SYNTHESIS OF 4-DEMETHOXY-11-DEOXY-ANALOGS OF DAUNOMYCIN AND ADRIAMYCIN

Sir:

In our study of new anthracycline analogues, after modification of the sugar moiety had afforded the very active 4'-O-THP-adriamycin\(^1\), our attention then focussed on the structural difference between aklavinone and daunomycinone (or adriamycinone). The 11-deoxy derivatives of daunomycin and adriamycin appeared to be crucial compound for understanding structure-activity relationships in this series.

Recently the 11-deoxy analogues were independently isolated as microbial metabolites by ARCAMONE et al. and reported to have activity against P388 and weak activity against L1210\(^2,3\). Here we describe the synthesis and antitumor activity of 4-demethoxy-11-deoxy-daunomycin (9) and -adriamycin (17), and their diastereomers.

The starting tetraline (1, mp 63.5°C) was prepared from 5-methoxy-2-tetralone by reaction with ethynyl magnesium bromide at 20°C for 22 hours, followed by hydrolysis with aq. H\(_2\)SO\(_4\) and mercuric oxide at 20°C for 27 hours.

The FRIEDEL-CRAFTS acylation of 1 with phthalic anhydride was conducted according to the literature\(^4\); a mixture of 1 (230 mg), phthalic anhydride (230 mg), NaCl (460 mg) and AlCl\(_3\) (2.3 g) was melted at 180°C for 5 minutes to give, after column chromatography (benzene - ethyl acetate, 20:1), the adduct 2 in 31% yield; mp 208-214°C (dec.); NMR (DMSO-d\(_6\)): \(\delta 2.28\) (s, 3H, COCH\(_3\)), 7.38 (s, 1H, H-11), 12.78 (s, 1H, OH-6).

Treatment of 2 (100 mg) with ethylene glycol (TsOH, benzene, reflux, 3 hours) gave the ethylene ketal, which was brominated with bromine and a,a'-azo-bis-isobutyronitrile in a mixture of CHCl\(_3\) and C\(_6\)H\(_6\) at 20°C for 5 hours, followed by hydrolysis with 8 N hydrochloric acid in acetone at 20°C for 18 hours to give the 7-hydroxy derivatives 3 (41 mg) and 3' (24 mg); TLC (benzene - EtOH, 10:1): R\(_f\) 0.33 and 0.25. 3: mp 199-207°C (dec.), NMR (CDCl\(_3\)): \(\delta 2.98\) and 3.28 (AB-q, each 1H, J=18 Hz, CH\(_2\)-10), 3.65 (m, 1H, OH-7), 4.60 (s, 1H, OH-9), 5.34 (m, 1H, H-7), 7.60 (s, 1H, H-11), 13.24 (s, 1H, OH-6). Compound 3' was convertible into 3 by reaction with 60% HClO\(_4\) in dioxane.

The glycal 4, mp 144-148°C, \([\alpha]^{26}_{D}\)-100° (c 0.5, acetone), was prepared in 80% yield from 1,4-bis-O-(p-nitrobenzoyl)-N-trifluoroacetyldaunomycin\(^6\) in two steps: (1) 4 x HCl acetone, 20°C, 10 hours; (2) TsCl, pyridine, 80°C, 18 hours\(^6\).

Compound 3 (20 mg) reacted with 4 (80 mg) in the presence of TsOH (1 mg) in benzene at 20°C for 93 hours to give, after preparative TLC (benzene - EtOAc, 4:1), all possible isomer: 5 (8 mg), 6 (4 mg), 7 (11 mg) and 8 (4 mg); TLC (benzene - EtOAc, 4:1): R\(_f\) 0.32, 0.28, 0.18 and 0.35, respectively. 5: mp 153-156°C, \([\alpha]^{24}_{D}\)-125° (c 0.2, acetone); 6: mp 142-145°C, \([\alpha]^{25}_{D}\)+87.5° (c 0.2, acetone); 7: mp 148-152°C, \([\alpha]^{25}_{D}\)-225° (c 0.2, acetone); 8: mp 190-195°C, \([\alpha]^{25}_{D}\)-138° (c 0.2, acetone). In the NMR spectra, the signals due to their anomeric protons showed that 5 (\(\delta\) 5.68, broad s) and 7 (\(\delta\) 5.60, broad s) are \(\alpha\)-anomers, while 6 (\(\delta\) 5.15, dd, J=2 and 10 Hz) and 8 (\(\delta\) 5.52, dd, J=2 and 10 Hz) are \(\beta\)-anomers\(^7\).

