International Journal of Pharmaceutics, 82 (1992) 29–37

IJP 02674

Research Papers

Stabilization of daunorubicin and 4-demethoxydaunorubicin on complexation with octakis(2,6-di-*O*-methyl)-γ-cyclodextrin in acidic aqueous solution

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(Received 24 June 1991)
(Accepted 27 September 1991)

Key words: Daunorubicin; 4-Demethoxydaunorubicin; Degradation; Inclusion complex; Octakis(2,6-di-*O*-methyl)-γ-cyclodextrin; Stabilization

Summary

The effects of octakis(2,6-di-*O*-methyl)-γ-cyclodextrin (DM-γ-CyD) on the chemical stability of the anthracycline antibiotics daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr) in acidic aqueous media have been investigated. As determined from the analysis of inclusion complexation of anthracyclines with CyDs, DM-γ-CyD displayed the highest stabilizing ability, followed in descending order by 3-hydroxypropyl-γ-CyD > γ-CyD > hydroxyethyl-γ-CyD, whilst octakis(2,3,6-tri-*O*-methyl)-γ-CyD showed no effect. Nevertheless, 4-demethoxyDr formed a much more stable inclusion complex with DM-γ-CyD; surprisingly, the effect of stabilization by DM-γ-CyD is significantly smaller compared with Dr. ^{1}H-NMR data indicate that the aglycone region of the anthracycline molecule is included within the DM-γ-CyD cavity.

Introduction

Daunorubicin (Dr, Fig. 1) is one of the most widely employed anthracycline antibiotics in clinical use (Arcamone, 1984). However, like other glycosides, Dr is susceptible to hydrolytic degradation in aqueous solutions (Beijnen et al., 1985, 1986). Some of the present authors have succeeded in improving the chemical stability of anthracyclines and mitomycin C by using cyclodextrins (CyDs), in particular γ-CyD, which has the largest cavity size (Bekers et al., 1988, 1989, 1990). In our series of studies on this area, we have recently shown that among various γ-CyD homologues, octakis(2,6-di-*O*-methyl)-γ-CyD (DM-γ-CyD) provides the greatest extent of stabilization against degradation of mitomycin C (Suenaga et al., 1990). In this work, the effectiveness of DM-

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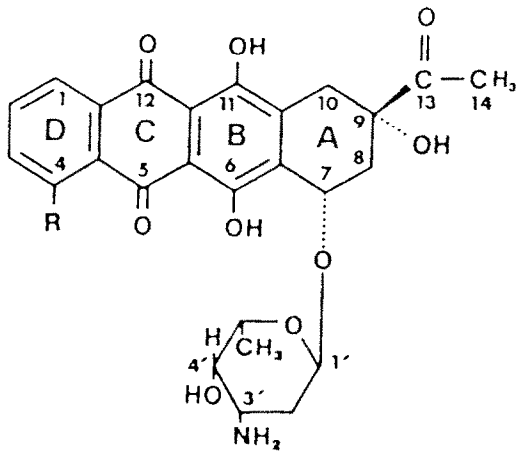


Fig. 1. Structures of daunorubicin (R=OCH_3) and 4-demethoxydaunorubicin (R=H).

γ-CyD in preventing the hydrolysis of Dr to the aglycone was investigated, with the aim of improving the chemical stability of Dr. Moreover, the degradation of 4-demethoxydaunorubicin (4-demethoxyDr, Fig. 1) was studied in order to gain insight into the mechanism of stabilization of Dr. In addition, the mode of interaction of Dr and 4-demethoxyDr with DM-γ-CyD was monitored in relation to the degree of stabilization by DM-γ-CyD employing both ^{1}H-NMR and fluorescence spectroscopy.

