14-ESTERS OF 7-*O*-(3,4-DI-*O*-ACETYL-2,6-DIDEOXY-α-L-*LYXO*-HEXOPYRANOSYL)ADRIAMYCINONE: SYNTHESIS AND ANTITUMOR ACTIVITY*

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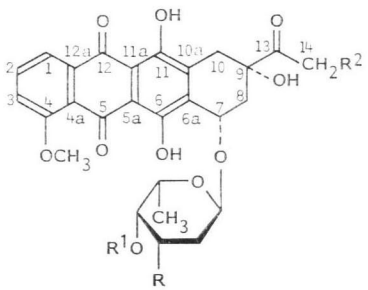
A range of 14-esters (**8**~**13**) of the title compound, 3′-deamino-3′-hydroxydoxorubicin 3′,4′-diacetate (**5**), has been synthesized by nucleophilic substitution of the corresponding 14-bromide (**6**) by the appropriate sodium carboxylate salts. Antitumor activities were determined *in vivo* in the murine P388 lymphocytic leukemia assay and compared with those of the 14-hydroxy and 14-acetoxy analogs.

Recent publications from this laboratory have described 3′-deamino-3′-hydroxy analogs of daunorubicin[2], doxorubicin[3], and their 14-substituted derivatives[1,4]. This series of compounds is of interest because of their antitumor activity, their low toxicity, and their possible significance in consolidating our understanding of the mechanism whereby the anthracycline antibiotics exert their anticancer activity. Work from this laboratory has already demonstrated that the presence of a 3′-amino group is not essential for manifestation of biological activity.

It is known that esters of doxorubicin (**2**) display significant antitumor activity[5]. In this context, it is noteworthy that the 14-*O*-acetyl derivative (**7**) of 3′-deamino-3′-hydroxydoxorubicin 3′,4′-diacetate (**5**) showed high activity (T/C 261), close to that of the 14-hydroxy derivative itself (T/C 269)[4].

This retention of activity upon esterification, combined with altered solubility parameters and possible differences in pharmacological characteristics, makes the 14-esters of anthracycline antibiotics significant analogs having possible clinical potential.

The foregoing rationalize prompted the present synthesis of a series of 14-esters (**8**~**13**) of 3′-deamino-3′-hydroxydoxorubicin 3′,4′-diacetate.



	R	R^1	R^2
1	NH_2	H	H
2	NH_2	H	OH
3	OH	H	H
4	OAc	Ac	H
5	OAc	Ac	OH
6	OAc	Ac	Br
7	OAc	Ac	$OCOCH_3$
8	OAc	Ac	$OCOC_2H_5$
9	OAc	Ac	$OCOC_3H_7$
10	OAc	Ac	$OCOC_4H_9$
11	OAc	Ac	$OCOC_5H_{11}$
12	OAc	Ac	$OCOC_7H_{15}$
13	OAc	Ac	$OCOC_9H_{19}$

Chemical Synthesis

Compounds **8**~**10** were prepared from the previously described[3] 14-bromo-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-daunomycinone (**6**) by treatment in acetone with the sodium salts of the appropriate carboxylic

* For a preliminary report, see reference 1.

Table 1. ^{13}C NMR chemical-shift data (δ) for compounds **8**~**13**[a].

