ARUGOMYCIN, A NEW ANTHRACYCLINE ANTIBIOTIC

III. BIOLOGICAL ACTIVITIES OF ARUGOMYCIN AND ITS ANALOGUES OBTAINED BY CHEMICAL DEGRADATION AND MODIFICATION

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Biological activities of arugomycin and its analogues obtained by chemical degradation and modification were evaluated. Differences in the sugar moieties affected their biological activities including induction of differentiation of mouse Friend erythroleukemia cells and mouse myeloid leukemia cells, antitumor activities against sarcoma S-180, Ehrlich ascites carcinoma and P388 leukemia, and cytotoxicity against murine leukemia cells. Some relationships were found between the sugar moieties and biological activities.

Arugomycin (AGM) is an anthracycline antibiotic produced by *Streptomyces violaceochromogenes*. It induces differentiation of mouse Friend erythroleukemia cells and shows antitumor activities against sarcoma S-180, Ehrlich carcinoma and P388. It possesses two sugar chains at C-7 and C-4′ of the aglycone, arugorol. The former chain consists of L-diginose (DIG), 2-deoxy-L-fucose (deFUC), DIG and 4-*O*-fumaryl-L-diginose, and the latter chain contains deFUC, DIG and L-decilonitrose (DEC). During the structural elucidation of AGM reported in the preceding papers[1,2], we obtained several analogues of AGM with different sugar moieties. In view of the important role of the sugar part for the biological activities of anthracycline antibiotics[3], it was of interest to compare the biological activities of AGM with 11 structural analogues and other anthracyclines (Figs. 1 and 2).

Materials and Methods

AGM, AG1, AG2, AG3, AG4 and AG6

These compounds were prepared as reported in the previous papers[1,2].

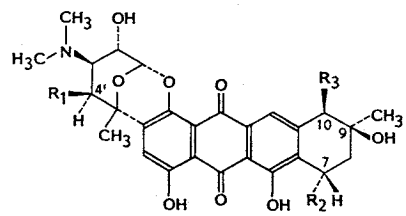
AG7

Degradation of AGM (200 mg) in 100 ml of a 0.01 N NaOH aqueous solution at 25°C for 60 minutes, followed by preparative silica gel TLC with chloroform - methanol (10 : 1) gave a defumaryl compound of AGM, named AG7 (38 mg).

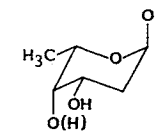
AG7 Acid

Hydrolysis of AGM (200 mg) in 100 ml of a 0.4 N LiOH aqueous solution at 25°C for 4 hours and acidification to pH 3.0 with 1 N HCl gave AG7 acid with a free carboxylic acid at C-10. AG7 acid (37 mg, Table 1) was recovered from the reaction mixture by Diaion HP-20 column chromatography (3×15 cm) with methanol, and preparative silica gel TLC with chloroform - methanol (10 : 1).

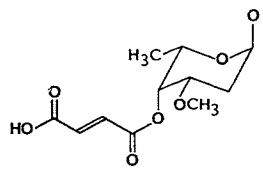
Fig. 1. Structures of arugomycin and its analogues.



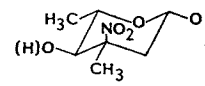
DIG



deFUC



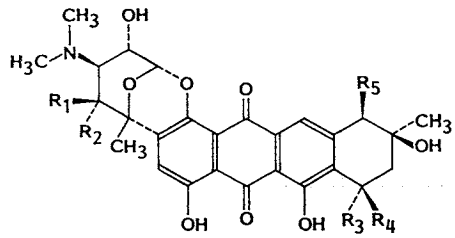
fDIG



DEC

AG1	R_1=deFUC-DEC	R_2=OH	R_3=$COOCH_3$
AG2	R_1=deFUC-DEC-DIG	R_2=DIG	R_3=$COOCH_3$
AG3	R_1=deFUC-DEC	R_2=DIG	R_3=$COOCH_3$
AG4	R_1=deFUC-DEC-DIG	R_2=DIG-deFUC	R_3=$COOCH_3$
AG6	R_1=deFUC-DEC-DIG	R_2=H	R_3=$COOCH_3$
AG7	R_1=deFUC-DEC-DIG	R_2=DIG-deFUC-DIG-DIG	R_3=$COOCH_3$
decAG7	R_1=deFUC-DEC-DIG	R_2=DIG-deFUC-DIG-DIG	R_3=H
AG7 acid	R_1=deFUC-DEC-DIG	R_2=DIG-deFUC-DIG-DIG	R_3=COOH
AGM	R_1=deFUC-DEC-DIG	R_2=DIG-deFUC-DIG-fDIG	R_3=$COOCH_3$

Fig. 2. Structures of arugorol analogues and menogarol.



