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_2SO_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): δ 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 α,α'-azo-bis-isobutyronitrile in a mixture of $CHCl_3$, CCl_4 and water 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): Rf 0.33 and 0.25. **3**: mp 199~207°C (dec.), NMR ($CDCl_3$): δ 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 acetone at room temperature for 3 hours. Treatment of **3** with 2,2-dimethoxypropane and TsOH in dioxane gave the acetonide, NMR ($CDCl_3$): δ 1.14 and 1.51 (each s, each 3H, CH_3 of acetonide), 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 acetonide, indicating that **3** was the desired mixture of 7(*S*),9(*S*)- and 7(*R*), 9(*R*)-isomers having *cis* hydroxyl groups.

The glycal **4**, mp 144~148°C, $[\alpha]_D^{26}$ −100° (*c* 0.5, acetone), was prepared in 80% yield from 1,4-bis-O-(*p*-nitrobenzoyl)-N-trifluoroacetyldaunosamine[5] in two steps: (1) 4 N 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): Rf 0.32, 0.28, 0.18 and 0.35, respectively. **5**: mp 153~156°C, $[\alpha]_D^{24}$ −125° (*c* 0.2, acetone); **6**: mp 142~145°C, $[\alpha]_D^{25}$ +87.5° (*c* 0.2, acetone); **7**: mp 148~152°C, $[\alpha]_D^{25}$ −225° (*c* 0.2, acetone); **8**: mp 190~195°C, $[\alpha]_D^{25}$ −138° (*c* 0.2, acetone). In the NMR spectra, the signals due to their anomeric protons showed that **5** (δ 5.68, broad s) and **7** (δ 5.60, broad s) are α-anomers, while **6** (δ 5.15, dd, J=2 and 10 Hz) and **8** (δ 5.20, dd, J=2 and 10 Hz) are β-anomers[7].

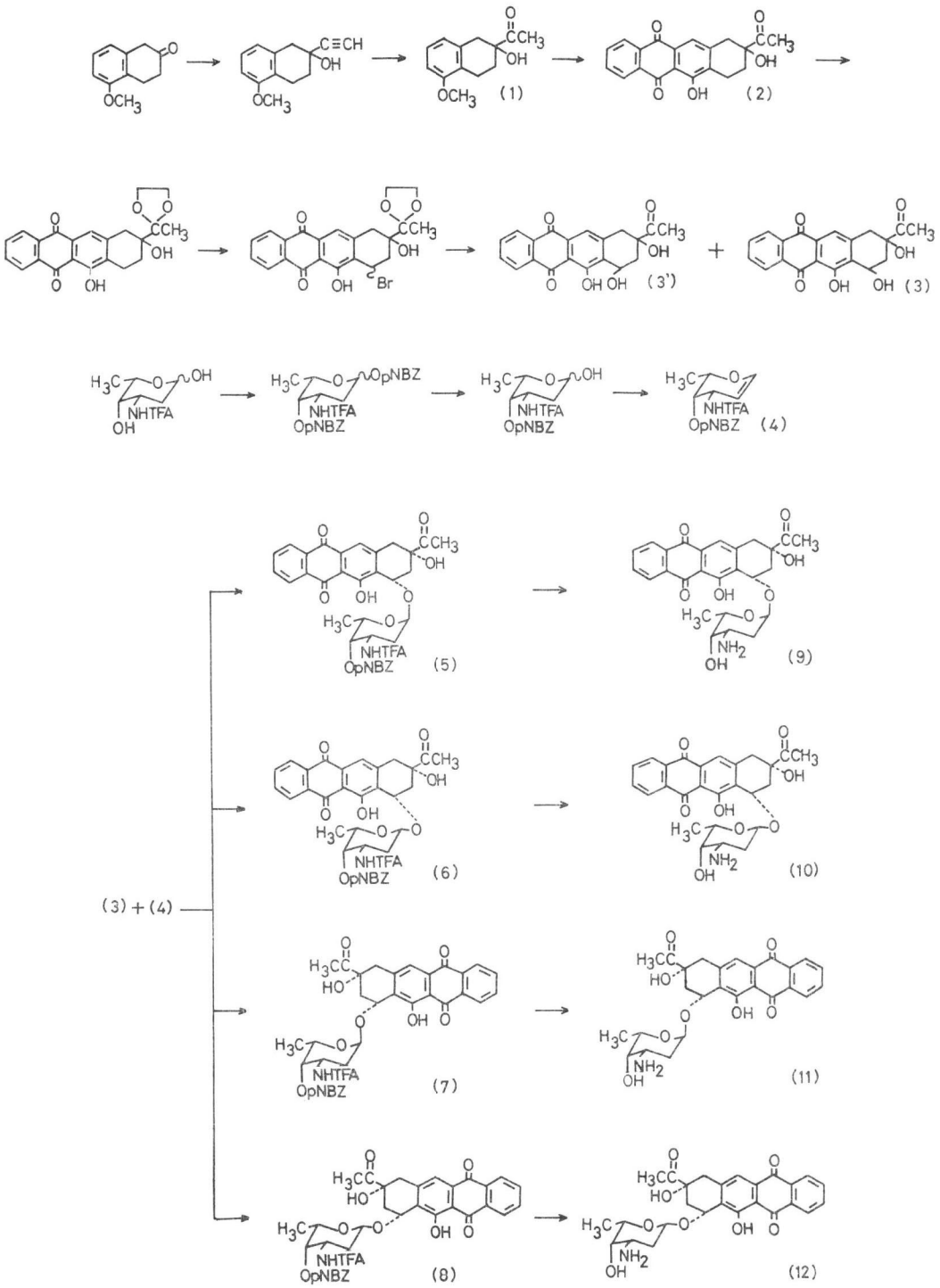
Deprotection of **5** with 10% aqueous K_2CO_3 solution in methanol at 8°C for 12 hours gave the daunomycin analogue **9** as a yellow powder in 94% yield. Similarly, **6**, **7** and **8** gave **10**, **11** and **12** in 87%, 92% and 86% yields. **9**: mp 202~212°C (dec.), $[\alpha]_D^{24}$ +50° (*c* 0.1, MeOH), CD: $[\theta]_{285}^{max}$ -1.08×10^4 (MeOH), Rf 0.40 (TLC: $CHCl_3$ - MeOH - AcOH, 40: 10: 1); **10**: mp 160~172°C (dec.). $[\alpha]_D^{22}$ +500° (*c* 0.1, MeOH), CD: $[\theta]_{287}^{max}$ -1.52×10^4 (MeOH), Rf 0.31; **11**: mp 180~195°C (dec.), $[\alpha]_D^{26}$ −150° (*c* 0.1, MeOH), CD: $[\theta]_{287}^{max}$ $+2.28\times10^4$ (MeOH), Rf 0.35; **12**: mp 185~195°C (dec.), $[\alpha]_D^{22}$ −400° (*c* 0.1, MeOH), CD: $[\theta]_{287}^{max}$ $+1.16\times10^4$ (MeOH), Rf 0.38.

