SYNTHESIS AND ANTITUMOR ACTIVITY OF 4'-*O*-ACYLANTHRACYCLINES

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(Received for publication September 14, 1991)

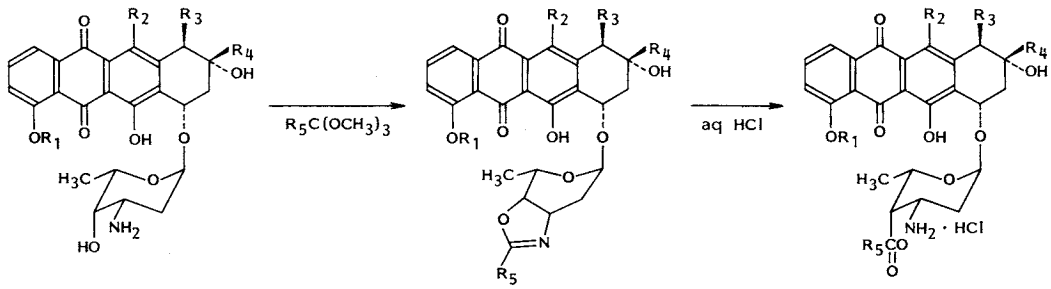
4'-*O*-Acyl derivatives of doxorubicin, daunorubicin, 13-deoxocarminomycin, 13-deoxo-10-hydroxycarminomycin, 13-deoxo-11-deoxycarminomycin were synthesized through the formation and mild acid hydrolysis of 2-oxazoline intermediates. The antitumor activity of these 4'-*O*-acyl derivatives against P388 leukemia was similar to or more effective than the parent anthracyclines.

In the continuing effort to elucidate the structure-activity relationships of anthracycline antibiotics, and to obtain analogs which have stronger antitumor activity, lower toxicity, or both[1], it has been demonstrated that some analogs modified at C-4' position, such as 4'-*O*-THP-doxorubicin[2] and 4'-epidoxorubicin[3], possess improved activity along with decreased toxicity compared with adriamycin itself. This fact prompted us to synthesize 4'-*O*-acylanthracyclines[4]. In this paper, we report the synthesis and antitumor activities of 4'-*O*-acetyl analogs of doxorubicin[5], daunorubicin[6], 13-deoxocarminomycin[7], 13-deoxo-10-hydroxycarminomycin (oxaunomycin)[8] and 13-deoxo-11-deoxycarminomycin[9] as their hydrochlorides. We also report the synthesis of 4'-*O*-butyroyl, valeroyl and benzoyl derivatives of 13-deoxocarminomycin to elucidate the relationship between the lipophilicity index of their acyl moiety and antitumor activity.

Chemical Synthesis

Treatment of daunorubicin (**1b**) with trimethyl orthoacetate under reflux conditions gave a 2-methyl-2-oxazoline derivative (**2b**) in 92% yield[10]. The ^{1}H NMR spectrum showed the anomeric proton as a dd (J=6.6 and 9.2 Hz) at 5.40 ppm. These J values suggested a pseudo-boat conformation for the sugar ring. The signals assigned to 3'-H and 4'-H, which were observed as a br d at 4.34 ppm and as a dd at 4.42 ppm, clearly confirmed the structure of **2b**. Mild acid hydrolysis of the oxazoline (**2b**) gave 4'-*O*-acetyldaunorubicin hydrochloride (**3b**) in 77% yield. The signals at 2.24 ppm and 5.13 ppm observed in ^{1}H NMR spectrum of **3b** obviously indicated that the 4'-hydroxyl group was acetylated. Hydrolysis under alkaline condition or drastic acid hydrolysis gave *N*-acetyldaunorubicin or a mixture of **1b** and daunomycinone, respectively. Also, other 4'-*O*-acylanthracyclines were synthesized according to the manner

† Deceased.



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	R_1	R_2	R_3	R_4	R_5
a	CH_3	OH	H	$COCH_2OH$	CH_3
b	CH_3	OH	H	$COCH_3$	CH_3
c	H	OH	H	CH_2CH_3	CH_3
d	H	OH	OH	CH_2CH_3	CH_3
e	H	H	H	CH_2CH_3	CH_3
f	H	OH	H	CH_2CH_3	$(CH_2)_2CH_3$
g	H	OH	H	CH_2CH_3	$(CH_2)_3CH_3$
h	H	OH	H	CH_2CH_3	Ph

described above. For every 2-oxazoline or 4′-*O*-acyl derivative thus obtained, similar charactaristic signals for **2b** and **3b** are observed on their ^{1}H NMR spectrum, which allowed assignment of the structures illustrated in the figure.