7-Dis-*O*-arugorol	R_1=OH	R_2=H	R_3=OCH_3	R_4=H	R_5=$COOCH_3$
7-Dis-*O*-arugol	R_1=OH	R_2=H	R_3=OCH_3	R_4=R_5=H	
7-Con-*O*-arugol	R_1=OH	R_2=R_3=H		R_4=OCH_3	R_5=H
Menogarol	R_1=H	R_2=OH	R_3=H	R_4=OCH_3	R_5=H

decarbomethoxy AG7 (decAG7)

AG7 acid (30 mg) readily lost carbon dioxide in a DMF solution at room temp to give decAG7 in a similar manner to nogalamycin[4,5]. Pure decAG7 (18 mg, Table 1) was recovered from the reaction solution by removing DMF under reduced pressure and purification by silica gel column

Table 1. Physico-chemical properties of AG7, MeAGM, decAG7 and AG7 acid.

	AG7	MeAGM	decAG7	AG7 acid
Appearance	Orange powder	Orange powder	Orange powder	Orange powder
Molecular formula	$C_{76}H_{110}O_{34}N_2$	$C_{81}H_{114}O_{37}N_2$	$C_{74}H_{108}O_{32}N_2$	$C_{75}H_{108}O_{34}N_2$
MW (FD-MS) *m/z*	1,596	1,708	1,538	1,582
MP (°C, dec)	212~215	214~217	195~198	134~137
$[\alpha]_D^{25}$ (*c* 0.1, $CHCl_3$ - CH_3OH, 2:1)	+100.1°	+219.2°	+156.0°	+99.8°
Elemental analysis				
Found (Calcd)	C 57.03 (57.21), H 6.98 (6.95), N 1.83 (1.76)	C 57.32 (56.97), H 6.68 (6.73), N 1.56 (1.64)	C 57.93 (57.80), H 6.95 (7.08), N 1.89 (1.82)	C 56.78 (56.95), H 6.94 (6.86), N 1.69 (1.77)
UV λ_{max} nm (ε)				
in CH_3OH	234 (51,900), 257 (23,900), 290 (9,100), 465 (15,900)	234 (53,500), 257 (25,100), 289 (16,600), 478 (14,200)	235 (71,500), 258 (34,600), 289 (11,500), 478 (20,800)	236 (43,800), 262 (22,700), 479 (13,300)
in CH_3OH +0.1 N NaOH	238 (45,200), 290 (8,000), 540 (13,200)	238 (42,700), 285 (15,900), 540 (12,000)	245 (64,600), 295 (11,500), 545 (20,800)	

chromatography (2×30 cm) with chloroform - methanol - water (25:1:0.01).

Methyl Ester of AGM

An ethereal solution of diazomethane was added to 10 ml of a chloroform - methanol (9:1) solution containing AGM (200 mg), and the reaction mixture was then evaporated under reduced pressure. The residue was separated by preparative silica gel TLC with chloroform - methanol (10:1) and gel filtered on a Sephadex LH-20 column (2×70 cm) with chloroform - methanol (1:1) to give a methyl ester of AGM (MeAGM, 28 mg, Table 1).

7-Dis-*O*-methylarugorol

7-Dis-*O*-methylarugorol† (21 mg) was obtained by methanolysis of AGM (200 mg) in 3 ml of 5% hydrogen chloride - methanol in a sealed tube at 85°C for 5 hours and by purification by Diaion HP-20 column chromatography (3×15 cm) with methanol, silica gel column chromatography (2×30 cm) with chloroform - methanol - water (10:1:0.01) and Sephadex LH-20 column chromatography (2×70 cm) with chloroform - methanol (1:1). Its structure with oxygen atoms at C-7 and C-9 in *trans* location was determined by ^1H and ^{13}C NMR spectral analysis.

