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*

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The 14-azido-, 14-thiocyanato-, 14-acetoxy-, and 14-acetylthio- derivatives of 7-O-(3,4-di-O-acetyl-2,6-dideoxy-α-L-Lyxo-hexopyranosyl)daunomycinone were synthesized by displacement reactions conducted on the corresponding 14-bromide. The in vivo antitumor activities of the products were compared with that of the 14-hydroxyl derivative in the murine P-388 lymphocytic leukemia assay. The 14-acetoxy derivative was highly active and of low toxicity; the other products showed negligible or low activities.

Work from this laboratory has shown3) that the analogue of daunorubicin (daunomycin, 1, NSC-82151) in which the 3'-amino group has been replaced by hydroxyl (3'-desamino-3'-hydroxydaunorubicin, 3, NSC-284682) and also its 3',4'-diacetate 4 (NSC-283158) retain high in vivo antitumor activity in a range of standard test-systems in mice, and manifest much lower toxicity, including cardiotoxicity, than the parent antibiotic 1. Likewise, the 14-hydroxylated analogue of 4 (compound 5, NSC-307990)2), which may be regarded as the 3',4'-diacetate of a doxorubicin (adriamycin, 2, NSC-123127) congener in which the 3'-amino group has been replaced by hydroxyl, displays3) high in vivo antitumor activity in a wide range of murine screens; its activity is higher than that of 3 and 4, and its acute toxicity is much lower than that of doxorubicin (2).

Based on the observation that hydroxylation at position 14 leads to enhancement of biological activity5,6) (2 is more active than 1; 5 is more active3) than 4), the present study was initiated to provide, on a comparative basis, data for the effect on biological activity of halogen, nitrogen, sulfur, and acyloxy substituents at C-14 in structure 4. It is shown that activity is markedly attenuated or abolished by bromo, azide, thiocyanato, or acetyltio groups at C-14, but full activity and low toxicity are retained in the 14-acetoxy derivative (9).

* For a preliminary report of this work, see reference 1.
Chemical Synthesis

Compounds 7~10 variously substituted at C-14 were prepared from the readily available\(^6\) 14-bromo-7-O-(3,4-di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)daunomycinone (6) by treatment\(^7\) with sodium (or potassium) azide, thiocyanate, acetate, and thioacetate, respectively. The reactions were conducted at \(\sim 25°C\) in dry acetone (3:1 acetone-ethanol with potassium thioacetate to increase the solubility). The reactions leading to azide 7, thiocyanate 8, and thioacetate 10 were complete in \(<0.5\) hour; the (less nucleophilic) acetate ion predictably\(^8,9\) took longer (\(\sim 36\) hours at \(25°C\), 1 hour at boiling point) for the conversion into acetate 9.

The structures assigned to compounds 7~10 were evident from analytical data (see Experimental section) and from \(^1\)H NMR (Table 1), \(^13\)C NMR (Table 2), and IR spectral data. The azide 7 showed characteristic IR absorption at \(2120\) cm\(^{-1}\) and the \(^13\)C signal of C-14 in 7 (at \(54.6\) ppm) is \(\sim 20\) ppm upfield of its position\(^4\) in the precursor bromide 6. Thiocyanate 8 had a characteristic IR band at \(2160\) cm\(^{-1}\), and its \(^13\)C NMR spectrum showed an additional carbon signal (SCN) at \(111.2\) ppm; the chemical shift of C-14 was \(40.5\) ppm. The protons at C-14 were nonequivalent and resonated as an AB pattern with chemical shifts of \(4.52\) and \(4.40\) ppm, respectively.

The 14-acetate 9 showed the anticipated 3-proton OAc resonance (2.20 ppm) and the associated \(^13\)C carbonyl-group signal (170.3 ppm), and the chemical shift of C-14 (66.1 ppm) is in line with expectation. The thiolacetate 10 showed its carbonyl band (1695 cm\(^{-1}\)) as expected\(^10,11\) at lower wavenumber than that for the oxygenated analogue. Likewise, the thiolacetate group gave a \(^1\)H NMR signal (2.40 ppm) at lower field\(^12\) than the acetoxy group. The protons at C-14 in 10 resonated at relatively high field (4.39 and 4.08 ppm), as did C-14 (35.9 ppm). The thiolacetate group showed \(^13\)C signals at 194.4 (C=O) and 30.2 (CH\(_3\)COS) ppm.

