THE ABSOLUTE STRUCTURES OF THP-ADRIAMYCINS

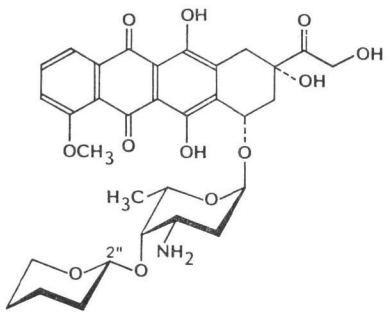
Sir:

We have synthesized diastereomeric 4′-*O*-tetrahydropyranyl (THP)-adriamycins[1] (**1** and **2**) from adriamycin and named them THP-adriamycins-(b) and -(a) respectively. Compound **1** was endowed with stronger antitumor activity and lower toxicity in mice than adriamycin[2]. The antitumor effect and low toxicity have been confirmed by Phase II study.

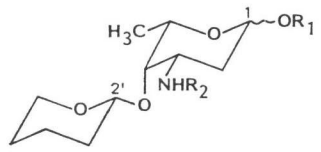
In this communication, the absolute structures of **1** and **2** are disclosed.

Treatment of **1** (hydrochloride: 100 mg) with cytochrome-C reductase[3] (50 mg) and β-nicotinamide adenine dinucleotide (reduced form: 400 mg) in a potassium phosphate buffer (pH 7.2; 150 ml) at 37°C for 2 hours under nitrogen gave 7-deoxyadriamycinone as precipitate. After removal of the solvent, the THP-daunosamine (**3**) which was in the supernatant was purified by column chromatography on CG 50 (NH_4^+) resin with water. The colorless solid (26 mg) gave Rf 0.49 on silica gel TLC ($CHCl_3$ - MeOH - conc NH_4OH, 40: 10: 1). The THP derivative **3** was *N*-acylated with *p*-bromobenzoyl chloride (25 mg) in pyridine (4 ml) followed by

Chart 1. THP-adriamycins and their derivatives.



1



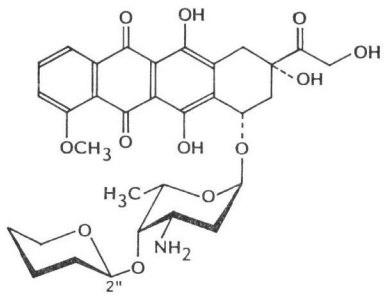
3 $R_1 = H$, $R_2 = H$

4α $R_1 = CO$ (α-Anomer)

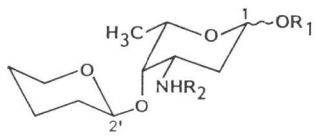
$R_2 = CO$ Br

4β $R_1 = CO$ (β-Anomer)

$R_2 = CO$ Br



2



5 $R_1 = H$, $R_2 = H$

6α $R_1 = CO$ (α-Anomer)

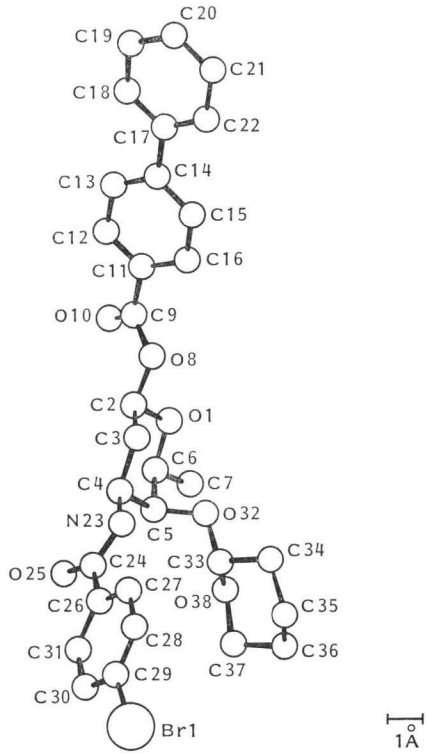
$R_2 = CO$ Br

6β $R_1 = CO$ (β-Anomer)

$R_2 = CO$ Br

Fig. 1. Molecular structure of **4β** drawn by PLUTO* program.

* PLUTO "Cambridge Crystallographic Database", Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England (1983).



O-acylation with *p*-phenylbenzoyl chloride (40 mg) and pyridine (2 ml) to give a mixture of 3-*N*-*p*-bromobenzoyl-1-*O*-*p*-phenylbenzoyl-4-*O*-(2′-tetrahydropyranyl)-α- and β-L-daunosamines (**4α** and **4β**). These α and β anomers were readily separated on a preparative silica gel TLC plate (Rf 0.57 and 0.51) with hexane - ethyl acetate (3: 2). After elution from the plate, needles of **4α** were obtained from dichloromethane - hexane (1 mg). Physico-chemical properties: mp 165~168°C; $[\alpha]_D^{24}$ −140° (*c* 0.15, acetone); ^{1}H NMR (90 MHz, acetone-d_6): δ 6.49 (1H, broad singlet, H-1). Prisms of **4β** were obtained from dichloromethane - hexane (4.2 mg). Physico-chemical properties: mp 182~183°C; $[\alpha]_D^{22}$ +17° (*c* 0.4, acetone); ^{1}H NMR (400 MHz, acetone-d_6): δ 4.58 (1H, dd, *J*=2 and 7 Hz, H-2′), 6.10 (1H, dd, *J*=2 and 10 Hz, H-1).

The X-ray crystallographic study of **4β** revealed the absolute configuration at C-2′ (Chart 1) to be the *R*-configuration as described below.