Antitumor Activity

Growth inhibitory effects of 4′-*O*-acyl analogs (**3a**～**3h**) on P388 *in vitro* were tested in comparison with their parent anthracyclines (**1a**～**1e**). The results are summarized in Table 1. Except for **3e**, 4′-*O*-acetyl derivatives had much less potency. 4′-*O*-Butyroyl, valeroyl, and benzoyl derivatives (**3f**, **3g** and **3h**) were more potent than 4′-*O*-acetyl analog (**3c**). Tables 2 and 3 show antitumor activity of the parent compounds and 4′-*O*-acyl analogs against P388 leukemia in CDF_1 mice, respectively. Although 4′-*O*-acetyl derivatives showed much less potency on P388 *in vitro*, they were shown to have similar or better activity compared with their parent anthracyclines. Higher acyl analogs (**3f** and **3g**) or an aromatic acyl analog (**3h**) showed similar activity to **3c**.

Table 1. Growth inhibitory effect on P388 *in vitro*.

Compound	IC_{50} (ng/ml)	Compound	IC_{50} (ng/ml)
1a	26.3	**3c**	33.0
1b	52.0	**3d**	27.5
1c	12.2	**3e**	150
1d	2.2	**3f**	24.0
1e	171	**3g**	20.1
3a	120	**3h**	12.0
3b	91.2		

IC_{50} values were determined in day 3 culture.

The antitumor activity of 2-oxazolines was also examined and the results are shown in Table 4. As can be seen, 2-methyl, 2-propyl, and 2-butyl-2-oxazolines (**2c**, **2f** and **2g**) were active against P388 leukemia, but 2-phenyl oxazoline (**2h**) was not.

Experimental

MP's were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-140 polarimeter. IR spectra were recorded with a Jasco A-3 spectrophotometer. ^{1}H NMR spectra were recorded at 500 MHz with a Jeol GX-500 spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane. Doxorubicin and daunorubicin were purchased

Table 2. Antitumor activity of the parent anthracyclines against P388 leukemia in CDF_1 mice.

Compound	Route	Dose (mg/kg)	T/C (%)	Compound	Route	Dose (mg/kg)	T/C (%)
1a	iv	1.8	123	**1d**	iv	0.125	112
		2.6	140			0.25	131
		4.0	179			0.5	207
		8.9	190			1	252
		13.3	240			2	188
		20	143	**1e**	iv	4	109
1b	iv	2.5	107			8	141
		5	136			16	158
		10	163			32	88
		20	143				
1c	ip	0.125	132				
		0.25	149				
		0.5	143				
		1	156				
		2	127				

P388 leukemia cells (10^6/mouse) were inoculated ip on day 0. The compounds were administered ip or iv on days 1 and 5.

Table 3. Antitumor activity of 4′-*O*-acylanthracyclines against P388 leukemia in CDF_1 mice.

Compound	Route	Dose (mg/kg)	T/C (%)	Compound	Route	Dose (mg/kg)	T/C (%)
3a	iv	2	126	**3e**	iv	4	138
		4	124			8	157
		8	188			16	221
		16	240			32	145
3b	iv	2	107	**3f**	ip	2	146
		4	110			4	190
		8	140			8	270
		16	219			16	98
3c	ip	1	143	**3g**	ip	2	159
		2	143			4	184
		4	190			8	224
		8	273			16	154
3d	iv	0.25	108	**3h**	ip	2	134
		0.5	142			4	153
		1	188			8	199
		2	263			16	195

P388 leukemia cells (10^6/mouse) were inoculated ip on day 0. The compounds were administered ip or iv on days 1 and 5.

from Aldrich. 13-Deoxocarminomycin, 13-deoxo-10-hydroxycarminomycin and 13-deoxo-11-deoxycarminomycin were obtained from fermentation broths[11]. Synthetic procedure and physico-chemical data of a 2-methyl-2-oxazoline derivative of doxorubicin (**2a**) and 4′-*O*-acetyldoxorubicin hydrochloride (**3a**) were reported previously[4].

