IMPROVED ANTITUMOR EFFECTS OF 3'-DEAMINO-3'-MORPHOLINO DERIVATIVES OF PIRARUBICIN

KEIICHI AJITO, DAISHIRO IKEDA,* CHISATO NOSAKA, KEIKO KOMURO
SHINICHI KONDO and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication June 25, 1990)

The preparation and antitumor effects of 3'-deamino-3'-morpholino derivatives of pirarubicin are described. Di-*N*-alkylation of pirarubicin with bis(2-iodoethyl)ether gave 3'-morpholino-pirarubicin, which was converted into its 13-tosylhydrazone, 13-deoxy derivative and 13-(*S*)- and 13-(*R*)-dihydro derivatives. Intraperitoneal administration to murine tumors indicated that the effective dose ranges of the compounds having sp^3 carbon at C-13 position were broader than those of the compounds having sp^2 carbon. By oral administration, 13-(*S*)-dihydro isomer was more effective than 13-(*R*)-dihydro isomer.

Semisynthetic antitumor anthracycline, pirarubicin[1] (**1**) was marketed in 1988. It shows excellent antitumor activities and low cardiotoxicity in the clinical treatments. As a part of our efforts to prepare related compounds having improved therapeutic properties, we already reported that *N*-salicylidene analogs of **1** exhibited the interesting *in vivo* antitumor effects.[2] Now, we wish to report the results of our studies on syntheses and biological evaluations of several morpholino analogs of **1**, such as 3'-deamino-3'-morpholino (**2**), 13-tosylhydrazone (**3**), 13-deoxy (**4**), 13-(*S*)- and 13-(*R*)-dihydro (**5** and **6**). Furthermore, the configurations of C-13 position of **5** and **6** have been completely assigned to be *S* for **5** and *R* for **6** by using ^{1}H NMR experiments.

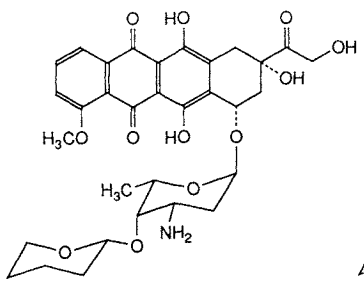
3'-Deamino-3'-morpholino analogs were known as highly potent anthracyclines.[3~6] Among them, the effective dose ranges of MX, MX2 and MY5 were rather broad compared with those of morpholino analogs of doxorubicin, daunorubicin and carminomycin. We considered that those differences of the antitumor effects were dependent on the structural differences at C-13 position between the MX group and the doxorubicin group.

The carbonyl function of C-13 of anthracyclines is one of the principal site of *in vivo* antitumor activity. The conversion of the carbonyl function of C-13 position of **2** into hydrazone, methylene and hydroxyl functions might display biological properties different from the parent compound.

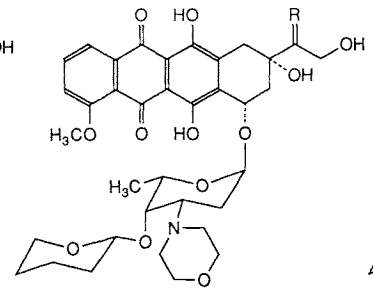
Chemistry

Di-*N*-alkylation of amino group of **1** with bis(2-iodoethyl)ether and triethylamine in *N*,*N*-dimethylformamide (DMF) provided 4-morpholinyl derivative **2** in 56% yield. Reductive *N*-alkylation of **1** also gave **2** in relatively low yield along with by-products.