Anal Calcd for $C_{30}H_{38}O_{12}N$: C 60.10, H 5.55, N 2.34.
Found: C 60.01, H 5.42, N 2.23.

MP 202~204°C (dec); $[\alpha]_D^{29}$ +490.1° (*c* 0.16, CH_3OH); field desorption mass spectra (FD-MS) *m/z* 600 (MH^+); UV λ_{max}^{MeOH} nm (ε) 236 (43,100), 260 (20,300), 476 (33,300); ^1H NMR (CD_3OD) δ 1.55 (3H, s, 9-CH_3), 1.64 (3H, s, 6'-CH_3), 2.08 (1H, d, J=14.0 Hz, 8-H_a), 2.38 (1H, br d, J=14.0 Hz, 8-H_b), 2.49 (6H, s, NCH_3), 2.96 (1H, br d, J=11.8 Hz, 3'-H), 3.56 (3H, s, OCH_3), 3.62 (3H, s, $COOCH_3$), 4.04 (1H, br s, 4'-H), 4.37 (1H, dd, J=4.0 and 11.8 Hz, 2'-H), 4.56 (1H, br s, 7-H), 5.85 (1H, d, J=4.0 Hz, 1'-H), 6.56 (1H, s, 11-H), 7.15 (1H, s, 3-H); ^{13}C NMR (CD_3OD) δ 149.1 (C-1), 140.8 (C-2), 123.8 (C-3), 157.3 (C-4), 115.5 (C-4a), 191.5 (C-5), 114.7 (C-5a), 163.1 (C-6), 132.5 (C-6a), 76.9 (C-7), 34.6 (C-8), 70.4 (C-9), 58.4 (C-10), 149.2 (C-10a), 120.1 (C-11), 134.4 (C-11a), 180.9 (C-12), 115.5 (C-12a), 98.8 (C-1'), 69.2 (C-2'), 62.8 (C-3'), 73.0 (C-4'), 78.0 (C-5'), 22.9 (C-6'), 59.2 (OCH_3), 52.8 ($COOCH_3$), 43.5 (NCH_3).

7-Dis-*O*-methylarugol and 7-Con-*O*-methylarugol

Methanolysis (85°C, 5 hours in a sealed tube) of decAG7 (200 mg) in 3 ml of 5% hydrogen chlo-

† For the dis/con nomenclature, see ref 4.

Table 2. Physico-chemical properties of 7-con-*O*-methylarugol and 7-dis-*O*-methylarugol.

	7-Con-*O*-methylarugol	7-Dis-*O*-methylarugol
Appearance	Orange powder	Orange powder
Molecular formula	$C_{28}H_{31}O_{10}N$	$C_{28}H_{31}O_{10}N$
MW (FD-MS, MH^+) *m/z*	542	542
MP (°C, dec)	200~203	200~203
Elemental analysis		
Found (Calcd)	C 61.87 (62.10),	C 62.34 (62.10),
	H 5.81 (5.77),	H 5.71 (5.77),
	N 2.48 (2.51)	N 2.44 (2.51)
$[\alpha]_D^{29}$	+469° (*c* 0.1, CH_3OH)	+426.7° (*c* 0.12, CH_3OH)
UV λ_{max} nm (ε)		
in CH_3OH	235 (26,000), 258 (13,000), 290 (4,300), 475 (7,000)	236 (39,500), 260 (19,600), 476 (12,500)
in CH_3OH +0.01 N HCl	235 (26,800), 260 (12,300), 292 (4,100), 470 (8,800)	
in CH_3OH +0.01 N NaOH	238 (22,100), 295 (3,800), 542 (8,600)	

ride - methanol gave a mixture of two compounds, 7-dis-*O*-methylarugol (12 mg) and 7-con-*O*-methylarugol (11 mg), the latter possessed oxygen atoms at C-7 and C-9 in a *cis* relationship. They were separated by Diaion HP-20 column chromatography (3×15 cm) with methanol after washing the column with water, and silica gel column chromatography (2×30 cm) with chloroform - methanol - water (10:1:0.01). Physico-chemical properties of 7-dis-*O*-methylarugol and 7-con-*O*-methylarugol are summarized in Table 2 and NMR spectral data are shown in Table 3.