A 2-Methyl-2-oxazoline Derivative of Daunorubicin (**2b**)

A solution of daunorubicin (49.5 mg, 0.088 mmol) in trimethyl orthoacetate (5 ml) was stirred under reflux for 2 hours, and evaporated. The residue was purified by column chromatography on silica gel (Wakogel C-200, 10 g) with chloroform-methanol (100:1) as an eluent to give **2b** (44.8 mg, 0.081 mmol, 92% yield) after crystallization from chloroform-hexane; mp 207~209°C (dec); $[\alpha]_D^{27}$ +24° (*c* 0.2, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.40 (1H, dd, J=6.6 and 9.2 Hz, 1′-H), 4.42 (1H, dd, J=1.8 and 10.4 Hz, 4′-H), 4.34 (1H, br d, J=10.4 Hz, 3′-H), 4.01 (1H, m, 5′-H), 2.04 (3H, s, C-CH_3).

Anal Calcd for $C_{28}H_{29}NO_{10}$: C 63.15, H 5.30, N 2.54.
Found: C 62.84, H 5.32, N 2.31.

Table 4. Antitumor activity of 2-oxazoline derivatives against P388 leukemia in CDF_1 mice.

Compound	Dose (mg/kg)	T/C (%)	Compound	Dose (mg/kg)	T/C (%)
2a	2	157	**2f**	2	109
	4	170		4	124
	8	186		8	154
	16	193		16	183
2b	2	115	**2g**	2	111
	4	121		4	117
	8	134		8	138
	16	146		16	172
2c	0.25	136	**2h**	2	115
	0.5	140		4	109
	1	155		8	101
	2	164		16	106
	4	154			

P388 leukemia cells (10^6/mouse) were inoculated ip on day 0. The compounds were administered ip on days 1 and 5.

A 2-Methyl-2-oxazoline Derivative of 13-Deoxocarminomycin (**2c**)

2c was prepared from 13-deoxocarminomycin (**1c**) in the same manner as above described in 79% yield; mp 131~132°C (dec); $[\alpha]_D^{27}$ −3° (*c* 0.3, chloroform).

Anal Calcd for $C_{28}H_{29}NO_9$: C 64.24, H 5.58, N 2.68.
Found: C 64.00, H 5.66, N 2.39.

A 2-Methyl-2-oxazoline Derivative of 13-Deoxo-10-hydroxycarminomycin (**2d**)

2d was prepared from 13-deoxo-10-hydroxycarminomycin (oxaunomycin) (**1d**) in 77% yield; mp 183~186°C (dec); $[\alpha]_D^{25}$ −28° (*c* 0.12, chloroform).

Anal Calcd for $C_{28}H_{29}NO_{10}$: C 62.33, H 5.42, N 2.60.
Found: C 62.68, H 5.65, N 2.41.

A 2-Methyl-2-oxazoline Derivative of 13-Deoxo-11-deoxycarminomycin (**2e**)

2e was prepared from 13-deoxo-11-deoxycarminomycin (**1e**) in 63% yield; mp 116~118°C (dec); $[\alpha]_D^{27}$ −10° (*c* 0.24, chloroform).

Anal Calcd for $C_{28}H_{29}NO_8$: C 66.26, H 5.76, N 2.76.
Found: C 66.55, H 5.91, N 2.52.

A 2-Propyl-2-oxazoline Derivative of 13-Deoxocarminomycin (**2f**)

2f was prepared from 13-deoxocarminomycin (**1c**) and trimethyl orthobutylate in 55% yield; mp 114~117°C (dec); $[\alpha]_D^{24}$ −204° (*c* 0.1, chloroform).

Anal Calcd for $C_{30}H_{33}NO_9$: C 65.33, H 6.03, N 2.54.
Found: C 65.58, H 6.16, N 2.26.

A 2-Butyl-2-oxazoline Derivative of 13-Deoxocarminomycin (**2g**)

2g was prepared from 13-deoxocarminomycin (**1c**) and trimethyl orthovalerate in 31% yield; mp 108~110°C (dec); $[\alpha]_D^{24}$ −400° (*c* 0.05, chloroform).