Similarly, the other THP-adriamycin (**2**, 100 mg) was converted through the corresponding THP-daunosamine **5** [Rf 0.42 on silica gel TLC ($CHCl_3$ - MeOH - conc NH_4OH, 40: 10: 1)] into a mixture of 1-*O*-benzoyl-3-*N*-*p*-bromobenzoyl-4-*O*-(2′-tetrahydropyranyl)-α- and β-L-daunosamines (**6α** and **6β**). These were separated on a preparative silica gel TLC plate (Rf

Fig. 2. Molecular structures of **6α** drawn by PLUTO program.

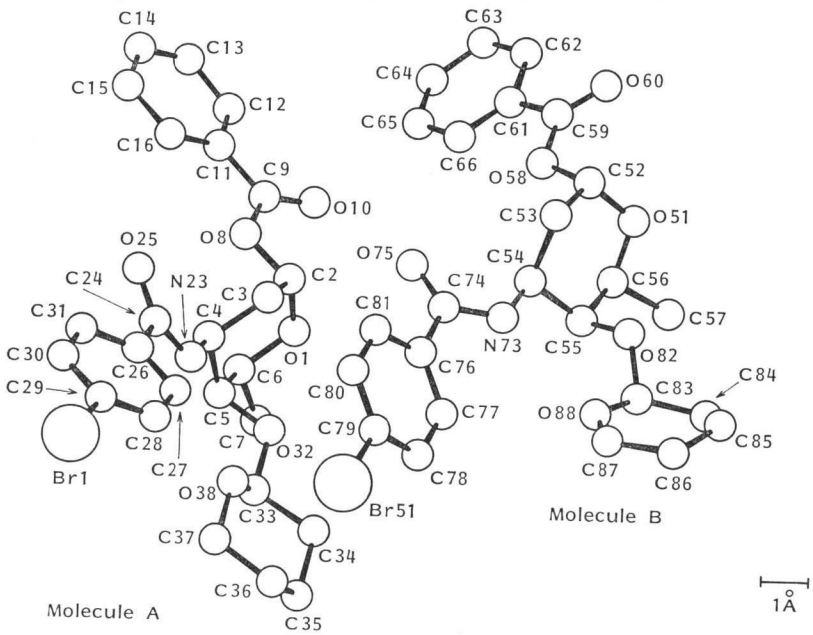


Table 1. Crystal data and the process of structure determination.

	4β	**6α**
Formula	$C_{31}H_{32}NO_6Br$	$C_{25}H_{28}NO_6Br$
MW	594.5	518.4
Crystal system	Monoclinic	Triclinic
Space group	$P2_1$	P1
Lattice parameters		
a (Å)	13.901 (7)	9.749 (5)
b	11.042 (6)	15.157 (8)
c	9.550 (5)	8.853 (5)
α (°)		93.64 (5)
β	104.35 (5)	107.82 (5)
γ		87.04 (5)
V (Å^3)	1,420	1,242
Z	2	2
Dcal (gcm^{-3})	1.391	1.386
Radiation	CuKα (monochromated by graphite)	
Intensity measurement		
2θ range (°)	6~140	6~150
No. of planes		
Theoretical	2,803	5,010
Observe as above 2σ (I)	1,740	3,781
No. of refined atoms	39 (C, N, O, Br)	66 (C, N, O, Br)
Final R value (%)	6.68	6.74
No. of Bijvoet pairs		
Accounted	74	237
Agreed with the absolute configuration	71	222

0.68 and 0.52) with hexane - ethyl acetate (1: 1). After elution from the plate, plates of **6α** were obtained from acetone - hexane (2 mg). Physico-chemical properties: mp 140~143°C; $[\alpha]_D^{24}$ −151° (c 0.3, acetone); ^{1}H NMR (400 MHz, acetone-d_6): δ 4.63 (1H, dd, J=2 and 8 Hz, H-2′), 6.44 (1H, broad singlet, H-1). Compound **6β** was precipitated from acetone - hexane (4.2 mg). Physico-chemical properties: mp 80~84°C; $[\alpha]_D^{22}$ −6.3° (c 0.3, acetone); ^{1}H NMR (90 MHz, acetone-d_6): δ 6.07 (1H, dd, J=3 and 9 Hz, H-1).

The absolute configuration at C-2′ (Chart 1) of **6α** was determined to be the *S*-configuration by the X-ray crystallographic study as follows.

The lattice parameters and intensity data were measured on a Philips PW 1100 diffractometer using graphite-monochromated CuKα radiation. The crystal data and the process of the structure determination are summarized in Table 1*.

The molecular structures of **4β** and of the two crystallographically independent molecules of **6α** are shown in Figs. 1 and 2.

The corresponding bond lengths and angles in these three molecules agreed well with each other and also with the expected values for the chemical structures. Most of the differences were comparable with their estimated standard deviation's (0.02 Å and 1°) except for those involving C84, C85, C87 and O88 atoms in molecule B of **6α** which underwent large thermal vibrations (B_{eq}'s of these four atoms ranged from 14 to 20 Å^2 compared with about 6 Å^2 for other atoms).

Consequently, THP-adriamycin-(b) (**1**) which showed a strong antitumor effect against L-1210 *in vivo* was determined to have (2″*R*)-configuration and THP-adriamycin-(a) (**2**) was confirmed to have (2″*S*)-configuration, respectively, as depicted in Chart 1.

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* The atomic parameters, bond lengths and bond angles have been sent to the Cambridge Crystallographic Data Centre. The list of observed and calculated structure factors may be obtained from one of the authors (H.N.) upon request.

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(Received May 10, 1984)

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