Experimental

Materials

Dr was kindly provided by Meiji Seika Co., Ltd (Tokyo). 4-DemethoxyDr was a generous gift from Dr S. Penco (Farmitalia, Milan, Italy). The anthracyclines were supplied as hydrochloride salts. γ-CyD and 2,3,6-tri-*O*-methyl-γ-CyD (TM-γ-CyD) were donated by Nihon Shokuhin Kako Co., Ltd (Tokyo, Japan), being used after recrystallization from water. Various γ-CyD derivatives, except DM-γ-CyD, such as 3-hydroxypropyl-γ-CyD (3-HP-γ-CyD), 2,3-dihydroxypropyl-γ-CyD (2,3-DHP-γ-CyD) and hydroxyethyl-γ-CyD (HE-γ-CyD) were supplied by Wako Chemical Co., Ltd (Osaka, Japan). DM-γ-CyD was prepared according to the method of Tanimoto et al. (1990). All other materials and solvents were of special reagent grade. Deionized distilled water was used throughout the experiments.

Buffer solutions

For the kinetic studies, perchloric acid solutions (pH range 1.0–3.0) were used. Ionic strength ($\mu = 0.3$) was maintained constant for each degradation solution, adjustment being made via addition of NaCl. In the analysis of anthracycline degradation, where the influences of CyD and dioxane were investigated, various amounts of CyD and/or dioxane were added to the present buffer solution and the pH then adjusted to the desired value. These solutions were freshly prepared before use.

Kinetic measurements

The kinetic experiments were performed at a constant temperature of 50°C in a thermostat water bath, with protection of the reaction vials from light. Degradation was initiated by the addition of 30 μl of a stock solution of anthracycline in water (3.5×10^{-3} M) to 3 ml of a preheated buffer solution, yielding an initial concentration of 3.5×10^{-5} M. In order to prevent adsorption of anthracyclines and degradation products to glass walls, the solutions were kept in stoppered polypropylene test tubes.

For the construction of log k_{obs}-pH profiles, the DM-γ-CyD concentration was kept constant at 2.0×10^{-3} M, whereas for evaluation of K_s and k_{cat}, the level of DM-γ-CyD was varied from 0 to 5.0×10^{-3} M. At appropriate time intervals, samples were withdrawn and assayed for undegraded anthracycline using HPLC. The samples were stored at −20°C until analysis. Anthracycline degradation was not observed during storage under such conditions for a period of at least 3 months.

Apparatus and analytical procedures

HPLC was carried out using a 655A-11 pump and F-1000 fluorescence spectrophotometer (both from Hitachi, Tokyo, Japan) with λ(excitation) = 495 nm and λ(emission) = 550 nm. Determination of undegraded anthracycline was based on peak area measurements using a Hitachi D-2000

chromato-integrator (Tokyo, Japan). Separation was conducted on a LiChrosorb RP-8 column (7 μm in 4 mm $\times$ 25 cm, Cica-Merck, Tokyo, Japan), with 0.02 M sodium chloride (pH 2.0)/acetonitrile (40:60, v/v) as mobile phase at a flow rate of 0.8 and 0.9 ml/min for Dr and 4-demethoxyDr, respectively. Standard curves for anthracycline showed good linearity ($r > 0.999$) over the concentration range of interest (3.5×10^{-6}–3.5×10^{-5} M). All chromatographic analyses were carried out at ambient temperature. The parent compounds and degradation products were satisfactorily separated using the above-described HPLC.

Fluorescence spectra were recorded on a Jasco FP-770 spectrofluorometer (Tokyo, Japan).

400 MHz ^{1}H-NMR spectra were registered in D_2O (pH 6.5) or 0.1 M KD_2PO_4 (pH 4.0) at 30°C using a JNM-GX 400 spectrometer (Jeol, Tokyo, Japan). An average of 100 accumulations with 32768 data points were made at a sweep width of 6000 Hz. The ^{1}H chemical shifts were assigned values based on an external standard of sodium 2,2-dimethyl-2-silapentane-5-sulfonate with an accuracy of ± 0.0015 ppm.

Results and Discussion

Kinetic studies

The degradation of Dr or 4-demethoxyDr as well as that of other anthracyclines follows a pseudo first-order kinetic pathway as mentioned previously (Bekers et al., 1988, 1990). The presence of CyD does not affect this kinetic behavior, altering neither the number nor the kind of degradation products. Therefore, it is concluded that the degradation mechanism for both anthracyclines is the same irrespective of whether CyD is present.