Anal Calcd for $C_{31}H_{35}NO_9$: C 65.83, H 6.24, N 2.48.
Found: C 66.00, H 6.32, N 2.17.

A 2-Phenyl-2-oxazoline Derivative of 13-Deoxocarminomycin (**2h**)

2h was prepared from 13-deoxocarminomycin (**1c**) and trimethyl orthobenzoate in 41% yield; mp 215~218°C (dec); $[\alpha]_D^{24}$ −576° (*c* 0.15, chloroform).

Anal Calcd for $C_{33}H_{31}NO_9$: C 67.68, H 5.34, N 2.39.
Found: C 68.02, H 5.45, N 2.03.

4'-*O*-Acetyldaunorubicin Hydrochloride (**3b**)

A mixture of **2b** (44.8 mg, 0.081 mmol) and 1 N hydrochloric acid (0.15 ml) in acetone (5 ml) was stirred at room temperature for 2.5 hours. Then, diethyl ether (100 ml) was added to the mixture, and the reddish precipitates were collected. Recrystallization from methanol and diethyl ether gave **3b** (38.1 mg, 0.063 mmol, 77% yield) as a red powder; mp 160~163°C (dec); $[\alpha]_D^{24}$ +224° (*c* 0.2, methanol); IR (KBr) cm^{-1} 1750 ($OCOCH_3$); ^1H NMR ($CDCl_3$, CD_3OD) δ 2.24 (3H, s, $OCOCH_3$), 5.13 (1H, s, 4'-H).

Anal Calcd for $C_{29}H_{32}NO_{11}Cl$: C 57.48, H 5.32, N 2.31.
Found: C 57.83, H 5.55, N 2.17.

4'-*O*-Acetyl-13-deoxocarminomycin Hydrochloride (**3c**)

3c was prepared from **2c** in 73% yield; mp 172~173°C (dec); $[\alpha]_D^{27}$ +233° (*c* 0.22, methanol).

Anal Calcd for $C_{28}H_{32}NO_{11}Cl$: C 58.18, H 5.58, N 2.42.
Found: C 58.26, H 5.81, N 2.11.

4'-*O*-Acetyl-13-deoxo-10-hydroxycarminomycin Hydrochloride (**3d**)

3d was prepared from **2d** in 83% yield; mp 169~172°C (dec); $[\alpha]_D^{25}$ +216° (*c* 0.14, methanol).

Anal Calcd for $C_{28}H_{32}NO_{12}Cl$: C 56.62, H 5.43, N 2.35.
Found: C 56.87, H 5.59, N 2.27.

4'-*O*-Acetyl-13-deoxo-11-deoxycarminomycin Hydrochloride (**3e**)

3e was prepared from **2e** in 47% yield; mp 169~170°C (dec); $[\alpha]_D^{27}$ +122° (*c* 0.24, methanol).

Anal Calcd for $C_{28}H_{32}NO_{10}Cl$: C 59.84, H 5.74, N 2.49.
Found: C 60.12, H 5.96, N 2.33.

4'-*O*-Butyroyl-13-deoxocarminomycin Hydrochloride (**3f**)

3f was prepared from **2f** in 79% yield; mp 152~155°C (dec); $[\alpha]_D^{24}$ +222° (*c* 0.2, methanol).

Anal Calcd for $C_{30}H_{36}NO_{11}Cl$: C 59.45, H 5.99, N 2.31.
Found: C 59.11, H 6.06, N 2.08.

4'-*O*-Valeroyl-13-deoxocarminomycin Hydrochloride (**3g**)

3g was prepared from **2g** in 74% yield; mp 149~152°C (dec); $[\alpha]_D^{24}$ +198° (*c* 0.2, methanol).

Anal Calcd for $C_{31}H_{38}NO_{11}Cl$: C 60.05, H 6.18, N 2.26.
Found: C 60.38, H 6.21, N 2.02.

4'-*O*-Benzoyl-13-deoxocarminomycin Hydrochloride (**3h**)

3h was prepared from **2h** in 66% yield; mp 165~167.5°C (dec); $[\alpha]_D^{24}$ −24° (*c* 0.2, methanol).

Anal Calcd for $C_{33}H_{34}NO_{11}Cl$: C 61.92, H 5.35, N 2.19.
Found: C 62.21, H 5.41, N 2.01.

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