other position (see experimental part). Solvent used was the same as shown in Table 1.

Table 3. Inhibitory activity of **4**~**6**, **1** and doxorubicin on growth of culture L1210 leukemia cells.

Compound	IC_{50} (μ g/ml)
	Growth
10-*epi*-11-deoxyoxaunomycin (**4**)	0.037
11-Deoxyoxaunomycin (**5**)	0.009
10-*epi*-oxaunomycin (**6**)	0.48
Oxaunomycin (**1**)	0.006
Doxorubicin	0.05

Cultured L1210 leukemia cells (5×10^4 cells/ml) were exposed for 48 hours to the drugs and the viable cells were counted by coulter counter. IC_{50} is expressed as a drug concentration required to inhibit by a 50% of control growth of cultured L1210 cells.

ones of the parent compounds, indicating that the carboxyl group is replaced by a hydroxyl group. The chemical shifts of 10-H for **4** and **6** shifted to upper fields when compared to **5** and **1**, respectively. The relative stereochemistry of **4**~**6** was examined by NOE experiments. NOEs were observed between 10-H and 13-CH_2 for **4**, **6**, and the aglycone of **4** (10-*epi*-11-deoxy-β-rhodomycinone, **7**), although it was not observed for **5** and **1**. The coupling constants, $J_{8\text{-Hb},7\text{-H}}$ for **4** and $J_{8\text{-Ha},7\text{-H}}$ for **6** were larger than those of **5** and **1**, respectively. These observations suggest that **4**~**6** are 10-*epi*-11-deoxyoxaunomycin, 11-deoxyoxaunomycin, and 10-*epi*-oxaunomycin, respectively.

To elucidate the absolute stereochemistry, the configuration at C-9 of **3** was confirmed as follows. Treatment of **3** with CH_2N_2/ether gave a methylated compound (**8**) which was identified as 11-deoxy-13-deoxo-10-methoxycarbonylcarminomycin by MS and NMR analyses. Acid hydrolysis of **8** in 0.1 N HCl at 90° for 30 minutes afforded a yellow aglycone (**9**). ^1H and ^{13}C NMR data and moleculacr mass of **9** were identical to those of aklavinone[11] which has the stereochemical configuration of 7(*S*), 9(*R*) and 10(*R*). Furthermore, $[\alpha]_D^{25}$ values of **9** (+130°, $CHCl_3$) was in good agreement with that of aklavinone (+125°, $CHCl_3$). These data demonstrated the configuration of **3** is 7(*S*), 9(*R*) and 10(*R*). The stereochemistry of **1** had been established to be 7(*S*), 9(*R*) and 10(*R*)[3]. From all these findings, the chemical structures of **4**~**6** were determined as shown in Fig. 1.

The antitumor activity of **1** and **4**~**6** *in vitro* against cultured L1210 cells was examined and the results are shown in Table 3. The cytotoxicities (IC_{50} values) of **5** and **6** were low when compared with that of **1**. Especially, the configuration of C-10 greatly affected the reduced cytotoxicity. **4** also showed decreased cytotoxicity when compared with **5**. These results suggest that the epimerization of the hydroxyl group at C-10 plays an important role for the cytotoxic properties of this family of compounds. PENCO *et al.* reported the effects of conformations of ring A on antitumor activities in mice[12]. They found that 10(*S*)-methoxydaunorubicin had no activity on P388 lymphocytic leukemia although 10(*R*)-methoxydaunorubicin exhibited activity. This fact is similar to our observations. The coupling constants between 7-H and 8-CH_2 for **1** and **6** were almost the same as those of 10(*R*)- and 10(*S*)-methoxydaunorubicinones, respectively. Alternatively, the coupling constants for **4** were different from those for **6**, indicating that the conformation of ring A for **4** is distorted. Interestingly, the comparison of IC_{50} values between **4** and **6** showed that the hydroxyl group at C-11 has no effect toward the reduction of cytotoxicity; since it is known that the absence of C-11 hydroxyl group results in considerably decreased cytotoxicity against cultured tumor cells[9,13,14], we believe that the distorted ring A in **4** counterbalances the absence of hydroxyl group at C-11. Since **4** showed a moderate activity, equivalent to adriamycin, we expect it to be an improved oxaunomycin derivative with high antitumor activity *in vivo*.