The standard deviation (SD) in the observed rate constant (k_{obs}) was determined for Dr and 4-demethoxyDr at pH 1.5 ($\mu = 0.3$) in the absence of DM-γ-CyD. The values of $k_{obs} \pm$ SD for Dr and 4-demethoxyDr were $3.5 \pm 0.2 \times 10^{-5}$ s^{-1} ($n = 6$) and $3.5 \pm 0.1 \times 10^{-5}$ s^{-1} ($n = 6$), respectively. On the other hand, in the presence of DM-γ-CyD (3×10^{-3} M), the respective values for Dr and 4-demethoxyDr were $8.7 \pm 0.1 \times 10^{-6}$ s^{-1} ($n = 4$) and $1.1 \pm 0.002 \times 10^{-5}$ s^{-1} ($n = 4$). All other rate constants are the average of duplicate measurements.

TABLE 1

Influence of various γ-cyclodextrin (γ-CyD) derivatives on daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr) degradation at pH 1.5 ($\mu = 0.3$) and 50°C

CyD	k_{obs} (s^{-1})	
	Dr	4-DemethoxyDr
Blank	3.6×10^{-5}	3.6×10^{-5}
γ-	2.1×10^{-5}	2.0×10^{-5}
2,6-DM-γ-	8.5×10^{-6}	9.8×10^{-6}
2,3,6-TM-γ-	2.8×10^{-5}	3.4×10^{-5}
3-HP-γ-	1.7×10^{-5}	1.7×10^{-5}
2,3-DHP-γ-	2.5×10^{-5}	2.1×10^{-5}
HE-γ	2.3×10^{-5}	2.1×10^{-5}

[Dr] or [4-demethoxyDr] = 3.5×10^{-5} M, [CyD] = 3.5×10^{-3} M. 2,6-DM-, 2,6-dimethyl-; 2,3,6-TM-, 2,3,6-trimethyl-; 3-HP-, 3-hydroxypropyl-; 2,3-DHP-, 2,3-dihydroxypropyl-; HE-, hydroxyethyl-.

The influence of various γ-CyD derivatives on the degradation of Dr and 4-demethoxyDr was studied at pH 1.5 ($\mu = 0.3$) and 50°C. Table 1 summarizes the k_{obs} values. It is clear that TM-γ-CyD exerts virtually no stabilizing effect on Dr and 4-demethoxyDr degradation, while HP-, γ-, HE- and DHP-γ-CyD result in a significant positive influence on the stability of both anthracyclines. The strongest stabilizing effect was found to be exerted by DM-γ-CyD. All subsequent experiments were therefore conducted using DM-γ-CyD as the host molecule.

The complexation of anthracycline with DM-γ-CyD and the degradation reaction of the free drug and the inclusion complex are expressed as (Colter et al., 1964):

$$\frac{[\text{CyD}]}{k_0 - k_{obs}} = \frac{[\text{CyD}]}{k_0 - k_{cat}} + \frac{1}{K_s(k_0 - k_{cat})}$$

where K_s denotes the formation constant of the inclusion complex, with k_0 and k_{cat} being the respective rate constants for degradation of the free drug and the guest molecule in the complex. By plotting $[\text{CyD}]/(k_0 - k_{obs})$ vs [CyD], Line-

TABLE 2

Influence of octakis(2,6-di-O-methyl)-γ-cyclodextrin on rate constants (k_{cat}) and stability constants (K_s) for the degradation of daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr) at pH 1.5 ($\mu = 0.3$) and 50°C

Anthracycline	k_0 (s^{-1})	k_{cat} (s^{-1})	K_s (M^{-1})	k_0/k_{cat}
Dr	3.6×10^{-5}	4.4×10^{-6}	1960	8.2
4-DemethoxyDr	3.6×10^{-5}	9.0×10^{-6}	3690	4.0