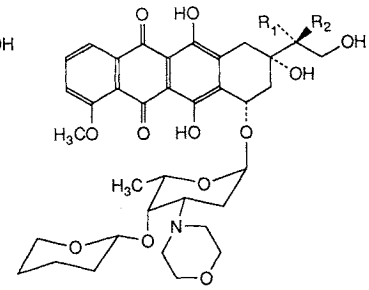
The conversion of carbonyl function at C-13 position of **2** to corresponding methylene derivative was accomplished by the mild reduction of 13-tosylhydrazone **3** with catecholborane.[7] A large number of methods were reported concerning the preparation of methylene derivatives from carbonyl compounds, but the reduction procedures generally utilized strong acids, bases or large excess of hydride reagents. Treatment of **2** with *p*-toluenesulfonylhydrazide in anhydrous methanol under refluxing afforded



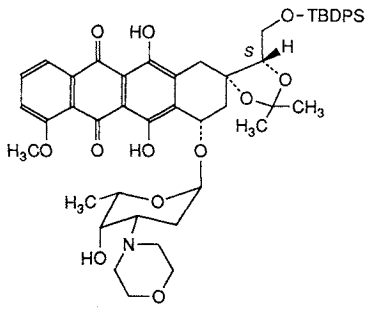
1



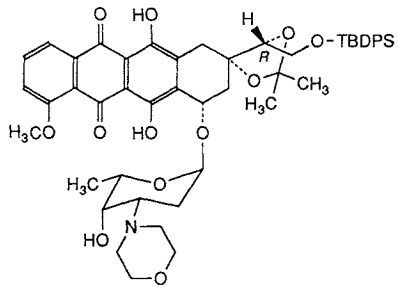
2 R=O
3 R=NNHTs
4 R=H_2



5 R_1=H R_2=OH; 13-(*S*)
6 R_1=OH R_2=H; 13-(*R*)



7



8

TBDPS: *tert*-Butyldiphenylsilyl.

13-tosylhydrazone (**3**) in 69% yield. The hydroboration of **3** with 1.1 eq of catecholborane in anhydrous chloroform at $-10°C$ for 20 minutes, followed by the elimination with sodium acetate trihydrate under a gentle reflux gave 13-deoxy derivative **4** in 13% yield.

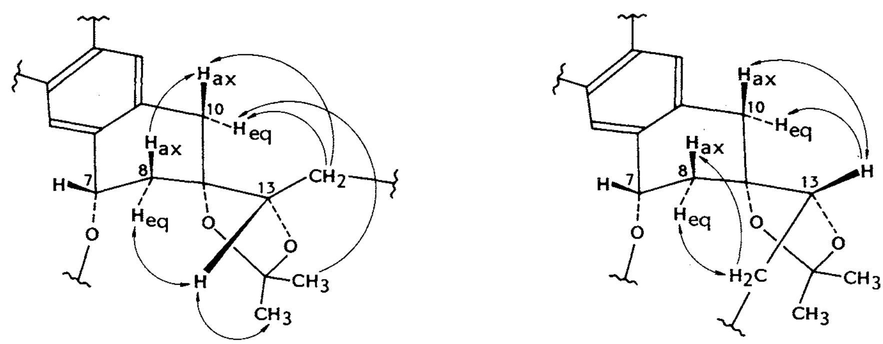
It has been known that 13-carbonyl function of doxorubicin and its analogs was extensively reduced in mammalian tissues to give the corresponding 13-dihydro derivatives and that these metabolites had significant antitumor activity. When **2** was treated with sodium cyanoborohydride and trifluoroacetic acid in chloroform and methanol at room temperature, two diastereomeric isomers of 13-dihydro derivatives **5** and **6** were obtained. In ^1H NMR spectrum of the obtained isomeric mixture, signals of each isomer were observed in 1 : 1 ratio. Each isomer was obtained as the pure form by the precise silica gel chromatography and crystallization. The diastereomeric mixture was chromatographed on silica gel column to give a slightly less polar isomer **5** and the mixture with enriched proportion of isomer **6**, which was crystallized from acetonitrile to afford pure **6**. In ^1H NMR spectrum, the methine proton of C-13 position of pure **5** appeared at δ 3.53 as a broad singlet and that of **6** at δ 3.69 as a broad singlet. In order to determine the stereochemistry of C-13 position, the derivatizations of **5** and **6** were carried out. The treatment of **5** with *tert*-butylchlorodiphenylsilane and imidazole in DMF subsequently with 2,2-dimethoxypropane and *p*-toluenesulfonic acid gave the de-*O*-tetrahydropyranylated 14-*O*-*tert*-butyldiphenylsilyl-13-dihydro-9,13-*O*-isopropylidene derivative **7**. The isomer **6** was transformed to **8** by the same procedure. The isomeric mixture of **5** and **6** (1 : 1) was also converted to silyl-acetonide. The obtained mixture was chromatographed on the preparative silica gel TLC to yield a pure **7** and **8**.