For consolidation of all signal assignments for the glycon portion of compounds 7~10, the \(^1\)H and \(^13\)C NMR spectra of a suitable model compound, methyl 3,4-di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranoside\(^4\) (11) were recorded, and the corresponding spectral data are incorporated in Tables 1 and 2.

Biological Activity

The importance of the 14-substituent for biological activity in the natural anthracyclines is underscored by comparing daunorubicin (1) with its 14-hydroxylated analogue, doxorubicin (2), which is more active\(^6,14\) as an antitumor agent. Likewise, comparison of 3'-desamino-3'-hydroxydaunorubicin diacetate (4) with its 14-hydroxy analogue 5 again shows the latter to be the more active\(^9\). As illustrated by the \(in\ vivo\) test data for the murine P-388 screen given in Table 3, introduction of other substituents at C-14 in the parent structure 4 markedly modifies activity. Although hydroxylation at C-14 (compound 5) increases activity, introduction of bromide (compound 6), or acetyltio (compound 10) completely abolishes detectable activity. Introduction of azide (compound 7) or thiocyanate (compound 8) causes a notable decrease in activity; these compounds manifested no activity at 50 mg/kg, but marginal activity was evident at higher doses (125~200 mg/kg). Only in the case of the 14-acetoxy derivative 9 was high activity retained; this compound exhibited activity comparable to that of 3'-desamino-3'-hydroxydoxorubicin diacetate (5) and demonstrably greater than that of the non-14-hydroxylated analogue 4. It is very probable that nonspecific esterases bring about conversion \(in\ vivo\) of the acetyl-
Table 1. $^1$H NMR spectra data for 14-substituted 7-O-(3,4-di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)daunomycinones (7–10) and methyl 3,4-di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranoside (11).a