The CD spectra of **9** and **10** showed curves similar to that of daunomycin ($[\theta]_{287}^{max}$ -1.72×10^4 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*)-α- and β-glycosides, and **11** and **12** are 7(*R*),9(*R*)-α- and β-glycosides[8].

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

Bromination (NBS, THF, 20°C, 6 hours) of **2**

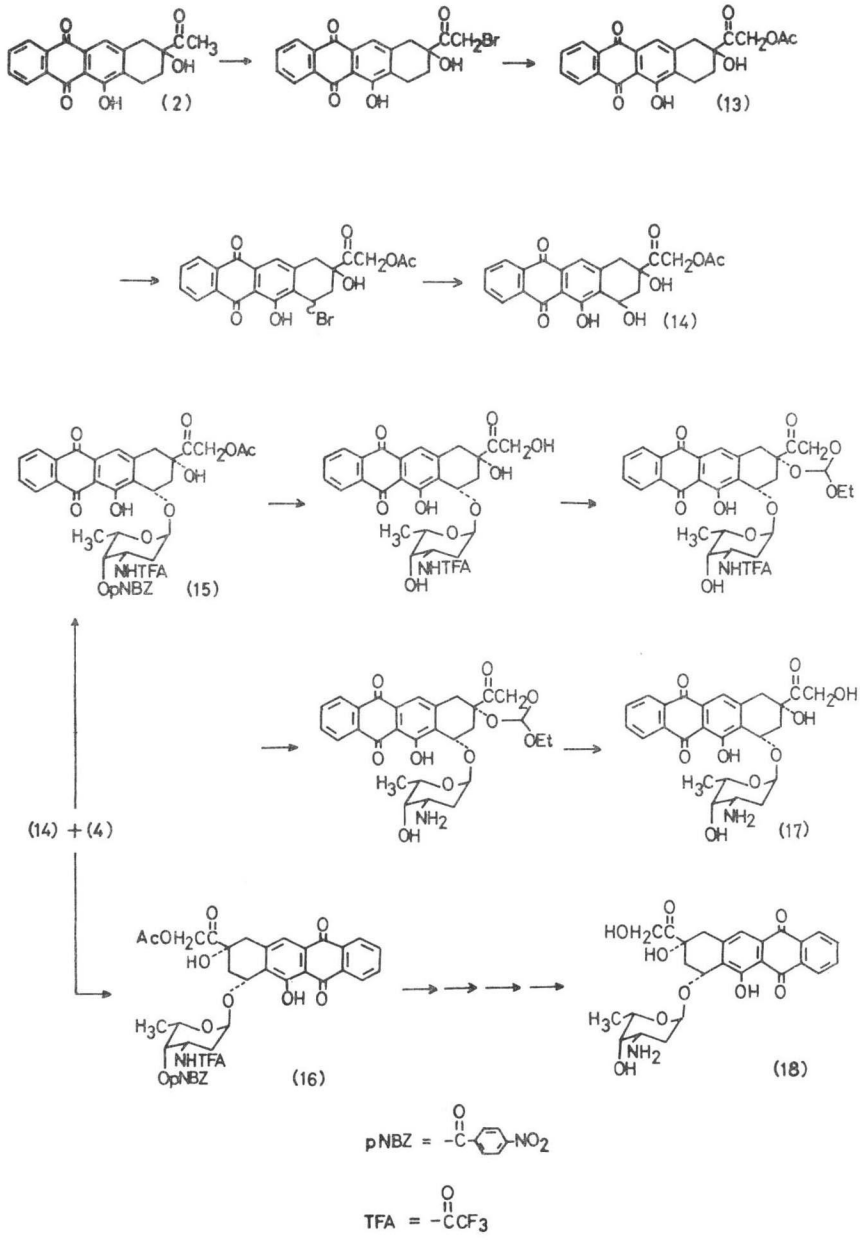
Fig. 1.