Based on ^1H NMR experiments of **7** and **8**, the absolute configurations of C-13 position of **5** and **6**

Table 1. ^{1}H NMR data of **7** and **8**.

Protons	**7** (δ, multiplicity, J=Hz)	$\Delta\delta$	**8** (δ, multiplicity, J=Hz)	$\Delta\delta$
O–C(CH$_3$)$_2$–O (CH$_3$)	1.32, s		1.42, s	
(CH$_3$)	1.44, s		1.42, s	
8-H_{ax}	2.15, dd, 15, 5.5	0.12	1.99, dd, 14.5, 5.5	0.20
8-H_{eq}	2.27, br d, 15		2.19, br d, 14.5	
10-H_{ax}	2.63, d, 18	0.59	2.75, d, 18.5	0.55
10-H_{eq}	3.22, br d, 18		3.30, br d, 18.5	
13-H	3.97, br dd, 6.5, 5		4.21, br dd, 7.5, 5.5	
14-H_h	3.82, dd, 9.5, 6.5		3.79, dd, 10.5, 7.5	
14-H_l	4.02, dd, 9.5, 5		3.98, dd, 10.5, 5.5	

Fig. 1. NOE experiments of **7** and **8**.



7 **8**

were determined. ^{1}H NMR spectral data of the selected protons of **7** and **8** were listed in Table 1. PENCO *et al.*[8] utilized the differences of chemical shifts ($\Delta\delta$ value) between 8-H_{ax} and 8-H_{eq}, and between 10-H_{ax} and 10-H_{eq} of 13-dihydroanthracyclinones to determine the absolute configuration of C-13 positions. In our case, however, as shown in Table 1 $\Delta\delta$ values of 8-H_2 and 10-H_2 in **7** and **8** could not give the clear evidence for the configuration of C-13 position. In further NMR studies, the NOE experiments of **7** and **8** clarified the configuration of C-13 position. Fig. 1 shows the results of the differential spectra of NOE in **7** and **8**. NOEs were observed between 13-H and 8-H_{eq} in **7**. On the other hand, NOEs in **8** were observed between 13-H and 10-H_{ax}, and 13-H and 10-H_{eq}. These ^{1}H NMR experiments showed that the absolute configuration of 13-position of **7** was *S* and that of **8** was *R*. Thus, **5** has *S* configuration at C-13 position and **6** has *R* configuration. Configurations of some 13-dihydroanthracyclines were reported as follows: C-13 positions of actinomycete products, rubeomycins **B** and **B$_1$** were *R* configurations,[9] while those of urine metabolites, such as 13-dihydroadriamycin, -daunomycin and -idarubicin were *S* configurations.[8] The stereochemistry of C-13 position of 13-dihydroidarubicin was confirmed to be *S* by the total synthesis.[10]

Antitumor Activity

Table 2 represents the cytotoxicity of derivatives **2**~**6** against P388 and doxorubicin-resistant P388 (P388/ADR) cells. The *in vivo* antitumor activity of **2**~**6** were evaluated in the murine L1210 leukemia. The results of the intraperitoneal and oral administrations are shown in Figs. 2 and 3, respectively.

The effective dose ranges of **4**, **5** and **6** by ip administration were broader than those of **2** and **3** (Fig.

Fig. 2. Antitumor activity of **1**~**6** against L1210 leukemia (ip).

■ **1**, ▲ **2**, ● **3**, △ **4**, ○ **5**, □ **6**.
* Loss of body weight of animals (>2 g) was observed.