<table>
<thead>
<tr>
<th>Compound</th>
<th>H-1 $^b$ ($J_{3',2'}$)</th>
<th>H-2</th>
<th>H-3$^c$ ($J_{2'a,3'a}$)</th>
<th>H-1′ $^b$ ($J_{3'a,3'}$)</th>
<th>H-7</th>
<th>H-4′ $^c$ ($J_{2'a,3'}$)</th>
<th>H-3$^d$ ($J_{2'a,3'}$)</th>
<th>9-OH</th>
<th>H-14A</th>
<th>H-14B</th>
<th>H-5‘$^d$ ($J_{2'a,3'}$)</th>
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<tbody>
<tr>
<td>7</td>
<td>8.05 bd (7.7)</td>
<td>7.79 app. t</td>
<td>7.40 bd (8.2)</td>
<td>5.63 bd (3.4)</td>
<td>5.33 bs</td>
<td>5.23 bs (2.7)$^b$</td>
<td>5.04 ddd (12.0) (4.7)</td>
<td>−4.50 s, 3H→</td>
<td>4.19 bq</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.04 bd (7.7)</td>
<td>7.79 app. t</td>
<td>7.40 bd (7.8)</td>
<td>5.64 bd (2.8)</td>
<td>5.35 bs</td>
<td>5.23 bs</td>
<td>5.05 ddd (17.0)</td>
<td>4.69 s</td>
<td>4.52 d (14.0)</td>
<td>4.40 d</td>
<td>4.20 bq</td>
</tr>
<tr>
<td>9</td>
<td>8.03 dd (7.7)</td>
<td>7.78 app. t</td>
<td>7.39 dd (8.5)</td>
<td>5.61 bd (3.6)$^e$</td>
<td>5.29 m</td>
<td>5.24 bd (2.9)$^b$</td>
<td>5.06 ddd (12.5) (5.1)</td>
<td>4.48 s</td>
<td>5.33 d (18.0)</td>
<td>5.10 d</td>
<td>4.25 bq</td>
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<tr>
<td>10</td>
<td>8.03 bd (7.7)</td>
<td>7.77 app. t</td>
<td>7.39 bd (8.6)</td>
<td>5.63 bd (3.4)</td>
<td>5.29 m</td>
<td>5.24 bs (2.6)$^b$</td>
<td>5.08 ddd (12.9) (5.0)</td>
<td>4.54 s</td>
<td>4.39 d (17.6)</td>
<td>4.08 d</td>
<td>4.31 bq</td>
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<tr>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.86 dd (3.7)</td>
<td>—</td>
<td>5.17 nm</td>
<td>5.22 ddd (12.2) (5.4) (J$^{2'a,3'}$)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.04 qd (1.2)</td>
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<tr>
<th>Compound</th>
<th>H-10e $^b$ ($J_{6,10e}$)</th>
<th>H-10ax</th>
<th>H-8e $^b$ ($J_{6,8a}$)</th>
<th>H-8ax $^b$ ($J_{6,8a}$)</th>
<th>H-2’a $^b$ ($J_{2'a,3'}$)</th>
<th>H-2’e $^c$ ($J_{2'a,3'}$)</th>
<th>H-6’ $^d$ ($J_{2'a,3'}$)</th>
<th>6-OH, 11-OH</th>
<th>OMe</th>
<th>OAc</th>
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<tr>
<td>7</td>
<td>3.27 bd (&lt;1.5)</td>
<td>2.98 d (18.9)</td>
<td>3.24 bd (14.6)</td>
<td>2.22 dd (3.9)</td>
<td>2.16−1.79 m</td>
<td>1.22 d (6.9)</td>
<td>13.22, 13.99 s</td>
<td>4.09 s</td>
<td>1.94, 2.17 s</td>
<td></td>
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<tr>
<td>8</td>
<td>3.29 bd</td>
<td>2.94 d (18.9)</td>
<td>3.23 bd (14.2)</td>
<td>2.25 dd (3.9)</td>
<td>2.09 m</td>
<td>1.87 m</td>
<td>1.23 d (6.5)</td>
<td>13.20, 13.98 s</td>
<td>4.09 s</td>
<td>1.94, 2.17 s</td>
</tr>
<tr>
<td>9</td>
<td>3.28 dd (1.5)</td>
<td>3.00 d (18.8)</td>
<td>2.47 bd (14.3)</td>
<td>2.20−1.98 m (13.1)$^e$</td>
<td>1.86 bdd (13.1)$^d$</td>
<td>1.23 d (6.6)</td>
<td>13.20, 13.97 s</td>
<td>4.08 s</td>
<td>1.94, 2.17, 2.20 s</td>
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<tr>
<td>10</td>
<td>3.31 bd</td>
<td>3.01 d (18.9)</td>
<td>2.52 bd (14.4)</td>
<td>2.20−1.81 m</td>
<td>1.24 d (6.5)</td>
<td>13.22, 13.98 s</td>
<td>4.08 s</td>
<td>1.94, 2.17, 2.40 s</td>
<td></td>
<td></td>
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<tr>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.04 dt</td>
<td>1.84 ddd (12.5) (J$^{2'a,3'}$) (5.4) (J$^{2'a,3'}$)</td>
<td>1.15 d (6.6)</td>
<td>—</td>
<td>3.34 s</td>
<td>1.98, 2.15 s</td>
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</table>

a Spectra recorded at 200 MHz in chloroform-$d$. Spin couplings (Hz) are given in parentheses. Signal multiplicities: app, apparent; b, broadened; d, doublet; m, multiplet; n, narrow; q, quartet; s, singlet; t, triplet.

b Coupling constants were measured from spectra decoupled at: $^b$ H-5’ or H-2’e; $^c$ H-2’e; $^d$ H-3’; $^e$ H-4’; $^f$ H-5’. $^s$ Ac.
ated compounds 5 and 9 into the same, active product. The differential biological-transport properties
of 5, 9, and their common deacetylation product may, however, result in significant differences in the
physiological behavior of the three compounds as potential agents for use in cancer chemotherapy.

The work here underscores the significance of the oxygenated substituent at C-14 for antitumor
activity in these anthracyclines, and demonstrates that introduction of halogen, nitrogen, or sulfur
at this position is unlikely to lead to compounds of antitumor activity higher than that of the 14-O-
substituted derivatives.