weaver-Burk plots are obtained. If k_0 is known, then k_{cat} can be calculated from the slope and K_s from the intercept of the straight line. Inclusion complex formation may have an accelerative or decelerative effect on the reactivity of the guest molecule, depending on the nature of the reaction and the orientation of the guest molecule within the CyD cavity (Vanetten et al., 1966; Uekama et al., 1979; Tabushi, 1982). Table 2 lists the kinetic parameters characterizing the Dr-DM-γ-CyD and 4-demethoxyDr-DM-γ-CyD complexes. In acidic medium (pH < 4.0), Dr and 4-demethoxyDr included in DM-γ-CyD appear to undergo degradation at a slower rate compared to the free drug. Interestingly, 4-demethoxyDr, with a more lipophilic aglycone moiety, forms a much more stable inclusion complex with DM-γ-CyD (see K_s in Table 2), however, the k_0/k_{cat} ratio in this case is much lower. The discrepancy between the stabilization effect and the magnitudes of the stability constants can be explained on the basis of the different orientation and disposition of the two guest molecules within the DM-γ-CyD cavity.

Log k_{obs}-pH profiles for the degradation of Dr-DM-γ-CyD and 4-demethoxyDr-DM-γ-CyD were compared with a similar profile for the free drug in the pH range 0.5–3.0 (Fig. 2). For both anthracyclines, a plot of log k_{obs} vs pH yields a straight line with a slope of −1. This implies specific proton catalysis for hydrolytic degradation. As can be seen from Fig. 2, the inhibitory effects of DM-γ-CyD on the degradation of Dr and 4-demethoxyDr are independent of pH over this pH interval. This is consistent with the fact that the degree of protonation of the sugar amino

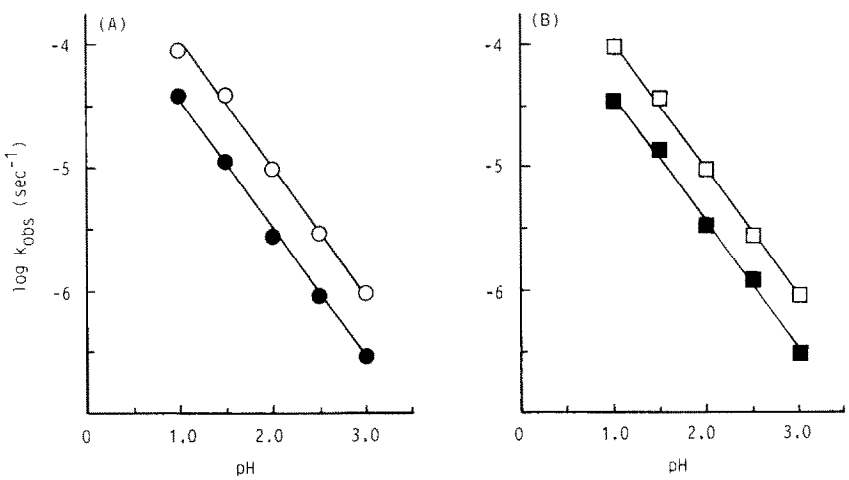


Fig. 2. Variation in the rate of degradation of (A) daunorubicin (Dr) and (B) 4-demethoxydaunorubicin (4-demethoxyDr) as a function of pH in the absence and presence of octakis(2,6-di-*O*-methyl)-γ-cyclodextrin (DM-γ-CyD) (2.0×10^{-3} M) at 50°C; (○) Dr alone (3.5×10^{-5} M), (●) Dr with DM-γ-CyD, (□) 4-demethoxyDr alone (3.5×10^{-5} M), (■) 4-demethoxyDr with DM-γ-CyD.

TABLE 3

Second-order rate constants for the proton-catalyzed degradation of daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr) at 50°C in the absence (k_H) and presence of octakis(2,6-di-O-methyl)-γ-cyclodextrin (DM-γ-CyD) (k_H^{CyD})

Anthracycline	k_H ($mol^{-1}\ s^{-1}$)	k_H^{CyD} ($mol^{-1}\ s^{-1}$)
Dr	1.2×10^{-3}	3.7×10^{-4}
4-DemethoxyDr	1.0×10^{-3}	3.5×10^{-4}

[Dr or 4-demethoxyDr] = 3.5×10^{-5} M, [DM-γ-CyD] = 2.0×10^{-3} M.

function remains unchanged and, consequently, no variation in the mode of inclusion can be expected at all pH values examined in this study.

