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Preliminary communication

Adriamycin analogs hydroxylated at C-3′: synthesis and antitumor activity*

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The antitumor drugs daunorubicin (**1**), adriamycin (**2**), and carminomycin (**3**) have been extensively documented[1,2]; their clinical efficacy is well established, but a cumulative, dose-related cardiotoxicity and mutagenic properties remain drawbacks in their use. In a quest for active, but less toxic analogs[3], we have synthesized[1,2] the 3′-hydroxy analog (**4**) of **1**, and have shown[2] that it, and also its 3′,4′-diacetate (**5**), demonstrate high antitumor activity, but lower toxicity than **1**. As adriamycin (**2**) is a more potent drug than daunorubicin (**1**), the synthesis and evaluation of the corresponding adriamycin analogs (**9** and **12**), remained a significant goal, and this work is the subject of the present communication.

Three alternative approaches to **9** were envisaged: (*a*) from adriamycinone by sequential protection of the 14-hydroxyl group, glycosylation at O-7, and deprotection; (*b*) from 7-*O*-(3,4,-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinone[2] (**5**) by bromination at C-14, followed by hydrolysis of the 14-bromide; and (*c*) by successive bromination of daunomycinone at C-14, glycosylation at O-7, and hydrolysis of the 14-bromide. It is shown that route (*a*) produces only mediocre yields of **9** in a tedious synthesis, and route (*b*) gives very poor results, whereas route (*c*) is effective and affords excellent yields in all steps to the biologically active diacetate **12**.

For route (*a*), adriamycinone was obtained by acid hydrolysis[4] of adriamycin (**2**) and converted into its 14-(*p*-anisyldiphenylmethyl) ether[5]. This product was treated with 2 molar equivs. of 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl chloride[6] (**13**) under Koenigs–Knorr conditions[2] (yellow mercuric oxide and mercuric bromide) in the presence of powdered molecular sieve 3A in dry dichloromethane, for 24 h at 25°, to give a mixture containing two red products (t.l.c.). The excess of **13** was decomposed by addition of methanol, inorganic material was filtered off, and the products were resolved on a column of silica gel G that was eluted with 3:1 ether–petroleum ether (to remove sugar contaminants) and then with acetone to give red material, which was resolved on a second column with 4:1 benzene–acetone as the eluant. The first fractions

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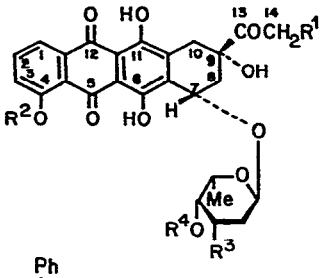
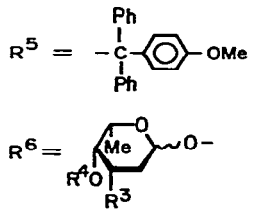
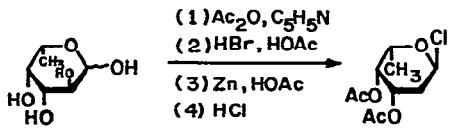


	R^1	R^2	R^3	R^4
1	H	Me	NH_2	H
2	OH	Me	NH_2	H
3	H	H	NH_2	H
4	H	Me	OH	H
5	H	Me	OAc	Ac
6	OR^5	Me	OAc	Ac
7	OR^6	Me	OAc	Ac
8	OR^5	Me	OH	H
9	OH	Me	OH	H
10	OR^5	Me	OH	H
11	Br	Me	OAc	Ac
12	OH	Me	OAc	Ac





13

afforded the desired 14-*O*-(*p*-anisyldiphenylmethyl)-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinone* (**6**) in 70% yield, m.p. 147–148° (chloroform–hexane), $[\alpha]_D^{22}$ + 115° (*c* 0.01, chloroform); n.m.r. (acetone-d_6): δ 5.44 (bs, W_h 7 Hz, H-1′α), 7.30–7.70 (aryl), 6.92 (d, $J_{2,3}$ 9 Hz, H-3), 4.9–5.1 (m, H-3′,4′,7), 4.45 (broad s, 2 H, H-14), 4.2 (m, H-5′), 3.94 (s, 3 H, OCH_3), 2.74 (broad s, 2 H, H-10), 2.06, 2.09 (2 s, OAc), 1.8–2.0 (m, H-2′,8), and 0.94 (d, 3 H, $J_{5',6'}$ 5 Hz, H-6′); ν_{max}^{KBr} 2.87 (OH), 5.70 (OAc, *C*-acetyl), 6.16, and 6.30 μm (chelated quinone).

The second fraction afforded the 7,14-bis(glycosyl)ated derivative **7** in 17% yield; m.p. 148–149° (acetone–hexane), $[\alpha]_D^{22}$ + 54° (chloroform). The ^{1}H-n.m.r. spectrum of **7** (chloroform-*d*) indicated that the product had the sugar residue α-L-linked at O-7, but was probably a mixture of α- and β-L-substituted anomers at O-14; no signals attributable to the *p*-(anisyldiphenylmethyl) group were present. Compound **7** is probably formed by 14-deprotection of either **6** or the protected adriamycinone, with subsequent glycosylation. Trial experiments with only 1 equiv. of **13** at ~ 25 or 0° still showed some formation of **7**. No comparable 14-deprotection was reported[5] in a synthesis of adriamycin (**2**) employing 2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α,β-L-*lyxo*-hexopyranosyl chloride. Compound **7** could be *O*-deacetylated to give **10**.