Differentiation of Friend Erythroleukemia Cell

Friend cells (1×10^5 cells/ml) were cultured (37°C, 4 days, CO_2 atmosphere) in an Eagle minimum essential medium (MEM) containing 10% fetal calf serum (FCS) with various concentrations of the test compounds. Then 100 μl of 0.5% *o*-dianisidine solution was added to 1 ml of the cultured media and the numbers of stained cells were counted under a microscope (×200). The stained cells were defined as hemoglobin positive cells differentiated from Friend erythroleukemia cells.

Differentiation of Mouse Myelocytic Leukemia Cells

Mouse myelocytic leukemia (M1) cells (1×10^5 cells/ml) were incubated in an EM† medium (37°C, 2 days, CO_2 atmosphere) containing 10% FCS with various concentrations of samples. The cultured cells were collected, transferred into serum free EM medium containing 1 μm latex beads and incubated for 14 hours in a CO_2 incubator. The cells, cultured in serum free EM medium were collected, washed with saline - phosphate buffer (PBS), suspended in 1 ml of PBS, and finally stained with eosin Y to observe the cells which contained latex beads. The cells taking up latex into themselves were judged to be differentiated M1 cells with recovered phagocytic activities.

Inhibition of DNA and RNA Synthesis in L-5178 Y Cells

L-5178 Y cells (5×10^5 cells/ml) were suspended in an Eagle MEM medium supplemented with 10% horse serum, 0.05% asparagine and 0.05% glutamine. Then 0.01-ml quantities of each ethanol solution of samples at various concentrations were added to 1 ml of the suspended culture medium. After shaking for 1 hour at 37°C, 0.05 μCi/ml of [2-^{3}H]uridine or [2-^{3}H]thymidine was added to the culture medium and incubated for 2 hours at 37°C. The cultured media were then filtered through a 0.45 μm membrane filter and the cells were washed twice with 2 ml of saline and then with 1 ml of 5% trichloroacetic acid and dried. The uptake of radioactivity was measured by a liquid scintillation counter. Inhibitory activities of samples are expressed as 50% inhibitory concentration (IC_{50}).

† EM medium is a modified Eagle MEM containing the twice amount of vitamins and amino acids.

Table 3. 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectral data of 7-con-*O*-methylarugol and 7-dis-*O*-methylarugol.