C atom	Compound						C atom	Compound					
	8	**9**	**10**	**11**	**12**	**13**		**8**	**9**	**10**	**11**	**12**	**13**
1	119.8	119.7	119.7	119.7	119.8	119.7	13	206.6	206.5	206.4	206.3	206.4	206.4
2	135.7	135.6	135.6	135.6	135.6	135.6	14	65.8	65.7	65.7	65.7	65.7	65.7
3	118.5	118.4	118.5	118.5	118.5	118.5	OMe	56.6	56.6	56.6	56.6	56.6	56.6
4	161.0	161.0	161.0	161.0	161.0	161.0	1′	101.1	101.1	101.1	101.1	101.1	101.1
6	156.2	156.1	156.1	156.1	156.1	156.1	2′	29.7	29.7	29.7	29.8	29.8	29.7
11	155.6	155.5	155.6	155.6	155.6	155.6	3′	66.4	66.4	66.4	66.4	66.4	66.4
5	186.9	186.7	186.8	186.8	186.8	186.7	4′	69.4[b]	69.4[b]	69.5[b]	69.5[b]	69.5[b]	69.5[b]
12	186.5	186.4	186.4	186.4	186.4	186.4	5′	66.0	66.0	66.1	66.1	66.1	66.1
4a	120.8	120.6	120.8	120.8	120.9	120.8	Me-5′	16.6	16.5	16.6	16.6	16.6	16.6
5a	111.4	111.3	111.4	111.4	111.4	111.4	OAc	20.8[c]	20.8[c]	20.7	20.7	20.7	20.7
11a	111.3	111.2	111.3	111.3	111.3	111.3	OAc			20.6	20.6	20.6	20.6
6a	135.4	135.3	135.4	135.4	135.4	135.3	C=O	170.6	170.5	170.5	170.4	170.4	170.4
10a	134.0	133.9	133.9	133.9	134.0	133.9	C=O	169.9	169.9	169.8	169.7	169.7	169.7
12a	133.5	133.4	133.5	133.5	133.5	133.5	C=O	173.8	173.0	173.1	173.0	173.1	173.1
7	69.9[b]	69.9[b]	69.9[b]	69.9[b]	69.9[b]	70.0[b]		27.2	35.8	33.6[c]	33.9; 31.2	33.9; 31.6	33.9; 31.8
8	35.4	35.4	35.4	35.5	35.5	35.4	Alkyl	9.0	18.4	27.0	24.6	29.0; 28.9	29.4; 29.3[c]
9	77.2	77.0	77.2	77.2	77.2	77.2	Chain		13.6	22.2	22.3	24.9; 22.6	29.1; 24.9
10	33.6	33.5	33.6[c]	33.6	33.6	33.6				13.7	13.8	14.0	22.6; 14.0

[a] Chemical-shift assignments are based on off-resonance decoupling plus single frequency, selective heteronuclear decoupling and by comparison with literature values.[3,7,8] Assignments bracketed are not specifically differentiated.

[b] Assignments for C-4′ and C-7 may be interchanged.

[c] Signals of double intensity.

Table 2. Activity[a] of 14-*O*-acyl-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinones (**8**~**13**) against P388 lymphocytic leukemia in mice.[b]

Compound	NSC No.	Substituent at C-14	Dose (mg/kg)	T/C (%)[c]
7	335043	$OCOCH_3$	100.00	120[d]
			50.00	261, 175[d]
			25.00	177, 187[d]
			12.50	130, 157[d]
			6.25	121, 127[d]
			3.12	102
8	341640	$OCOC_2H_5$	100.00	117
			50.00	160
			25.00	155
			12.50	146
			6.25	120
9	341639	$OCO(CH_2)_2CH_3$	100.00	130
			50.00	221
			25.00	165
			12.50	136
			6.25	122
10	341641	$OCO(CH_2)_3CH_3$	100.00	136
			50.00	198
			25.00	117
			12.50	122
			6.25	122
11	341643	$OCO(CH_2)_4CH_3$	100.00	165
			50.00	183
			25.00	132
			12.50	108
			6.25	108
12	341642	$OCO(CH_2)_6CH_3$	100.00	202
			50.00	155
			25.00	117
			12.50	117
			6.25	108
13	341644	$OCO(CH_2)_5CH_3$	100.00	174
			50.00	149
			25.00	122
			12.50	108
			6.25	113

[a] Date obtained under the auspices of the National Cancer Institute, Division of Cancer Treatment, Drug Research and Development Branch.

[b] CDF_1 mice were injected i.p. with 10^6 P388 lymphocytic leukemia cells on day 0 and treated i.p. on days 5, 9, and 13 with the drug dose specified. Toxic deaths were not observed in any of the tests.

[c] Ratio of median survival-time, expressed as percent of untreated controls.

