3′-DEAMINO-3′-MORPHOLINO DERIVATIVES OF DAUNOMYCIN, ADRIAMYCIN AND CARMINOMYCIN

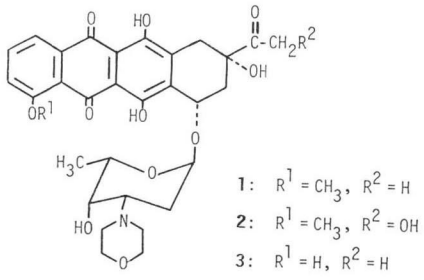
Sir:

In order to develop anthracyclines useful in cancer treatment, we have been studying the structure-activity relationships of anthracyclines and a variety of analogs including 4′-*O*-THP-adriamycin[1] and 4-demethoxy-11-deoxyadriamycin[2] have been synthesized.

Herein we describe the synthesis and properties of 3′-deamino-3′-morpholino-daunomycin (**1**), -adriamycin (**2**) and -carminomycin (**3**). Although it was our intention to defer publication up to the complete evaluation of their usefulness, the recent appearance[3] of a related study by ACTON *et al.* prompted disclosure of our results at this time. ACTON *et al.* applied BORCH's reductive alkylation to daunomycin and adriamycin, which produced their C-13 hydroxyl derivatives as byproducts as well as the desired 3′-morpholino derivatives, and reported that the 3′-morpholino derivative (**1**) of daunomycin was active at the 1/40 dose of adriamycin.

The synthesis is based on a new *N*-alkylation method to produce only the desired derivatives **1**, **2** and **3** in high yields, using bis(2-iodoethyl)-ether and triethylamine in DMF. The reagent, bis(2-iodoethyl)ether was readily obtained in a quantitative yield from commercially available bis(2-chloroethyl)ether and NaI[4]. Daunomycin hydrochloride (20 mg) was treated with bis(2-iodoethyl)ether (200 mg) and triethylamine (0.015 ml) in DMF (1 ml) at 20°C for 36 hours to give, after extraction (chloroform) and preparative TLC (chloroform - methanol, 9: 1), the corresponding derivative **1** in 87% yield; mp 139~145°C, $[\alpha]_D^{22}$ +325° (*c* 0.1, acetone), NMR ($CDCl_3$): 1.6~2.7 (m, 9H, H-2′, 3′, 8 and morpholino $CH_2\times2$), 1.38 (d, 3H, CH_3-6′), 2.40 (s, 3H, CH_3-14), 3.10 (AB-q, 2H, CH_2-10), 3.7 (m, 5H, H-4′ and morpholino $CH_2\times2$), 4.07 (dq, 1H, H-5′), *Anal.* found: C 62.01, H 6.12, N 2.10, calcd. for $C_{31}H_{35}NO_{11}$: C 62.30, H 5.90, N 2.34, FD-MS 597 (M^+).

Fig. 1.



Similarly, 3′-deamino-3′-morpholino-adriamycin (**2**) was synthesized from adriamycin in 85% yield; mp 154~160°C, $[\alpha]_D^{22}$ +300° (*c* 0.1, acetone), NMR ($CDCl_3$): 1.6~2.9 (m, 9H, H-2′, 3′, 8 and morpholino $CH_2\times2$), 1.37 (d, 3H, CH_3-6′), 3.10 (AB-q, 2H, CH_2-10), 3.7 (m, 5H, H-4′ and morpholino $CH_2\times2$), 3.97 (dq, 1H, H-5′), *Anal.* found: C 60.38, H 6.00, N 1.99, calcd. for $C_{31}H_{35}NO_{12}$: C 60.68, H 5.75, N 2.28, FD-MS 613 (M^+). Also, 3′-deamino-3′-morpholino-carminomycin (**3**) was obtained in 70% yield; mp 130~137°C, $[\alpha]_D^{22}$ +200° (*c* 0.1, acetone), Rf 0.91 (TLC: chloroform - methanol, 5: 1), NMR: 1.38 (d, 3H, CH_3-6′), 2.40 (s, 3H, CH_3-14), 3.12 (AB-q, 2H, CH_2-10), *Anal.* found: C 61.40, H 5.98, N 2.10, calcd. for $C_{30}H_{33}NO_{11}$: C 61.74, H 5.70, N 2.40, FD-MS 583 (M^+).

The effect of the derivatives **1** and **2** 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. The activity on nucleic acid synthesis is also shown in Table 1 as the ID_{50} (μg/ml) for [^{14}C]thymidine or [^{14}C]uridine incorporation into L1210 cells[5]. Both derivatives **1** and **2** were found to be much more active (5~10 times) in inhibiting L1210 cell proliferation and RNA synthesis than their parent antibiotics. The antitumor activity of **1** and **2** against L1210 leukemia was examined. The derivatives were given to CDF_1 mice intraperitoneally daily for 10 days starting 24 hours after the inoculation of 10^5 tumor cells. As shown in Table 2, their

Table 1. Inhibitory effects on growth, DNA and RNA synthesis in cultured L1210 cells.

	ID_{50} (μg/ml)		
	Growth (on day 2)	DNA	RNA
Daunomycin-HCl	0.036	0.3	0.18
Adriamycin-HCl	0.03	1.65	0.68
3′-Morpholino-DM (**1**)	0.003	0.28	0.03
3′-Morpholino-ADM (**2**)	0.006	0.2	0.05

Table 2. Antitumor activities (T/C % of the survival period) on L1210.

	Dose (μg/kg/day)											
	1250	625	312	200	156	100	78	50	25	12.5	6.25	3.12
Daunomycin-HCl	235	157	145		102		102					
Adriamycin-HCl	229	235	157		120		108					
3′-Morpholino-DM (**1**)				Toxic		205*		120	114	120	96	
3′-Morpholino-ADM (**2**)								Toxic	90*	151	108	108

1~9, i.p. * Toxic

Table 3. Rf values on TLC with chloroform-methanol (5: 1).

	Rf values
Daunomycin	0.07
Adriamycin	0.02
Aclacinomycin	0.88
1	0.89
2	0.78
3	0.91

effective dose ranges were very narrow, namely, the optimal dose of the daunomycin derivative **1** was only about 100 μg/kg, and that of the adriamycin derivative **2** was 12.5 μg/kg, indicating that these derivatives were very potent but have a strong toxicity. Derivatives **1** and **2** were also found to cause death by oral administration, when examined in CDF_1 mice: LD_{50} 1,000 μg/kg and 500 μg/kg, respectively. The acute toxicity by intraperitoneal injection was as follows: LD_{50} 500 μg/kg and 250 μg/kg for **1** and **2**. It indicates the possible absorption by oral administration.

Comparison of Rf values of these derivatives with that of orally active aclacinomycin[6], as shown in Table 3, suggests that Rf value more than approximately 0.7 may be a factor for the oral absorption of anthracyclines.

Acknowledgements

This work was supported by a grant-in-aid for cancer research from the Ministry of Education, Science and Culture, Japan.

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(Received October 8, 1981)

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