	7-Con-*O*-methylarugol	7-Dis-*O*-methylarugol
^{1}H NMR:		
	1.28 (3H, s, 9-CH_3)	1.37 (3H, s, 9-CH_3)
	1.64 (3H, s, 6′-CH_3)	1.70 (3H, s, 6′-CH_3)
	1.95 (1H, br d, J=14.2 Hz, 8-H_a)	2.04 (1H, br d, J=10.8 Hz, 8-H_a)
	2.40 (1H, br d, J=14.2 Hz, 8-H_b)	2.60 (6H, s, NCH_3)
	2.64 (6H, s, NCH_3)	2.66 (1H, br d, J=10.8 Hz, 8-H_b)
	3.08 (1H, dd, J=2.8, 12.0 Hz, 3′-H)	2.95 (1H, dd, J=2.8, 11.7 Hz, 3′-H)
	3.56 (3H, s, OCH_3)	3.44 (3H, s, OCH_3)
	4.16 (1H, d, J=2.8 Hz, 4′-H)	4.16 (1H, d, J=2.8 Hz, 4′-H)
	4.44 (1H, dd, J=3.4, 12.0 Hz, 2′-H)	4.40 (1H, dd, J=3.6, 11.7 Hz, 2′-H)
	4.64 (1H, s, 7-H)	4.44 (1H, br s, 7-H)
	5.92 (1H, d, J=3.4 Hz, 1′-H)	5.89 (1H, d, J=2.8, 11.7 Hz, 3′-H)
	6.48 (1H, s, 11-H)	6.69 (1H, s, 11-H)
	7.33 (1H, s, 3-H)	7.26 (1H, s, 3-H)
^{13}C NMR:		
	149.3 (C-1)	148.8 (C-1)
	141.0 (C-2)	140.8 (C-2)
	123.9 (C-3)	123.5 (C-3)
	157.4 (C-4)	157.5 (C-4)
	115.7 (C-4a)	115.7 (C-4a)
	191.8 (C-5)	191.8 (C-5)
	113.6 (C-5a)	113.9 (C-5a)
	162.4 (C-6)	162.5 (C-6)
	130.9 (C-6a)	131.7 (C-6a)
	76.5 (C-7)	76.0 (C-7)
	36.9 (C-8)	40.9 (C-8)
	69.3 (C-9)	69.6 (C-9)
	44.9 (C-10)	45.7 (C-10)
	147.1 (C-10a)	148.4 (C-10a)
	121.4 (C-11)	121.0 (C-11)
	134.0 (C-11a)	133.9 (C-11a)
	181.0 (C-12)	180.9 (C-12)
	115.7 (C-12a)	115.7 (C-12a)
	98.9 (C-1′)	98.8 (C-1′)
	69.1 (C-2′)	68.7 (C-2′)
	62.8 (C-3′)	62.8 (C-3′)
	72.7 (C-4′)	73.2 (C-4′)
	78.2 (C-5′)	78.2 (C-5′)
	22.9 (C-6′)	22.9 (C-6′)
	58.2 (OCH_3)	58.0 (OCH_3)
	43.4 (NCH_3)	42.8 (NCH_3)
	30.2 (9-CH_3)	29.4 (9-CH_3)

The spectra were taken in CD_3OD and the chemical shifts were expressed in ppm from internal TMS.

Antitumor Activity (*In Vivo*)

Experimental conditions for treating Ehrlich ascites carcinoma, sarcoma S-180 and P388 are shown in Tables 6, 7 and 8, respectively. AGM and its analogues were dissolved in 0.001 N NaOH aqueous solution.

Results

Differentiation of and Cytotoxicity against Friend Erythroleukemia cells, and Inhibition of DNA and RNA Synthesis in L-5178 Y Cells

AGM and its analogues induced differentiation of Friend erythroleukemia cells as shown in Table 4. AGM and AG7, both possessing seven sugar residues, were more cytotoxic than those with fewer sugar moieties. AG2, AG3, AG4, AG7, MeAGM and AGM showed stronger inhibitions of RNA synthesis than DNA synthesis. AG1, lacking the sugar chain at C-7, showed non-selective inhibition against DNA and RNA synthesis.

Table 4. Differentiation inducing activity of Friend erythroleukemia cells and inhibition of DNA and RNA syntheses in L-5178Y cell by arugomycin and its analogues.

Compound	Dose (μg/ml)	Benzidine positive cells (%)	Number of cells (cells/ml)	IC_{50} (μg/ml)		IC_{50} ratio on DNA/RNA
				RNA	DNA	
AGM	0.063	27.4	1.3×10^6	0.4	1.8	4.5
	0.125	75.4	0.5×10^6			
	0.25	15.3	0.4×10^6			
	0.50	0.0	0.0×10^6			
AG1	0.125	1.2	2.0×10^6	5.0	9.0	1.8
	0.25	7.3	1.3×10^6			
	0.50	17.6	1.2×10^6			
	1.25	75.7	0.4×10^6			
	2.50	25.0	0.1×10^6			
	5.0	0.0	0.0×10^6			
AG2	0.125	8.8	2.1×10^6	0.7	3.5	5.0
	0.25	67.0	1.3×10^6			
	0.50	91.9	0.6×10^6			
	1.25	15.4	0.6×10^6			
	2.50	0.0	0.0×10^6			
AG3	0.125	15.8	1.3×10^6	2.0	10.0	5.0
	0.25	35.8	1.4×10^6			
	0.50	76.1	0.2×10^6			
	1.25	0.0	0.0×10^6			
AG4	0.125	1.4	2.8×10^6	2.5	13.0	5.2
	0.25	22.6	1.3×10^6			
	0.50	76.5	1.2×10^6			
	1.25	0.0	0.0×10^6			
AG7	0.05	7.7	1.2×10^6	1.1	7.0	6.4
	0.10	59.7	1.2×10^6			
	0.20	14.8	0.1×10^6			
	0.50	0.0	0.0×10^6			
MeAGM	0.05	9.6	1.5×10^6	—	—	—
	0.10	51.5	5.8×10^6			
	0.20	15.0	1.0×10^6			
	1.00	0.0	0.0×10^6			
Control	—	0.5	2.0×10^6			

Benzidine positive cells are defined as those stained with *o*-dianisidine.