O-Deacetylation of **6** was effected with cold, 0.2M sodium hydroxide, to yield

*All new compounds gave acceptable elemental analyses, and mass and 100-MHz, ^{1}H-n.m.r. spectra consistent with the structures reported.

35% of the partially deprotected analog **8**, m.p. 152–153°, $[\alpha]_D^{22}$ + 80° (chloroform); n.m.r. ($CDCl_3$): δ 5.44 (bs, W_h 7.5 Hz, H-1′α), 3.80 (OMe), and 7.2–7.6 (aryl).

14-Deprotection of **8** with 4:1 acetic acid–water[5] gave "3′-hydroxyadriamycin" (**9**) in low yield; *m/e* (chemical ionization, isobutane): 337 (MH^+ – glycon – C-13,14 fragment) and 131 (glycosyl cation).

Approach (*b*) did not furnish a practical synthesis of **9** or **12**. Treatment of compounds[2] **4** or **5** with bromine under a variety of conditions failed to effect a high-yielding conversion into the corresponding 14-bromo derivatives; the reactions led to complex mixtures, and the principal reaction generally involved scission of the sugar residue.

The third approach, (*c*), provided the most satisfactory synthesis of the target compound as its diacetate **12**. 14-Bromodaunomycinone[5] was coupled with **13** under the same conditions[2] used for route (*a*) to afford 14-bromo-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl) daunomycinone (**11**) in yields of 75–94%; m.p. 152–156°, $[\alpha]_D^{25}$ + 119° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.63 (d, $J_{1',2'}$ 3.1, $J_{1',2''}$ <1 Hz, H-1′α), 4.09 (s, OCH_3), 2.18, 1.95 (s, OAc), and 1.21 (d, $J_{5',6'}$ 7.1 Hz, H-6′). For consistently high yields of **11**, it was necessary to use ~3 equiv. of **13**.

Displacement of the 14-bromo group from **11** was readily achieved by the action of 5% potassium carbonate in 2:1 oxolane–water at 0°, to give a 95% yield of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl) adriamycinone (**12**), m.p. 154–157° [from acetone–petroleum ether (b.p. 30–60°)], $[\alpha]_D^{25}$ + 138° (chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.61 (d, $J_{1',2''}$ 2.8 Hz, $J_{1',2''}$ <1 Hz, H-1′α), 13.98, 13.20 (s, phenolic OH), 4.09 (s, OCH_3), 2.18, 1.94 (s, OAc), and 1.21 (d, $J_{5',6'}$ 7.3 Hz, H-6′). *O*-Deacetylation of **12** did not afford good yields of **9** under a variety of conditions; loss

TABLE I

ANTITUMOR ACTIVITY *in vivo* OF ADRIAMYCIN AND DAUNORUBICIN ANALOGS AGAINST MURINE P-388 LYMPHOCYTIC LEUKEMIA[a]

Compound	*NSC Number*	*Dose, mg/kg*[b]	*T/C percent*[c]
Daunorubicin (**1**)	82151	8	151
Adriamycin (**2**)	123127	4	149
3′-Hydroxydaunorubicin[2] (**4**)	284682	200	192
3′-Hydroxydaunorubicin 3′,4′-diacetate[2] (**5**)	283158	200	186
7	311156	50, 12.5, 6.25, 3.13	100
11	307989	25	109
3′-Hydroxyadriamycin 3′,4′-diacetate (**12**)	307990	50	269
		50[d]	202[d]
		12.5[e]	203[e]
		12,5[f]	280[f]

[a] Data obtained by A. D. Little, Inc., under the auspices of DCT, NCI. [b] CDF_1 mice were injected i.p. with 10^6 P-388 cells on day 0, and treated i.p. on days 5, 9, and 13 with the drug dose specified. [c] Ratio (%) of median survival-time of treated mice to that of untreated controls; dose level giving maximum T/C. [d] In B-16 melanocarcinoma; single drug injection. [e] In L-1210 lymphoid leukemia; 9 daily injections. [f] In Lewis lung carcinoma.

of the sugar substituent was a major pathway under conditions[2] that afforded a high yield of **4** from its 3′,4′-diacetate **5**.

Compound **12** shows significant, *in vivo* activity against murine P-388 lymphocytic leukemia (see Table I) and is undergoing extensive, antitumor and toxicological screening. As the 3′-hydroxyl analog (**4**) of daunorubicin and its 3′,4′-diacetate (**5**) show[2] comparable antitumor activities, it is expected that **12** and its *O*-deacetylated analog **9** will have similar activities. Substitution for achievement of greater lipophilicity has caused enhancement[7] of activity in the daunorubicin series, and esterases may be expected to effect the *in vivo* conversion of **12** into **9** readily.

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