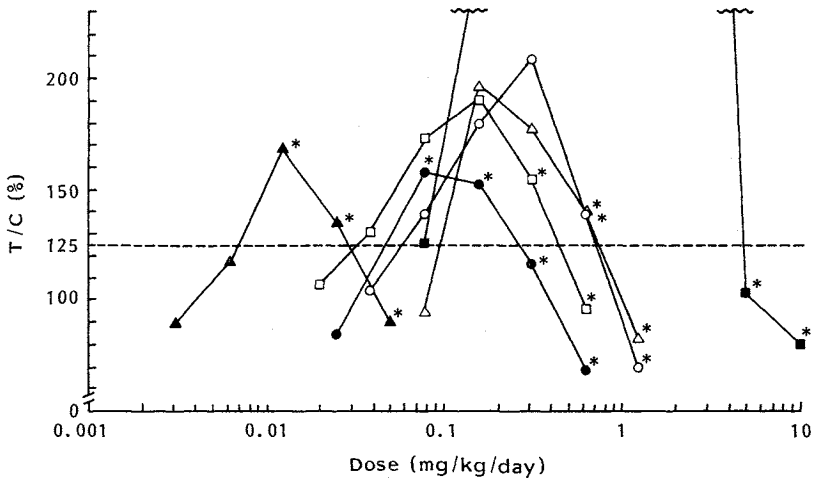
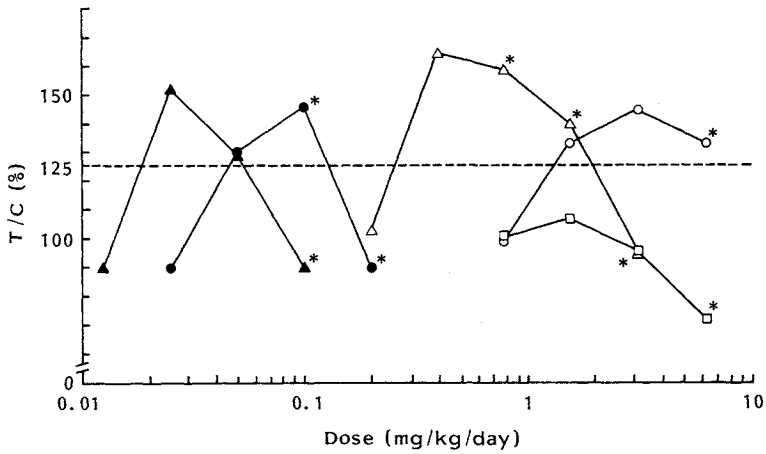


Fig. 3. Antitumor activity of **2**~**6** against L1210 (po)[a].

▲ **2**, ●, **3**, △ **4**, ○ **5**, □ **6**.
* Loss of body weight of animals (>2 g) was observed.



[a] Compound **1** was not active at doses of 12.5~50 mg/kg.

2). Although it was reported that rubidazone had a similar antitumor activity to the parent anthracycline,[11] 13-tosylhydrazone **3** did not show the improvement of the antitumor effects by ip administration. Both of 13-dihydro derivatives **5** and **6** exhibited nearly the same antitumor effects. By po administration, **5** showed a good activity but **6** showed no antitumor effect (Fig. 3). The configuration of C-13 position might affect the absorption of drugs from intestinal tract.

Table 2. Cytotoxicity against leukemia P388 and P388/ADR cells.

Compounds	IC_{50} (ng/ml)	
	P388	P388/ADR
1	5.4	130
2	54	110
3	730	>1,000
4	190	380
5	230	510
6	140	480

It was noteworthy that although all morpholino derivatives had weaker cytotoxicity than the parent compound **1** (Table 2), these derivatives showed *in vivo* antitumor effects at lower dose levels than that of **1**. The results showed that in morpholino derivatives of **1** the transformation of sp^2 carbon to sp^3 carbon at C-13 position improved the *in vivo* antitumor effects, even though the optimum dose level increased.

Experimental

General Methods

MP's were determined with a Yanagimoto micro melting point apparatus and were uncorrected. Optical rotations were measured on Perkin-Elmer 241 polarimeter at ambient temperature. Mass spectra were obtained on a Hitachi M-80H or a Jeol JMX-SX-102 mass spectrometers. ^{1}H NMR spectra were measured with a Varian EM-390 or a Jeol JNM-GX 400 NMR spectrometers in $CDCl_3$. TLC was performed on silica gel (Kieselgel 60 F_{254}, Merck) developed with a mixture of chloroform - methanol - 28% ammonium hydroxide (100 : 10 : 1) and Rf values were calculated.