**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. 1H NMR spectra were determined by Dr. O. Mols at 200 MHz
for solutions in chloroform-d with a Bruker WP-200 spectrometer. 13C Spectra were recorded by Dr. C.
COTTRELL at 20 MHz with a Bruker WP-80 instrument and by Dr. O. Mols at 50 MHz 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.
To a solution of 14-bromo-7-O-(3,4-di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)daunomycinonone 4 (345.8 mg, 0.5 mmole) in acetone (20 ml) was added sodium azide (156.7 mg, 2.4 mmole) and the mixture was stirred vigorously for 0.5 hour at -25°C. TLC (benzene - acetone, 6:1, developed twice) showed conversion of 4 into a single, slightly more-polar product (Rf 0.41). The mixture was poured into water (100 ml) and the product extracted with dichloromethane. The organic layer was washed with water, dried (magnesium sulfate) and evaporated under diminished pressure. Crystalization of the residue from acetone (sufficient to dissolve the sample), ethyl ether, and hexane gave a red solid that was dried at 50°C/0.3 mmHg; yield 290 mg (89%), m.p. 135°C, [α]D23 +230°, [α]D578 +284° (c 0.024, CHCl3); v (KBr) 3480 (OH), 2120 (N3), 1746 (C=O), 1622, and 1583 cm⁻¹ (H-bonded quinone).


7-O-(3,4-Di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-14-thiocyanatodaunomycinonone 8 (NSC-328006)

To a solution of the 14-bromide 6 (305.8 mg, 0.44 mmole) in acetone (20 ml) was added potassium thiocyanate (270 mg, 2.78 mmole) and the mixture was stirred vigorously for 0.5 hour at ~25°C. TLC (benzene - acetone, 6:1, developed twice) showed conversion of 6 into a single, slightly more-polar product (Rf 0.41). The mixture was poured into water (100 ml) and the product extracted with dichloromethane. The organic layer was washed with water, dried (magnesium sulfate) and evaporated under diminished pressure. Crystalization of the residue from acetone (sufficient to dissolve the sample), ethyl ether, and hexane gave a red solid that was dried at 50°C/0.3 mmHg; yield 290 mg (89%), m.p. 135°C, [α]D23 +230°, [α]D578 +284° (c 0.024, CHCl3); v (KBr) 3472 (OH), 2162 (SCN), 1745 (C=O), 1622, and 1583 cm⁻¹ (H-bonded quinone).

Anal. Calcd. for C32H31NO13S (669.667): C, 57.40; H, 4.67; N, 2.09; S, 4.79. Found: C, 57.19; H, 5.12; N, 1.95; S, 4.75.

To a vigorously stirred solution of the 14-bromide 6 (267.8 mg, 0.38 mmole) in acetone (20 ml)
was added sodium acetate (280 mg, 3.4 mmole). The reaction was complete after 36 hours at 25°C (TLC, benzene - acetone, 6: 1, one component, more polar than the substrate), and the product was isolated as for the two preceding reactions. The first crystallization afforded 9 as a red solid (165 mg), +202°, [a]_533 and the filtrate gave an additional 82 mg of 9, total yield 95 %; m.p. 141 -143°C, [α]_D^23 = +238 (c 0.02, CHCl_3); ν_κmax 3460 (OH), 1745 (C=O), 1621, and 1583 cm^-1 (H-bonded quinone).

Anal. Calcd. for C_{33}H_{34}O_{15}·0.5 H_2O (679.63): C, 58.32; H, 5.19.

Found: C, 58.31; H, 4.86.

14-Acetylthio-7-O-3,4-di-O-acetyl-2,6-dideoxy-a-L-Iyxo-hexopyranosyl)daunomycinone (10, NSC-327473)

The bromide 6 (175 mg, 0.25 mmole) was dissolved in a mixture of acetone (12 ml) and absolute ethanol (4 ml), potassium thioacetate (124 mg, 1.05 mmole) was added, and the mixture was stirred vigorously at ~25°C. After 10 minutes, TLC (benzene - acetone, 6: 1 developed twice) showed 6 to be absent and a single, more-polar (R_f 0.42) product had been formed. The mixture was poured into water (50 ml) and the product extracted with dichloromethane. The organic layer was washed with water, dried with magnesium sulfate, and evaporated, to afford a red oil (155.4 mg) that was crystallized from a little acetone plus ethyl ether and hexane. Compound 10 was obtained as a red solid that was dried for 5 hours at 65°C and 0.3 mmHg; yield 135.5 mg (78%), m.p. 140 -141°C, [α]_D^23 = -191°, [α]_D^23 = +205° (c 0.02, CHCl_3); ν_κmax 3483 (OH), 1748 (C=O), 1696 (SC=O), 1621, and 1582 cm^-1 (H-bonded quinone).

Anal. Calcd. for C_{33}H_{34}O_{14}S (686.695): C, 57.72; H, 4.99; S, 4.67.

Found: C, 57.44; H, 4.96; S, 4.67.

Acknowledgments

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