Plotting k_{obs} vs $[H^+]$ yielded straight lines ($r > 0.999$) for both anthracyclines irrespective of the presence of CyD. Their slopes were employed to determine the k_H values, as listed in Table 3. The presence of DM-γ-CyD results in lower k_H values, indicating that the anthracyclines are protected from attack by the hydrogens.

Fig. 3 illustrates the influence of the concentration of dioxane on k_{obs} for the case of Dr and 4-demethoxyDr degradation in a water-dioxane mixture at pH 1.5 ($\mu = 0.3$) and 50°C. It is evident that with increasing concentration of dioxane, which gives rise to greater hydrophobicity of the medium, the values of k_{obs} diminish exponentially. This effect is similar to that of DM-γ-CyD on k_{obs} for the degradation of Dr and 4-demethoxyDr. Probably, the hydrophobic environment of the CyD cavity is responsible for a conformational rearrangement in the anthracycline molecule which retards the rate of reaction.

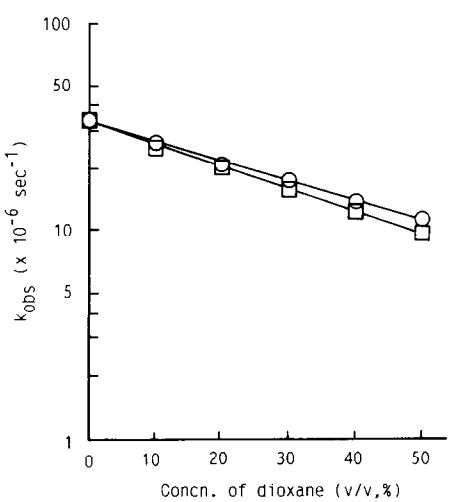


Fig. 3. Effect of dioxane concentration on the rate of degradation of daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr) in perchloric acid solution (pH 1.5, $\mu = 0.3$) at 50°C; (○) Dr, (□) 4-demethoxyDr.

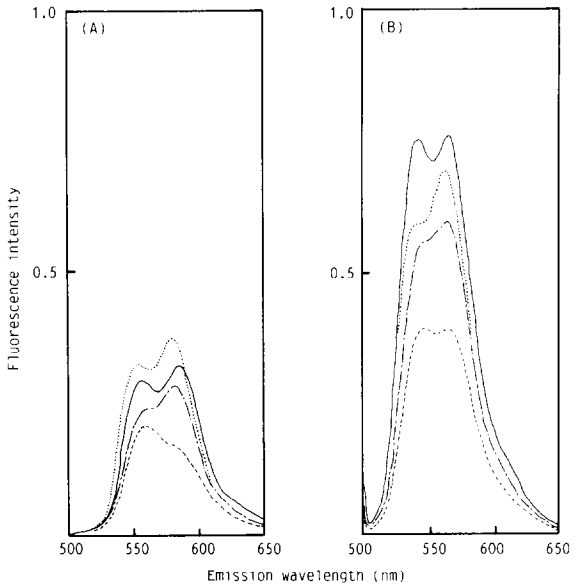


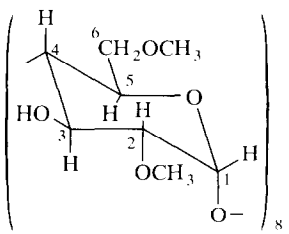
Fig. 4. Fluorescence spectra of (A) daunorubicin (Dr) and (B) 4-demethoxydaunorubicin (4-demethoxyDr) in the presence of octakis(2,6-di-*O*-methyl)-γ-cyclodextrin (DM-γ-CyD) in 0.15 M sodium phosphate buffer (pH 7.4) and in dioxane or MeOH at 25°C; (- - - - -) Dr or 4-demethoxyDr alone (1×10^{-5} M) in pH 7.4 buffer, (———) with DM-γ-CyD (2×10^{-3} M) in pH 7.4 buffer, (—·—·—) Dr or 4-demethoxyDr alone (1×10^{-5} M) in dioxane, (· · · · ·) Dr or 4-demethoxyDr alone (1×10^{-5} M) in MeOH. Excitation wavelength was 495 nm.