Satisfactory elemental analyses were obtained for all derivatives synthesized here.

Antitumor Activity *In Vitro*

P388 and P388/ADR leukemia cells, maintained on RPMI-1640 medium containing 10% fetal bovine serum and 10 μM of 2-hydroxyethyl disulfide, were incubated with target compounds for 72 hours. The number of viable cells was measured by the MTT assay and IC_{50} values were calculated.

Antitumor Activity *In Vivo*

L1210 leukemia cells (10^5) maintained by serial intraperitoneal passage in our institute were implanted intraperitoneally into female CDF_1 mice (20 $\pm$ 1 g) on day 0 and compounds were administered once daily on day 1 ~ 9. The survival period was observed for 60 days. T/C (%) was calculated for the median survival period of the treated group (T) of mice and that of the control group (C, survival period: 7 ~ 9 days). T/C values over 125% were judged to be active.

3'-Deamino-3'-morpholino Derivative (**2**)

To a suspension of pirarubicin (**1**, 1.45 g) in DMF (73 ml) containing triethylamine (1.1 ml) was added bis(2-iodoethyl)ether (8.7 g). The reaction mixture was stirred at room temperature overnight in the dark place. The resultant solution was concentrated to give a residue which was purified on silica gel column chromatography (chloroform→chloroform - ethanol, 60 : 1). The fractions containing **2** were concentrated with additional isopropyl ether to afford **2** (910 mg) in 56% yield: MP 155 ~ 162°C (dec); $[\alpha]_D$ +340° (*c* 0.01, chloroform); FD-MS *m/z* 698 $(M+H)^+$; Rf 0.73; ^{1}H NMR δ 1.35 (3H, d, 6'-H), 1.97 (1H, td, 2'-H), 2.14 (1H, dd, 8-H_{ax}), 3.06 (1H, d, 10-H_{ax}), 3.28 (1H, dd, 10-H_{eq}), 3.46 (1H, m, 6''-H), 3.63 (4H, t, $N(CH_2CH_2)_2O$), 3.96 (1H, s, 4'-H), 4.09 (3H, s, OCH_3), 4.76 (2H, s, 14-H), 5.12 (1H, dd, 2''-H), 5.30 (1H, br s, 7-H), 5.58 (1H, br d, 1'-H), 7.40 (1H, d, 3-H), 7.79 (1H, t, 2-H), 8.04 (1H, d, 1-H).

3'-Deamino-3'-morpholino-13-tosylhydrazone Derivative (**3**)

A mixture of **2** (239 mg) and *p*-toluenesulfonylhydrazide (128 mg) in anhydrous methanol (7 ml) was refluxed for 3.5 hours. After evaporation, the residue was purified on preparative TLC with chloroform - ethanol - toluene - ethyl acetate (20 : 2 : 5 : 5) to afford **3** (205 mg) in 69% yield: MP 156 ~ 160°C (dec); $[\alpha]_D$ +210° (*c* 0.01, chloroform); FD-MS *m/z* 866 $(M+H)^+$; Rf 0.46; ^{1}H NMR δ 1.26 (3H, d, 6'-H), 2.41 (3H, s, Ar-CH_3), 2.80 (1H, d, 10-H_{ax}), 3.18 (1H, d, 10-H_{eq}), 3.62 (4H, t, $N(CH_2CH_2)_2O$), 3.90 (1H, s, 4'-H), 4.06 (3H, s, OCH_3), 4.61 (2H, s, 14-H), 5.08 (1H, br s, 2''-H), 5.22 (1H, br s, 7-H), 5.57 (1H, br s, 1''-H).