Experimental

General

MP's were determined on a Kofler hot stage microscope and are uncorrected. UV spectra were recorded on a Hitachi U-3210 spectrophotometer and IR spectra (KBr pellet) on a Jasco FT/IR-5300 spectrometer. ^1H and ^{13}C NMR were recorded with a JEOL JNM-GSX400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts were expressed in δ values (ppm) using TMS as an internal standard and coupling constants

were given in J (Hz). Mass spectra were recorded with a Hitachi JEOL JMS-SX102A spectrometer. Specific rotations were recorded on a Jasco DIP-181 digital polarimeter. TLC analyses were performed on Silicagel 60 F_{254} (E. Merck) using solvent systems of $CHCl_3$-MeOH-H_2O-AcOH (30:10:0.4:0.2, system A) and $CHCl_3$-MeOH-concd NH_4OH (40:10:0.1, system B) as developing solvents.

HPLC Analysis

The concentration or purity of the products was determined using a reversed-phase HPLC method, which was performed on a Shimadzu HPLC system consisting of LC-6A pump, SPD-6AV detector, and Chromatopak C-R3A integrator with a analytical column, YMC-A312 (ODS) (150×6 mm i.d., Yamamura Chemical Laboratories Co., Ltd.). Solvent system of 30% CH_3CN (adjusted to pH 2.0 with phosphoric acid) was used as a mobile phase at a flow rate of 1.0 ml/minute, and UV absorbance was monitored at 254 nm. Retention times of **1**~**6** were 7.09, 5.81, 5.17, 6.51, 5.83, 12.03 minutes, respectively.

Photochemical Reaction of 2 and 3

2 or **3** (5 mg) was dissolved in 10 ml of 80% Me_2CO in 0.1 M citrate buffer (pH 3.5) and 5 mg of iodide were added to it. After adjusting to pH 5.2, the solution was exposed to a mercury lamp (UVL-400H-300P, Riko Science Industry; distance: 8 cm) in a water bath (25~30°C). The reaction solution (0.5 ml) was diluted with 2 ml of acidic water (pH 2.0, phosphoric acid) and 10 μl aliquote was injected for HPLC analysis.