Deprotection of 5 with 10% aqueous K\(_2\)CO\(_3\) solution in methanol at 8°C for 12 hours gave the daunomycin analogue 9 as a yellow powder in 94% yield. Similarly, 6, 7 and 8 gave 10, 11 and 12 in 87%, 92% and 86% yields. 9: mp 202-212°C (dec.), \([\alpha]^{25}_{D}\)+50° (c 0.1, MeOH), CD: \([\theta]^{25}_{D\text{max}}\)-1.08 x 10\(^4\) (MeOH), Rf 0.40; 10: mp 160-172°C (dec.), \([\alpha]^{25}_{D}\)+500° (c 0.1, MeOH), CD: \([\theta]^{25}_{D\text{max}}\)-1.52 x 10\(^4\) (MeOH), Rf 0.31; 11: mp 180-195°C (dec.), \([\alpha]^{25}_{D}\)-150° (c 0.1, MeOH), CD: \([\theta]^{25}_{D\text{max}}\)+2.28 x 10\(^4\) (MeOH), Rf 0.35; 12: mp 185-195°C (dec.), \([\alpha]^{25}_{D}\)-400° (c 0.1, MeOH), CD: \([\theta]^{25}_{D\text{max}}\)+1.16 x 10\(^4\) (MeOH), Rf 0.38.

The CD spectra of 9 and 10 showed curves similar to that of daunomycin (\([\theta]^{28}_{D\text{max}}\)-1.72 x 10\(^4\) in MeOH), but those of 11 and 12 gave curves opposite to those of 9 and 10, indicating that 9 and 10 had the same (S)-configuration at C-7 as does daunomycin; that is, 9 and 10 are 7(S),9(S)-\(\alpha\)- and \(\beta\)-glycosides, and 11 and 12 are 7(R),9(R)-\(\alpha\)- and \(\beta\)-glycosides\(^8\).

Similarly, 4-demethoxy-11-deoxy-adriamycin analogues were synthesized from 4 and 14.

Bromination (NBS, THF, 20°C, 6 hours) of 2 xypropane and TsOH in dioxane gave the acetone, NMR (CDCl\(_3\)): \(\delta 1.14\) and 1.51 (each s, each 3H, CH\(_3\) of acetone), 2.41 (s, 3H, COCH\(_3\)), 3.14 (s, 2H CH\(_2\)-10), 5.55 (t, 1H, H-7, J=3 Hz), but 3' did not give an acetone, indicating that 3 was the desired mixture of (S),9(S)- and 7(R), 9(R)-isomers having cis hydroxyl groups.
Fig. 1.

\[ \text{Compound 1} \rightarrow \text{Compound 2} \rightarrow \text{Compound 3} \rightarrow \text{Compound 4} \]

\[ \text{Compound 5} \rightarrow \text{Compound 6} \rightarrow \text{Compound 7} \rightarrow \text{Compound 8} \]

\[ \text{Compound 9} \rightarrow \text{Compound 10} \rightarrow \text{Compound 11} \rightarrow \text{Compound 12} \]
followed by acylation (KOH, acetone, 20°C, 8 hours) gave 13, mp 206–209°C (dec.), (CDCl₃): δ 2.22 (s, 3H, OAc), 5.14 (s, 2H, CH₂-14), 7.61 (s, 1H, H-11), 13.04 (s, 1H, OH-6), the benzyl position (C-7) of which was functionalized as described above to give the 4-demethoxy-11-deoxy-adriamycinone 14, mp 198–205°C (dec.). NMR (CDCl₃): δ 2.20 (s, 3H, OAc), 5.13 and 5.37 (AB-q, each 1H, CH₂-14), 5.42 (m, 1H, H-7), in 64% yield.

The aglycone 14 (32 mg) was condensed with 4 in a manner similar to that described above to give, after preparative TLC (benzene - EtOAc, 4:1), the glycosides 15 (12 mg) and 16 (12 mg) as major products. 15: mp 154–157°C, [α]₂₅ = −125° (c 0.2, acetone), Rf 0.36 (TLC: benzene -
EtOAc, 4: 1); 16: mp 155 -160°C, [α]D +225° (c 0.2, acetone), Rf 0.21.