3'-Deamino-13-deoxy-3'-morpholino Derivative (**4**)

Hydrazone **3** (205 mg) was dissolved in chloroform (4 ml) and the solution was cooled at −10°C. To the chilled solution was added catecholborane (28 μl) and the resultant solution was kept at −10°C for

20 minutes. After addition of sodium acetate trihydrate (97 mg) at −10°C the mixture was refluxed for 1 hour and extracted with chloroform (20 ml). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to give a residue which was purified on preparative TLC with chloroform - methanol - 28% ammonium hydroxide (100 : 10 : 1). 13-Deoxy derivative **4** (21 mg) was afforded in 13% yield: MP 126~145°C (dec); $[\alpha]_D$ +260° (*c* 0.01, chloroform); FD-MS *m/z* 684 $(M+H)^+$; Rf 0.71; ^{1}H NMR δ 1.38 (3H, d, 6'-H), 1.87 (1H, dt, 13-H), 1.96 (1H, td, 2'-H_{ax}), 2.04 (1H, td, 13-H), 2.55 (1H, br d, 8-H_{eq}), 2.58 (1H, d, 10-H_{ax}), 3.42 (1H, br dd, 10-H_{eq}), 3.46 (1H, m, 6''-H), 3.62 (4H, t, $N(CH_2CH_2)_2O$), 3.93 (1H, br s, 4'-H), 3.99 (2H, q, 14-H), 4.07 (1H, q, 5'-H), 4.09 (3H, s, OCH_3), 5.11 (1H, dd, 2''-H), 5.26 (1H, br dd, 7-H), 5.58 (1H, d, 1'-H), 7.39 (1H, br d, 3-H), 7.78 (1H, t, 2-H), 8.05 (1H, dd, 1-H). Unreacted **3** (20 mg) was recovered.

(13*S*)-3'-Deamino-13-dihydro-3'-morpholino Derivative (**5**) and (13*R*)-3'-Deamino-13-dihydro-3'-morpholino Derivative (**6**)

To a solution of **2** (908 mg) and trifluoroacetic acid (18 μl) in chloroform (9 ml) and anhydrous methanol (9 ml) was added $NaB(CN)H_3$ (163 mg) and the solution was allowed to stand for 1.5 hours at room temperature. After addition of triethylamine (34 μl) concentration of the solution gave a residue which was extracted with chloroform (36 ml). The organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to give crude products. These are purified on silica gel chromatography (chloroform - ethanol, 40 : 1) in a short column to yield a mixture (650 mg) of **5** and **6** (1 : 1) in 71% yield. The mixture was applied onto the second silica gel (150 g) column equilibrated with chloroform - ethanol (40 : 1). Pigments were developed with chloroform - ethanol (25 : 1) in 20 ml fractions. Fractions (Nos. 51~59) were concentrated with additional isopropyl ether to afford pure **5** (28.9 mg). Concentration of fractions (Nos. 96~115) gave a mixture (27.6 mg, **5** : **6** = 1 : 1.67) which was dissolved with 2.8 ml of acetonitrile. Keeping this solution at room temperature for 10 days pure **6** (12.3 mg) was obtained as a crystalline solid. **5**: MP 153~160°C (dec); $[\alpha]_D$ +280° (*c* 0.02, chloroform); FD-MS *m/z* 700 $(M+H)^+$; Rf 0.44; ^{1}H NMR δ 1.38 (3H, d, 6'-H), 1.96 (1H, td, 2'-H_{ax}), 2.63 (1H, d, 10-H_{ax}), 2.75 (1H, br d, 8-H_{eq}), 3.23 (1H, br d, 10-H_{eq}), 3.46 (1H, m, 6''-H), 3.53 (1H, br s, 13-H), 3.62 (4H, br t, $N(CH_2CH_2)_2O$), 4.08 (3H, s, OCH_3), 5.13 (1H, br t, 2''-H), 5.26 (1H, br s, 7-H), 5.58 (1H, br d, 1'-H), 7.38 (1H, d, 3-H), 7.77 (1H, t, 2-H), 8.01 (1H, d, 1-H).

6: MP 153~164°C; $[\alpha]_D$ +280° (*c* 0.02, chloroform); FD-MS *m/z* 700 $(M+H)^+$; Rf 0.44; ^{1}H NMR δ 1.37 (3H, d, 6'-H), 2.76 (1H, d, 10-H_{ax}), 3.30 (1H, br d, 10-H_{eq}), 3.45 (1H, m, 6''-H), 3.62 (4H, br t, $N(CH_2CH_2)_2O$), 3.69 (1H, br s, 13-H), 4.02 (1H br q, 5'-H), 4.08 (3H, s, OCH_3), 5.11 (1H, br t, 2''-H), 5.27 (1H, br s, 7-H), 5.58 (1H, br d, 1'-H), 7.38 (1H, d, 3-H), 7.77 (1H, t, 2-H), 8.02 (1H, d, 1-H).