followed by acylation (KOAc, acetone, 20°C, 8 hours) gave **13**, mp 206~209°C (dec.), ($CDCl_3$); δ 2.22 (s, 3H, OAc), 5.14 (s, 2H, CH_2-14), 7.61 (s, 1H, H-11), 13.04 (s, 1H, OH-6), the benzylic 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 ($CDCl_3$): δ 2.20 (s, 3H, OAc), 5.13 and 5.37 (AB-q, each 1H, CH_2-14), 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, $[\alpha]_D^{25}$ −125° (*c* 0.2, acetone), Rf 0.36 (TLC: benzene -

Fig. 2.



EtOAc, 4: 1); **16**: mp 155~160°C, $[\alpha]_D^{25}$ −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 K_2CO_3 (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 K_2CO_3 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.), $[\alpha]_D^{24}$ +75° (*c* 0.1, H_2O), Rf 0.14 (TLC: $CHCl_3$ - MeOH - AcOH, 20: 5: 1), NMR (D_2O) δ 5.30 (s, 2H, CH_2-14), 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.), $[\alpha]_D^{25}$ −125° (*c* 0.1, H_2O), Rf 0.10, NMR (D_2O): δ 5.34 (s, 2H, CH_2-14), 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 ($[\theta]_{292}^{max}$ -0.69×10^4) was similar to that of **17** ($[\theta]_{290}^{max}$ -0.54×10^4) but opposite to that of **18** ($[\theta]_{285}^{max}$ $+0.5\times10^4$ in H_2O), 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.

4-Demethoxy-11-deoxy-derivatives (**9** and **17**) of daunomycin and adriamycin inhibited L1210 cell proliferation to the same extent as did daunomycin (DM) and adriamycin (ADM), however, other diastereomers showed no or, weak activity.

The activity on nucleic acid synthesis is shown in Table 1 as the ID_{50} (μg/ml) for [^{14}C]-thymidine or [^{14}C]-uridine incorporation into L1210 cells. Compounds **9** and **17** showed strong activity and inhibited [^{14}C]-thymidine incorporation more than [^{14}C]-uridine incorporation.

The antitumor activity of **9** and **17** against L1210 leukemia was examined. The compounds were given to CDF_1 mice intraperitoneally daily for 10 days starting 3 hours after the inoculation of 10^5 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 L1210[3], 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 1. Inhibitory effects of 4-demethoxy-11-deoxydaunomycin and adriamycin on growth, DNA and RNA synthesis in cultured L1210 cells.

Compounds	ID_{50} (μg/ml)		
	Growth (on day 2)	DNA	RNA
Daunomycin (DM)	0.036	0.3	0.18
Adriamycin (ADM)	0.03	1.65	0.68
4-Demethoxy-11-deoxy DM			
7*S*, 9*S*, 1′α (**9**)	0.01	0.22	0.34
7*S*, 9*S*, 1′β (**10**)	0.51	3.1	5.4
7*R*, 9*R*, 1′α (**11**)	>2.5	5.4	9.0
7*R*, 9*R*, 1′β (**12**)	1.25	4.5	5.0
4-Demethoxy-11-deoxy ADM			
7*S*, 9*S*, 1′α (**17**)	0.03	0.64	0.8
7*R*, 9*R*, 1′α (**18**)	72.5	>10	>10

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

Compounds	mg/kg/day					
	5	2.5	1.25	0.6	0.3	0.15
Daunomycin	Tox.	138*	191	145	132	118
Adriamycin	189*	351	272	239	147	130
4-Demethoxy-11-deoxy DM						
7*S*, 9*S*, 1′α (**9**)		160	135	115	100	100
4-Demethoxy-11-deoxy ADM						
7*S*, 9*S*, 1′α (**17**)		177*	>310	278	167	142

1-9, ip. *: Tox.

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