Preparation of 4 and 5

Using 400 mg of **3** as a starting material, the photochemical reaction was carried out for 5 hours as described above. To the reaction solution were added 400 ml of H_2O, adjusted to pH 8.0, and the products were extracted with 800 ml of $CHCl_3$. The $CHCl_3$ layer was washed with 200 ml of saturated aqueous NaCl, concentrated *in vacuo* to a small volume, and precipitated with an excess of *n*-hexane to give 150 mg of crude powder containing **4** and **5**. This crude powder was applied to a column of silica gel (20 mm i.d., Wakogel C-200, 25 g) and eluted stepwise with $CHCl_3$-MeOH-concd NH_4OH (120:10:0.05, 80:10:0.1, 40:10:0.1). Each of crude fractions enriched in **4** and **5** was further purified by preparative HPLC (column: Capcell Pak C18 SG120, 5 μm, 250×30 mm i.d., Shiseido Co, Ltd.; mobile phase: 30% CH_3CN (adjusted to pH 2.0 with phosphoric acid), flow rate: 5 ml/minute, detection: UV at 254 nm). The fraction containing pure product was washed with toluene and the product was extracted with $CHCl_3$ at pH 8.0. The $CHCl_3$ layer was dried over anhydrous sodium sulfate and concentrated *in vacuo* to a small volume. To the concentrate, an excess of *n*-hexane was added to precipitate a yellowish orange powder. This procedure yielded 32 mg of **4** and 3 mg of **5**. **4**: MP 185~190°C (dec); $[\alpha]_D^{20}$ +131° (*c* 0.02, $CHCl_3$); IR (KBr) cm^{-1} 1671, 1622; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1\,cm}^{1\%}$) 229 (738), 258 (443), 433 (222); FAB-MS *m/z* 500 ($(M+H)^+$, $C_{26}H_{29}NO_9$); ^1H NMR ($CDCl_3$-CD_3OD, 20:1) δ 7.84 (1H, s, 11-H), 7.80 (1H, d, J=7.34 Hz, 1-H), 7.68 (1H, t, J=7.34 Hz, 2-H), 7.29 (1H, d, J=7.33 Hz, 3-H), 5.42 (1H, d, J=3.67 Hz, 1'-H), 5.11 (1H, br t, J=4.40 and 2.93 Hz, 7-H), 4.52 (1H, s, 10-H), 4.12 (1H, q, J=6.60 Hz, 5'-H), 3.49 (1H, br s, 4'-H), 3.03 (1H, br d, J=11.74 Hz, 3'-H), 2.23 (1H, dd, J=14.67 and 5.13 Hz, 8-Ha), 2.18 (1H, dd, J=14.68 and 2.93 Hz, 8-Hb), 1.7~1.83 (2H, 2'-CH_2), 1.7~1.8 (1H, 13-Ha), 1.63 (1H, m, J=7.34 Hz, 13-Hb), 1.33 (3H, d, J=5.86 Hz, 6'-CH_3), 1.06 (3H, t, J=7.34 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$-CD_3OD, 20:1) δ 192.65 (C-5), 181.75 (C-12), 162.43 (C-4), 161.52 (C-6), 147.73 (C-10a), 137.38 (C-2), 133.54* (C-6a), 133.12* (C-12a), 130.65 (C-11a), 124.85 (C-3), 121.76 (C-11), 120.23 (C-1), 115.87 (C-4a), 114.70 (C-5a), 101.11 (C-1'), 73.08 (C-9), 72.82 (C-10), 71.01 (C-7), 70.35 (C-4'), 67.45 (C-5'), 46.42 (C-3'), 33.50 (C-8), 32.86 (C-2'), 29.32 (C-13), 16.83 (C-6'), 6.55 (C-14), similar values asterisked may be interchanged; Rf value: 0.22 (system A), 0.15 (system B). **5**: MP 145~147°C (dec); $[\alpha]_D^{20}$ +70° (*c* 0.02, $CHCl_3$); IR (KBr) cm^{-1} 1630, 1607; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1\,cm}^{1\%}$) 230 (643), 257 (422), 435 (196); FAB-MS *m/z* 500 ($(M+H)^+$, $C_{26}H_{29}NO_9$); ^1H NMR ($CDCl_3$-CD_3OD, 20:1) δ 7.87 (1H, d, J=6.60 Hz, 1-H), 7.81 (1H, s, 11-H), 7.71 (1H, t, J=7.34 Hz, 2-H), 7.34 (1H, d, J=8.07 Hz, 3-H), 5.29 (1H, d, J=3.66 Hz, 1'-H), 4.94 (1H, s, 10-H), 4.93 (1H, 7-H), 4.07 (1H, q, J=6.60 Hz, 5'-H), 3.49 (1H, br s, 4'-H), 3.09 (1H, br d, J=12.47 Hz, 3'-H), 2.27 (1H, dd, J=14.67 and 5.14 Hz, 8-Ha), 2.18 (1H, d, J=13.94 Hz, 8-Hb), 1.87 (1H, m, J=7.34 Hz, 13-Ha), 1.84 (1H, t d, J=13.94 and 3.67 Hz, 2'-Ha), 1.72 (1H, m, J=7.34 Hz, 13-Hb), 1.67 (1H, dd, J=13.94 and 4.40 Hz, 2'-Hb), 1.35 (3H, d, J=6.60, 6'-CH_3), 1.11 (3H, t, J=7.34 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$-CD_3OD, 20:1) δ 187.86 (C-5), 187.57 (C-12), 162.66 (C-4), 162.18 (C-6), 142.99 (C-10a), 137.02 (C-2), 134.08* (C-6a), 133.21* (C-12a), 132.06 (C-11a), 125.17 (C-3), 120.48 (C-11), 119.62 (C-1), 116.15 (C-4a), 115.42 (C-5a), 98.53 (C-1'), 73.47 (C-7), 72.33 (C-9), 70.25 (C-4'), 67.70 (C-5'), 66.76 (C-10), 46.12 (C-3'), 33.71 (C-8), 33.20 (C-2'), 30.25 (C-13), 16.88 (C-6'), 6.56 (C-14), similar values asterisked may be interchanged; Rf value: 0.24 (system A), 0.20 (system B).