Friend cells at 1.0×10^5 cells/ml were incubated with various concentrations of AGM or its derivatives for 4 days.

—: Not tested.

Table 5. Growth inhibition and induction of phagocytic activity of M1 cells by arugomycin and its analogues.

Compound	Dose (μg/ml)	Number of cells ($\times 10^5$ cells/ml)	Phagocytic cells (%)
AGM	0.039	12.2	—
	0.078	13.0	—
	0.16	11.3	—
	0.31	11.1	4
	0.63	3.3	13
	1.3	0	—
AG1	0.31	15.1	—
	0.63	11.4	—
	1.3	5.6	—
	2.5	3.1	—
	5.0	0	—
AG2	0.020	14.4	—
	0.039	12.6	—
	0.078	8.9	—
	0.16	3.3	—
	0.31	0	—
AG4	0.039	12.5	—
	0.078	12.3	—
	0.16	12.8	—
	0.31	8.8	—
	0.63	1.8	—
	1.3	0	—
AG7	0.020	12.5	5
	0.039	6.6	28
	0.078	0.5	7
	0.16	0	—
MeAGM	0.010	12.4	3
	0.020	11.3	19
	0.039	5.6	25
	0.078	0	—
None	0	14.9	—

M1 cells were incubated with 1 μm latex for 14 hours and stained with eosin Y to observe phagocytic activity.
—: Not detected.

Growth Inhibition and Induction of Phagocytic Activity of M1 Cells

AGM, AG7 and MeAGM possessing the longer sugar chain at C-7 induced the phagocytic activity in M1 cells. AG1, AG2 and AG4 with one or two sugars at C-7 did not induce phagocytic activity, although AG2 showed strong growth inhibition activity comparable with that of AG7 and MeAGM as shown in Table 5.

Table 6. Antitumor activity of arugomycin and its analogues against Ehrlich ascites carcinoma.

Compound	Dose (mg/kg)	T/C (%)
AGM[a]	0.063	96
	0.13	107
	0.25	154
	0.50	189
	1.0	119
AG7[b]	0.50	205
	1.0	240
	2.0	270
	4.0	71
MeAGM[b]	0.50	88
	1.0	111
	2.0	234
	4.0	159

[a] Treatment: 1, 3, 5 days, ip; tumor inoculum, 1.0×10^6 ascites cells implanted ip; host, *dd*Y female mice, 8 animals/dose.
[b] Treatment: 1, 5 days, ip; tumor inoculum, 1.0×10^6 ascites cells implanted ip; host, ICR female mice, 8 animals/dose.

Table 7. Antitumor activity of arugomycin and other anthracyclines against sarcoma S-180 ascites tumor.

Compound	Dose (mg/kg)	T/C (%)	Survivors on day 60/total
Arugomycin	0.063	93	0/8
	0.13	126	0/8
	0.25	199	0/8
	0.50	>457	2/8
	1.0	>464	3/8
	2.0	79	0/8
Aclarubicin	2.0	103	0/8
	4.0	133	0/8
	8.0	>282	2/8
	16.0	79	0/8
Doxorubicin	1.0	>506	5/8
	2.0	>503	5/8
	4.0	>406	1/8
	8.0	>417	1/8
Daunorubicin	1.0	>409	2/8
	2.0	>262	2/8
	4.0	200	0/8
	8.0	101	0/8

Treatment: 1, 5 days, ip; tumor inoculum, 1.0×10^6 ascites cells implanted ip; host, ICR female mice, 8 animals/dose.

Table 8. Antitumor activity of arugomycin and its analogues against P388 leukemia.