(13*S*)-14-*O*-*tert*-Butyldiphenylsilyl-3'-deamino-13-dihydro-9,13-*O*-isopropylidene-3'-morpholinoadriamycin (**7**)

To a solution of **5** (20 mg) in DMF (1 ml) containing imidazole (2.9 mg) was added *tert*-butylchlorodiphenylsilane (9 μl), and the solution was kept at room temperature for **6** hours. After addition of water (5 μl) the mixture was concentrated to give a residue which was extracted with ethyl acetate (4 ml). The extract was washed with water, dried over anhydrous sodium sulfate and concentrated to give a 14-*O*-*tert*-butyldiphenylsilyl derivative. This was dissolved with 2 ml of acetone and 2,2-dimethoxypropane (0.1 ml) was added. After addition of anhydrous *p*-toluenesulfonic acid (6.1 mg) the solution was kept at room temperature for 30 minutes and poured into saturated aqueous sodium hydrogen carbonate solution (15 ml). After extraction with 15 ml of chloroform, the organic layer was washed with water, dried over anhydrous sodium sulfate and concentrated to give a residue which was purified on preparative TLC with chloroform - methanol (40 : 1). **7** (18 mg) was obtained in 70% yield. MP 186~189°C (dec); $[\alpha]_D$ +190° (*c* 0.02, chloroform); FAB-MS *m/z* 894 $(M+H)^+$; Rf 0.80. ^{1}H NMR δ 1.03 (9H, s, $C(CH_3)_3$), 1.32 and 1.44 (each 3H, s, $C(CH_3)_2$), 1.38 (3H, d, 6'-H), 1.72 (1H, br td, 2'-H_{ax}), 1.95 (1H, br dd, 2'-H_{eq}), 2.15 (1H, dd, 8-H_{ax}), 2.27 (1H, br d, 8-H_{eq}), 2.4~2.5, 2.6~2.7 (4H, $N(CH_2CH_2)_2O$), 2.63 (1H, d, 10-H_{ax}), 2.7 (1H, 3'-H), 3.22 (1H, br d, 10-H_{eq}), 3.63 (1H, br s, 4'-H), 3.69 (4H, br t, $N(CH_2CH_2)_2O$), 3.82 (1H, dd, 14-H), 3.97 (1H, br dd, 13-H), 4.02 (1H, dd, 14-H), 4.09 (3H, s, OCH_3), 4.24 (1H, br q, 5'-H), 5.02 (1H, br d, 7-H), 5.47 (1H, br d, 1'-H), 7.3~7.5 (7H, 3-H and 6H of phenyl), 7.6~7.7 (4H, phenyl), 7.78 (1H, t, 2-H), 8.05 (1H, dd, 1-H).

(13*R*)-14-*O*-*tert*-Butyldiphenylsilyl-3'-deamino-13-dihydro-9,13-*O*-isopropylidene-3'-morpholinoadriamycin (**8**)

Compound **6** was converted to **8** by the similar procedure as **5**. MP 125~131 °C (dec); $[\alpha]_D$ +140° (*c* 0.02, chloroform); FAB-MS *m/z* 894 $(M+H)^+$; Rf 0.81. ^1H NMR δ 1.03 (9H, s, $C(CH_3)_3$), 1.30 (3H, d, 6'-H), 1.42 (6H, s, $C(CH_3)_2$), 1.69 (1H, br td, 2'-H_{ax}), 1.92 (1H, br dd, 2'-H_{eq}), 1.99 (1H, dd, 8-H_{ax}), 2.19 (1H, br d, 8-H_{eq}), 2.4, 2.6~2.7 (4H, $N(CH_2CH_2)_2O$), 2.6~2.7 (1H, 3'-H), 2.75 (1H, d, 10-H_{ax}), 3.30 (1H, br d, 10-H_{eq}), 3.61 (1H, br s, 4'-H), 3.69 (4H, br t, $N(CH_2CH_2)_2O$), 3.79 (1H, dd, 14-H), 3.98 (1H, dd, 14-H), 4.09 (3H, s, OCH_3), 4.15 (1H, br q, 5'-H), 4.21 (1H, br dd, 13-H), 5.07 (1H, br d, 7-H), 5.63 (1H, br d, 1'-H), 7.3~7.5 (7H, 3-H, 6H of phenyl), 7.6~7.7 (4H, phenyl), 7.78 (1H, t, 2-H), 8.05 (1H, dd, 1-H).