Hydrolysis of 4

4 (15 mg) was dissolved in 5 ml of 0.1 N HCl and hydrolyzed at 90°C for 30 minutes. The resulting precipitate consisting mainly of the aglycone was purified by preparative TLC (Silicagel 60 F_{254}, E. Merck) using a developing solvent of $CHCl_3$-MeOH (15:1). The yellow band (Rf=0.38) was scraped from the plates and eluted with $CHCl_3$-MeOH (10:1). After washing with H_2O, the eluate was concentrated *in vacuo* to a small volume and precipitated with an excess of *n*-hexane to give 11-deoxy-10-*epi*-β-rhodomycinone (**7**) as a yellow powder (7 mg). **7**: MP 189~193°C (dec); $[\alpha]_D^{20}$ +86° (*c*

0.02, $CHCl_3$); IR (KBr) cm^{-1} 1672, 1622, 1574; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1\,cm}^{1\%}$) 228 (966), 258 (593), 432 (300); FAB-MS *m/z* 371 ($(M+H)^+$, $C_{20}H_{18}O_7$), 353 $(M+H-H_2O)^+$; ^1H NMR ($CDCl_3$-CD_3OD, 10:1) δ 7.81 (1H, dd, J=7.33 and 1.10 Hz, 1-H), 7.87 (1H, s, 11-H), 7.71 (1H, dd, J=8.43 and 7.69 Hz, 2-H), 7.31 (1H, dd, J=8.43 and 1.10 Hz, 3-H), 5.20 (1H, dd, J=5.13 and 3.66 Hz, 7-H), 4.51 (1H, s, 10-H), 2.25 (1H, dd, J=14.66 and 5.12, 8-Ha), 2.09 (1H, ddd, J=14.66, 3.66 and 0.73 Hz, 8-Hb), 1.78 (1H, m, 13-Ha), 1.61 (1H, m, J=7.33 Hz, 13-Hb), 1.07 (3H, t, J=7.32 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$-CD_3OD, 2:1) δ 192.77 (C-5), 181.82 (C-11), 162.43 (C-4), 160.82 (C-6), 147.45 (C-10a), 137.46 (C-2), 133.62* (C-6a), 132.83* (C-12a), 124.85 (C-3), 121.72 (C-11), 120.29 (C-1), 115.89 (C-4a), 114.72 (C-5a), 73.50 (C-9), 72.94 (C-10), 62.81 (C-7), 34.56 (C-8), 28.96 (C-13), 6.48 (C-14), similar values asterisked may be interchanged.