Compound	Dose (mg/kg)	T/C (%)	Compound	Dose (mg/kg)	T/C (%)
AGM	0.063	106	AG7 acid	2.0	104
	0.13	103		4.0	112
	0.25	82[a]		8.0	117
	0.50	64[a]		16.0	117
AG1	2.0	107		32.0	128
	4.0	107	decAG7	2.0	112
	8.0	118		4.0	119
	16.0	120		8.0	126
	32.0	124		16.0	126
AG2	1.3	100		32.0	151
	2.5	119	AG6	2.0	135
	5.0	116		4.0	137
	10.0	134		8.0	158
	20.0	135		16.0	147
	30.0	159		32.0	143
	40.0	86[a]	7-Dis-*O*-	1.0	96
AG3	0.5	109	methylarugorol	2.0	104
	1.0	115		4.0	111
	2.0	123		8.0	122
	4.0	138		16.0	124
	8.0	139		32.0	126
	16.0	158	7-Dis-*O*-	2.0	100
AG4	2.5	114	methylarugol	4.0	103
	5.0	115		8.0	106
	10.0	111		16.0	118
	20.0	81		32.0	121
AG7	0.5	113	7-Con-*O*-	2.0	121
	1.0	113	methylarugol	4.0	127
	2.0	126		8.0	130
	4.0	99[a]		16.0	140
Menogarol[4]	12.5	297		32.0	140

Treatment: 1, 5 days ip; tumor inoculum, 1.0×10^6 P388 cells implanted ip; host, CDF1 female mice, 8 animals/dose.

[a] Excessive weight loss (more than 20%) were observed due to toxicity of compound.

Antitumor Activity Against Ehrlich Ascites Carcinoma and Sarcoma S-180 Ascites Tumor

Antitumor activities against Ehrlich ascites carcinoma and sarcoma S-180 ascites tumor are shown in Tables 6 and 7, respectively. Activity of AGM against sarcoma S-180 was almost the same as those of aclarubicin and daunorubicin but weaker than that of doxorubicin.

Activities against P388 Leukemia

AG2, AG3 and AG6 showed stronger activities than the other tested compounds as shown in Table 8. 7-Con-*O*-methylarugol showed stronger activity than 7-dis-*O*-methylarugol. Menogarol showed stronger antitumor activity against P388 than 7-con-*O*-methylarugol, the C-4′ epimer of menogarol[4]. Excessive weight loss of the host was observed at relatively low dose level of AGM and AG7.

Discussions

Although AGM and all its analogues induced differentiation of Friend erythroleukemia cells (50~90%), only AGM, AG7 and MeAGM slightly induced differentiation of M1 cells. The presence of four sugars in the sugar chain at the C-7 position of the aglycone was important for inducing the differentiation of M1 cells. AGM showed stronger antitumor activity against allogenic murine tumors (sarcoma S-180 and Ehrlich ascites carcinoma) than syngenic murine tumor (P388 leukemia). AGM showed weak antitumor activity against P388, but AG2 and AG6 showed stronger activities against P388.

For M1 cell differentiation inducing activity, we observed some relationships between the differentiation inducing activity and sugar structures, indicating that the four sugars in the sugar chain at C-7 of the aglycone was necessary. On the other hand, the results of anticancer activity against P388 did not indicate any relationship between the activity and sugar structures.

Stereoisomerisms at C-7, C-9 and C-4′ of the aglycone were very important for the antitumor activity against P388. 7-Dis-*O*-methylarugol and 7-con-*O*-methylarugol are stereoisomers with the oxygen substituents *cis* and *trans* at C-7 and C-9, respectively. The *cis* compound, 7-con-*O*-methylarugol, showed stronger *in vivo* antitumor activity against P388 than the *trans* compound. Menogarol showed stronger antitumor activity against P388 than 7-con-*O*-methylarugol, the C-4′ epimer of menogarol. These results suggest that the stereoisomerism was very important for the biological activity of arugomycin derivatives and that the C-4′ epimerism was more significant than isomerization at C-7 and C-9. Microbial screens and the development of new chemical procedures, allowing the modification of both sugar and aglycone moieties of anthracycline antibiotics, might furnish the tools for more detailed structure-activity relationships.

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