[d] Data from a second series of tests.

acids. Complete substitution was achieved when the mixture was boiled under reflux for 1~3 hours. Each reaction gave essentially one product (TLC), and the analytical data fully support the assigned structures. The ^1H NMR spectra (200 MHz, $CDCl_3$), which are not reported here, confirmed in each instance the conversion of the 14-bromide into the corresponding ester. Except for the signals of the aliphatic side-chain, the spectra were essentially identical with that of the 14-acetate **7**, whose spectrum has been described in detail[4]. More characteristic are ^{13}C NMR spectra presented in Table 1, which

were very valuable for identifying and differentiating the esters.

Biological Activity

The *in vivo* antitumor activity of the esters (Table 2) does not show dramatic changes as compared with the activity of the 14-hydroxy analog **5** (NSC 307990)[3]. The optimal dose for the lower esters (**7**~**10**) is 50 mg/kg; at higher doses (100 mg/kg) the T/C value is markedly diminished. The esters also show significant activity at the lower dose-levels of 12.5 and 25 mg/kg, whereas the esters **12** and **13** are inactive or show only marginal activity at this level. In contrast, the higher esters (**12** and **13**) show their highest activity at dose levels of 100 mg/kg*. Activity at the 100 mg/kg dose level increases gradually through the series, reaching a maximum for the octanoate **12**.

Experimental

TLC was performed on precoated plastic sheets (0.2 mm) and glass plates (0.25 mm) of silica gel 60F-254 (E. Merck, Darmstadt, G.F.R.); zones of colorless compounds were detected by UV light and by spraying the plates with 0.1 M ceric sulfate in 2 M sulfuric acid, with subsequent heating. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded with a Perkin-Elmer 457 grating spectrophotometer. ^{1}H NMR spectra at 200 MHz and ^{13}C spectra at 50 MHz were recorded by Dr. O. MOLS for solutions in chloroform-*d* with a Bruker WP-200 spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane (δ=0.00). Elemental analyses were performed by Dr. O. MOLS.

7-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-14-*O*-propanoyladriamycinone (**8**, NSC 341640)

To a vigorously stirred solution of 14-bromo-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinone[3] (**6**, 184.9 mg, 0.267 mmol) in acetone (15 ml) was added sodium propanoate (140 mg, 1.46 mmol). The mixture was boiled under reflux for 2 hours, whereupon TLC (benzene - acetone, 6: 1) showed that **6** was absent. The mixture was poured into water and the product extracted with chloroform. The organic fraction was washed with water, dried with magnesium sulfate, and evaporated under diminished pressure. Crystallization of the residue from acetone, ethyl ether, and hexane gave a red solid that was dried at 50°C/0.3 mmHg; yield 140.8 mg (77%), mp 131~133°C, $[\alpha]_D^{25}$ +203° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1745 (C=O), 1618, and 1580 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{34}H_{36}O_{15}$ (684.657): C 59.65, H 5.30
Found: C 59.63, H 5.16

14-*O*-Butanoyl-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinone (**9**, NSC 341639)

To a solution of the 14-bromide[3] **6** (199.2 mg, 0.29 mmol) in acetone - ethanol (6: 1) was added sodium butanoate (232 mg, 2.11 mmol), and the mixture was boiled for 3.5 hours under reflux. The product was isolated as in the preceding experiment. Crystallization afforded **9** as a red solid (180 mg, 89%); mp 118~123°C, $[\alpha]_D^{25}$ +183° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1747 (C=O), 1620, and 1582 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{35}H_{38}O_{15}$ (698.684): C 60.17, H 5.48
Found: C 59.97, H 5.39

7-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-14-*O*-pentanoyladriamycinone (**10**, NSC 341641)

A solution of the bromide[3] **6** (184.6 mg, 0.27 mmol) in acetone (15 ml) containing sodium pentanoate (96 mg, 0.77 mmol) was boiled for 3.5 hours under reflux and the product isolated as in the preceding experiments. Evaporation gave 177.7 mg (93%) of a red solid which, for analytical and test purposes

* The highest dose tested. These compounds are under further investigation.