Methylation of **3**

To a solution of **3** (50 mg) in 15 ml of MeOH were added 5 ml of CH_2N_2/ether and stirred at 25°C for 30 minutes. After dilution with 10 ml of 1% $NaHCO_3$, the methylated product was extracted with 20 ml of $CHCl_3$ and concentrated *in vacuo* to dryness. The product was purified by preparative TLC using a developing solvent of $CHCl_3$-MeOH-H_2O-AcOH-concd NH_4OH (120:50:5:1:1). The yellow band was scraped from the plates and eluted with $CHCl_3$-MeOH (5:1). To the eluate was added H_2O, washed with $CHCl_3$ and extracted with $CHCl_3$ at pH 8.0. The organic layer was concentrated *in vacuo* to a small volume and precipitated with an excess of *n*-hexane to afford 15-*O*-methyl-D788-3 (**8**) as a yellow powder (39 mg). **8**: FAB-MS *m/z* 542 ($(M+H)^+$, $C_{28}H_{31}NO_{10}$); ^1H NMR ($CDCl_3$) δ 7.75 (1H, d, J=7.33 Hz, 1-H), 7.63 (1H, s, 11-H), 7.63 (1H, t, J=7.33 Hz, 2-H), 7.26 (1H, d, 3-H), 5.44 (1H, br s, 1'-H), 5.22 (1H, br s, 7-H), 4.11 (1H, q, J=7.34 Hz, 5'-H), 4.09 (1H, s, 10-H), 3.70 (3H, s, 16-CH_3), 3.49 (1H, br s, 4'-H), 3.12 (1H, br d, J=9.53 Hz, 3'-H), 2.51 (1H, dd, J=15.41 and 4.41 Hz, 8-Ha), 2.32 (1H, d, J=15.41 Hz, 8-Hb), 1.7~1.8 (3H, 13-Ha and 2'-CH_2), 1.50 (1H, m, J=7.33 Hz, 13-Hb), 1.33 (3H, d, J=6.60 Hz, 6'-H), 1.08 (3H, t, J=7.34 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$) δ 192.53 (C-5), 181.11 (C-12), 171.22 (C-15), 162.50 (C-4), 162.02 (C-6), 142.60 (C-10a), 137.26 (C-2), 133.38 (C-12a), 132.78 (C-6a), 131.19 (C-11a), 124.73 (C-3), 120.89 (C-11), 120.05 (C-1), 115.70 (C-4a), 114.56 (C-5a), 101.38 (C-1'), 71.61 (C-9), 71.00 (C-7), 70.31 (C-5'), 66.98 (C-4'), 57.05 (C-10), 52.48 (C-16), 46.33 (C-3'), 33.77 (C-8), 32.27 (C-2'), 32.07 (C-13), 16.83 (C-6'), 6.63 (C-14).

Hydrolysis of **8**

8 (30 mg) was dissolved in 10 ml of 0.1 N HCl and hydrolyzed at 90°C for 30 minutes. The aglycone was extracted with $CHCl_3$ and purified by preparative TLC using a developing solvent of $CHCl_3$-MeOH (20:1). The yellow band was scraped from the plates and eluted with $CHCl_3$-MeOH (10:1). After washing with H_2O, the eluate was concentrated *in vacuo* to a small volume and precipitated with an excess of *n*-hexane to give aklavinone (**9**) as a yellow powder (19 mg). **9**: MP 197~200°C (dec); $[\alpha]_D^{20}$ +125° (*c* 0.02, $CHCl_3$); IR (KBr) cm^{-1} 1734, 1674, 1624; UV $\lambda_{1\,cm}^{90\%\ MeOH}$ nm ($E_{max}^{1\%}$) 229 (501), 258 (294), 289 (116), 431 (149); FAB-MS *m/z* 413 ($(M+H)^+$, $C_{22}H_{20}O_8$); ^1H NMR ($CDCl_3$) δ 12.74 (1H, s), 11.97 (1H, s), 7.84 (1H, d, J=7.61 Hz, 1-H), 7.73 (1H, s, 11-H), 7.71 (1H, t, J=7.34 Hz, 2-H), 7.32 (1H, d, J=7.33 Hz, 3-H), 5.39 (1H, br s, 7-H), 4.10 (1H, s, 10-H), 3.71 (3H, s, 16-CH_3), 2.55 (1H, dd, J=14.68 and 5.14 Hz, 8-Ha), 2.27 (1H, d, J=14.67 Hz, 8-Hb), 1.73 (1H, m, J=7.34 Hz, 13-Ha), 1.58 (1H, m, J=7.33 Hz, 13-Hb), 1.10 (3H, t, J=7.34 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$) δ 192.84 (C-5), 181.27 (C-12), 171.27 (C-15), 162.66 (C-4), 161.14 (C-6), 142.62 (C-10a), 137.52 (C-2), 133.55 (C-12a), 132.86 (C-6a), 132.70 (C-11a), 124.85 (C-3), 121.36 (C-11), 120.29 (C-1), 115.76 (C-4a), 114.74 (C-5a), 71.69 (C-9), 62.60 (C-7), 56.71 (C-10), 52.52 (C-16), 34.81 (C-8), 32.39 (C-13), 6.69 (C-14).