Acknowledgments

We are grateful to Ms. MIEKO NAGASAWA and YUKO KARIYA, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., for the *in vitro* assay.

References

1) UMEZAWA, H.; Y. TAKAHASHI, M. KINOSHITA, H. NAGANAWA, T. MASUDA, M. ISHIZUKA, K. TATSUTA & T. TAKEUCHI: Tetrahydropyranyl derivatives of daunomycin and adriamycin. J. Antibiotics 32: 1082~1084, 1979
2) AJITO, K.; D. IKEDA, K. KOMURO, C. NOSAKA, N. WAKO, S. KONDO & T. TAKEUCHI: *N*-Salicylidene derivatives of pirarubicin. J. Antibiotics 42: 1133~1144, 1989
3) MOSHER, C. W.; H. Y. WU, A. N. FUJIWARA & E. M. ACTON: Enhanced antitumor properties of 3'-(4-morpholinyl) and 3'-(4-methoxy-1-piperidinyl) derivatives of 3'-deaminodaunorubicin. J. Med. Chem. 25: 18~24, 1982
4) TAKAHASHI, Y.; M. KINOSHITA, T. MASUDA, K. TATSUTA, T. TAKEUCHI & H. UMEZAWA: 3'-Deamino-3'-morpholino derivatives of daunomycin, adriamycin and carminomycin. J. Antibiotics 35: 117~118, 1982
5) ACTON, E. M.; G. L. TONG, C. W. MOSHER & R. L. WOLGEMUTH: Intensely potent morpholinyl anthracyclines. J. Med. Chem. 27: 638~645, 1984
6) UMEZAWA, H.; S. NAKAJIMA, H. KAWAI, N. KOMESHIMA, H. YOSHIMOTO, T URATA, A. ODAGAWA, N. OTSUKI, K. TATSUTA, N. ŌTAKE & T. TAKEUCHI: New morpholino anthracyclines, MX, MX2, and MY5. J. Antibiotics 40: 1058~1061, 1987
7) KABALKA, G. W. & J. D. BAKER, Jr.: A new mild conversion of ketones to the corresponding methylene derivatives. J. Org. Chem. 40: 1834~1835, 1975
8) PENCO, S.; G. CASSINELLI, A. BIGEVANI, P. ZINI, G. RIVOLA & F. ARCAMONE: Daunorubicin aldo-keto reductase: Enantioface differential reduction of the side-chain carbonyl group of antitumor anthracyclines. Correction of the stereochemistry at C(13) of 4-demethoxy-13-dihydrodaunorubicin. Gazz. Chim. Ital. 115: 195~197, 1985
9) OGAWA, Y.; H. MORI, N. YAMADA & K. KON: The absolute structures of rubeomycins A and A_1 (carminomycins II and III) and rubeomycins B and B_1 (4-hydroxybaumycinols A_1 and A_2). J. Antibiotics 37: 44~56, 1984
10) BROADHURST, M. J.; C. H. HASSALL & G. J. THOMAS: The total synthesis of 13(*R*)- and 13(*S*)-dihydro-4-demethoxydaunorubicin. Revision of stereochemistry of the microbial and mammalian reduction product of 4-demethoxydaunorubicin. Tetrahedron Lett. 25: 6059~6062, 1984
11) MARAL, R.; G. PONSINET & G. JOLLES: Experimental antitumor activity of a new-semisynthetic antibiotic, rubidazone (22050 R. P). C. R. Acad. Sci., Ser. D 275: 301~304, 1972