was crystallized from chloroform (4 ml), ethyl ether (10 ml), and an excess of hexane; mp 118~122°C, $[\alpha]_D^{25}$ +183° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1748 (C=O), 1620, and 1583 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{36}H_{40}O_{15}$ (712.711): C 60.67, H 5.66
Found: C 60.87, H 5.79

7-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-14-*O*-hexanoyladriamycinone (**11**, NSC 341643)

To a solution of the 14-bromide[8] **6** (170 mg, 0.246 mmol) in acetone (15 ml) was added sodium hexanoate (103 mg, 0.75 mmol) and the mixture was boiled for 1 hour under reflux. The product, a red solid (165.8 mg, 93%), was isolated as before; mp 120~125°C, $[\alpha]_D^{25}$ +194° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1749 (C=O), 1621, and 1584 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{37}H_{42}O_{15}$ (726.739): C 61.15, H 5.83
Found: C 60.86, H 5.72

7-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-14-*O*-octanoyladriamycinone (**12**, NSC 341642)

The bromide[8] **6** (134.9 mg, 0.195 mmol) plus sodium octanoate (200 mg, 1.20 mmol) in acetone (15 ml) was boiled for 1 hour under reflux and the product isolated as before: yield 134.3 mg (91%), mp 102~104°C, $[\alpha]_D^{25}$ +181° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1748 (C=O), 1619, and 1580 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{39}H_{46}O_{15}$ (754.793): C 62.06, H 6.14
Found: C 61.89, H 5.91

14-*O*-Decanoyl-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinone (**13**, NSC 341644)

The foregoing procedure was repeated with the 14-bromide[8] **6** (190 mg, 0.275 mmol) in acetone (15 ml) plus sodium decanoate (214.5 mg, 1.10 mmol) to give **13**; yield 201 mg (92%), mp 97~98°C, $[\alpha]_D^{25}$ +181° (*c* 0.01, chloroform); IR ν_{max}^{KBr} 1748 (C=O), 1620, and 1582 cm^{-1} (H-bonded quinone).

Anal. Calcd. for $C_{41}H_{50}O_{15} \cdot 0.5H_2O$: C 62.19, H 6.49
Found: C 62.26, H 6.49

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References

1) HORTON, D. & W. PRIEBE: Glycon- and C-14-modified, antitumor doxorubicin analogs. *In* H. S. EL KHADEM *Ed.*, Anthracycline Antibiotics. pp. 197~224, Academic Press, Inc., New York, 1982
2) FUCHS, E. F.; D. HORTON, W. WECKERLE & E. WINTER-MIHALY: Synthesis and antitumor activity of sugar-ring hydroxyl analogs of daunorubicin. J. Med. Chem. 22: 406~411, 1979
3) HORTON, D.; W. PRIEBE & W. R. TURNER: Synthesis and antitumor activity of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinone. Carbohydr. Res. 94: 11~25, 1981
4) HORTON, D. & W. PRIEBE: New adriamycin analogs. Synthesis and antitumor activity of 14-substituted 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinones. J. Antibiotics 34: 1019~1025, 1981
5) ARCAMONE, F.; G. FRANCESCHI, A. MINGHETTI, S. PENCO & S. REDAELLI: Synthesis and biological evaluation of some 14-*O*-acyl derivatives of adriamycin. J. Med. Chem. 17: 335~337, 1974
6) ARCAMONE, F.: Doxorubicin. Medicinal Chemistry Ser. 17: pp. 168~173, Academic Press, Inc., New York, 1982
7) ARNONE, A.; G. FRANZA, R. MONDELLI & A. VIGEVANI: ^{13}C NMR analysis of the antitumor antibiotics daunorubicin and adriamycin. Tetrahedron Lett. 1976: 3349~3350, 1976
8) WRIGHT, L. H.; J. A. CHAN, J. A. SCHROER & A. A. ASZALOS: A ^{13}C nuclear magnetic resonance study of *N*-acetyldaunorubicinol. J. Org. Chem. 42: 2344~2345, 1977