Preparation of **6**

The photochemical reaction of **2** (400 mg) was carried out in the same manner as described for **3**, and yielded 220 mg of crude powder of **6**. This crude powder was applied to a column of silica gel (20 mm i.d., Wakogel C-200, 25 g) and eluted stepwise with $CHCl_3$-MeOH-H_2O (100:10:0.5, 100:20:0.5) and $CHCl_3$-MeOH-H_2O-AcOH-concd NH_4OH (120:50:5:1:1). **6** was further purified by preparative TLC using a developing solvent of $CHCl_3$-MeOH-H_2O-AcOH-concd NH_4OH (120:50:5:1:1). The orange band was scraped from the plates and eluted with $CHCl_3$-MeOH (5:1). To the eluate was added a half volume of 0.1 N acetate buffer (pH 3.0), and **6** was reextracted with $CHCl_3$ at pH 8.0. The organic layer was washed with H_2O, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to a small volume. To the concentrate, an excess of *n*-hexane was added to precipitate **6** as a red orange powder (15 mg). **6**: MP 174~177°C (dec); $[\alpha]_D^{20}$ +17° (*c* 0.02, $CHCl_3$); IR (KBr) cm^{-1} 1601; UV $\lambda_{max}^{90\%}$ MeOH nm ($E_{1\,cm}^{1\%}$) 235 (969), 254 (515), 292 (185), 496 (338); FAB-MS *m/z* 516 ($(M+H)^+$, $C_{26}H_{29}NO_{10}$); ^1H NMR ($CDCl_3$-CD_3OD, 10:1) δ 7.85 (1H, d, J=8.07 Hz, 1-H), 7.71 (1H, t, J=8.07 Hz, 2-H), 7.30 (1H, d, J=8.80 Hz, 3-H), 5.42 (1H, br s, 1'-H), 4.93 (1H, t, J=5.87 Hz, 7-H), 4.74 (1H, s, 10-H), 4.14 (1H, q, J=6.60 Hz, 5'-H), 3.52 (1H, br s, 4'-H), 3.11~3.15 (1H, br m, 3'-H), 2.36 (1H, dd, J=13.94 and 5.87, 8-Ha), 2.08 (1H, dd, J=13.94 and 5.87 Hz, 8-Hb), 1.75~1.9 (2H, 2'-CH_2), 1.62 (1H, m, J=7.34 Hz, 13-Ha), 1.47 (1H, m, J=7.34 Hz, 13-Hb), 1.31 (3H, d, J=6.60 Hz, 6'-CH_3), 1.01 (3H, t, J=7.34 Hz, 14-CH_3); ^{13}C NMR ($CDCl_3$-CD_3OD, 10:1) δ 191.30 (C-5), 186.73 (C-12), 162.92 (C-4), 157.61* (C-11), 157.18* (C-6), 138.89 (C-10a), 137.66 (C-2), 136.89 (C-6a), 133.82 (C-12a), 125.32 (C-3), 120.18 (C-1), 116.45 (C-4a), 112.50 (C-5a), 112.08 (C-11a), 101.01 (C-1'), 72.19

(C-9), 71.57 (C-7), 70.33 (C-4'), 67.97 (C-5'), 67.91 (C-10), 46.97 (C-3'), 35.69 (C-8), 32.82 (C-2'), 30.51 (C-13), 17.24 (C-6'), 7.84 (C-14), similar values asterisked may be interchanged; Rf value: 0.18 (system A, in which 0.27 for **1**), 0.07 (system B, in which 0.24 for **1**).

Biological Activity

Inhibitory effects of the drugs on growth in murine leukemia L1210 cell culture were determined according to the method as described previously[15].

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