The NMR spectra of 15 (δ 5.69, broad s) and 16 (δ 5.60, broad s) showed that both were α-glycosides.

Mild hydrolysis of 15 (12.3 mg) with 10% aqueous K2CO3 (0.06 ml) solution in methanol (6 ml) at 0°C for 3 hours gave the de-O-acylated product, which was treated with triethyl orthoformate in the presence of TsOH to protect the hydroxyl groups at C9 and 14. Then, the resulting orthoformate was de-N-acylated with 10% aqueous K2CO3 solution (1 ml) in methanol (4 ml) at 8°C for 16 hours, followed by removal of the orthoformate group with 1% acetic acid to give the desired adriamycin analogue 17 in 46% total yield; monohydrochloride: mp 158 -170°C (dec.), [α]D +75° (c 0.1, H2O), Rf 0.14 (TLC: CHCl3 · MeOH · AcOH, 20: 5: 1), NMR (D2O) δ 5.30 (s, 2H, CH2-14), 5.94 (broad s, 1H, H-1'), 7.54 (s, 1H, H-11).

Direct de-N-acylation of 15 under more drastic conditions led to degradation of the C-9 side chain.

Similarly, the isomer 18 was obtained from 16 in 47% yield; monohydrochloride: mp 145 -155°C (dec.), [α]D +25° (c 0.1, H2O), Rf 0.10, NMR (D2O): δ 5.34 (s, 2H, CH2-14), 5.47 (narrow m, 1H, H-7), 5.90 (broad s, 1H, H-1', 7.48 (s, 1H, H-11)).

The CD curve of adriamycin ([θ]max292 -0.69 x 104) was similar to that of 17 ([θ]max292 +0.5 x 103 in H2O), indicating that 17 and 18 were 7(S),9(S)-α- and 7(R),9(R)-α-glycosides, respectively.

The effect of the compounds on cell proliferation of L1210 leukemia is shown in Table 1, which indicates the concentrations (µg/ml) required for 50% growth inhibition on day 2 of the culture.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Growth (on day 2)</th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daunomycin (DM)</td>
<td>0.036</td>
<td>0.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Adriamycin (ADM)</td>
<td>0.03</td>
<td>1.65</td>
<td>0.68</td>
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4-Demethoxy-11-deoxy derivative

<table>
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<tr>
<th>Compounds</th>
<th>ID50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S, 9S, 1α (9)</td>
<td>0.01</td>
</tr>
<tr>
<td>7S, 9S, 1β (10)</td>
<td>0.51</td>
</tr>
<tr>
<td>7R, 9R, 1α (11)</td>
<td>&gt;2.5</td>
</tr>
<tr>
<td>7R, 9R, 1β (12)</td>
<td>1.25</td>
</tr>
</tbody>
</table>

4-Demethoxy-11-deoxy

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ID50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S, 9S, 1α (17)</td>
<td>0.03</td>
</tr>
<tr>
<td>7R, 9R, 1α (18)</td>
<td>72.5</td>
</tr>
</tbody>
</table>

The antitumor activity of 9 and 17 against L1210 leukemia was examined. The compounds were given to CDF1 mice intraperitoneally daily for 10 days starting 3 hours after the inoculation of 10⁴ tumor cells. As shown in Table 2, the activity of the adriamycin analog 17 was stronger than that of the daunomycin analog 9. 11-Deoxyadriamycin has been reported to show only a weak effect on L1210⁶, but the compound 17 showed a strong effect.

From these findings, 4-demethoxy-11-deoxyadriamycin (17) appears to be an interesting compound worthy of further study.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mg/kg/day</th>
</tr>
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<tbody>
<tr>
<td>Daunomycin</td>
<td>138*</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>189*</td>
</tr>
<tr>
<td>4-Demethoxy-11-deoxy DM</td>
<td>160</td>
</tr>
<tr>
<td>4-Demethoxy-11-deoxy ADM</td>
<td>177*</td>
</tr>
</tbody>
</table>

1-9, ip. *: Tox.

Table 2. Antitumor effects (T/C, %) of 4-demethoxy-11-deoxydaunomycin and adriamycin on L1210.

Table 1. Inhibitory effects of 4-demethoxy-11-deoxydaunomycin and adriamycin on growth, DNA and RNA synthesis in cultured L1210 cells.

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References