Spectroscopic studies

Fig. 4 shows the fluorescence spectra of Dr and 4-demethoxyDr in the absence and presence of DM-γ-CyD in aqueous solution at pH 7.4. The spectra of anthracycline in dioxane and methanol are also compared with those in the case where DM-γ-CyD was added (Fig. 4). The fluorescence intensity of anthracycline was enhanced, displaying a blue shift upon addition of DM-γ-CyD. This tendency was particularly significant with 4-demethoxyDr. The fluorescence characteristics were

TABLE 4

Daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr)-induced ^1H chemical shifts of octakis(2,6-di-O-methyl)-γ-cyclodextrin



Proton	Δδ (ppm)	
	Dr	4-DemethoxyDr
H1	−0.1059	−0.1201
H2	0.0371	0.0401
H3	−0.0797	−0.0903
H4	−0.0608	−0.0681
H5	0.0848	0.0472
H6	0.0829	0.0706
C2-OCH_3	−0.0119	−0.0073
C6-OCH_3	−0.0522	−0.0559

Positive values denote downfield shifts.

observed to be similar in dioxane and methanol. These results suggest that 4-demethoxyDr may be located in the more hydrophobic environment of the DM-γ-CyD cavity as compared to Dr.

The ^1H-NMR spectra were used to estimate the mode of the inclusion of Dr and 4-demethoxyDr within the DM-γ-CyD cavity.

Demarco and Thakkar (1970) have shown that ^1H-NMR can provide evidence for the inclusion of aromatic substances into CyDs. Their reasoning was based on the expectation that, if inclusion takes place, the screening environment should be sensed by hydrogens on the inner surface (H3 and H5) but not by hydrogens on the outer surface (H1, H2 and H4).

The effects of addition of Dr and 4-demethoxyDr on the ^1H-NMR spectrum of DM-γ-CyD in aqueous solution were examined. Assignment of the ^1H-NMR signals of CyD was carried out on the basis of literature data (Wood et al., 1977). Table 4 lists the effects of Dr and 4-demethoxyDr on the ^1H chemical shifts of DM-γ-CyD. Here, $\Delta\delta$ is defined as $\delta_{complex} - \delta_0$, where $\delta_{complex}$ and δ_0 are the chemical shifts in the fully complexed and uncomplexed states, respectively. H3 and H5 experience large changes in shielding which are consistent with the inclusion of anthracyclines. In the presence of the anthracycline, H3 is shifted upfield while H5 is shifted downfield. The H6 protons which are located on the upper (C6) surface also show a downfield shift. Interestingly, the H5 and H6 protons which are located in the same region of the torus experience shielding changes in the same direction. The H1 and H4 protons, located on the exterior wall, both display upfield shifts. The H3 protons of CyD which are oriented toward the interior of the CyD cavity are shielded due to ring-current effects arising from the included aglycone moieties of anthracyclines. The relative magnitude and sign of $\Delta\delta$ for H3 clearly demonstrate that the aglycone regions are inserted into the CyD cavity in the same manner as observed in many other complexes (Bender and Komiyama, 1978), namely, from the secondary-hydroxyl side of CyD. The upfield shifts of H1 and H4 of CyD are indicative of the distortion of the CyD rings due to complex formation or to the interaction between anthracyclines and the cavity exterior. These observations are consistent with the notion that a complex is formed between DM-γ-CyD and anthracyclines. Wood et al. (1977) have also shown that the addition of *p*-iodoaniline to an α-CyD solution at acid pH results in an upfield shift for H3 and a downfield shift for H5. They explained the data as follows: the upfield shift of the H3 signal arises from the ring-current shielding effect in the benzene ring of the included *p*-iodoaniline and the downfield shift of H5 is due to the combined effect of the van der Waals deshielding by the iodine atom and ring-current effects.

It is also possible to explain these NMR data on the basis of two structures of the CyD complexes in aqueous solution, i.e., an association of the substrate external to CyD and an inclusion inside the ring. Anthracyclines self-associate in aqueous solution, the extent of this process being connected with the chemical structure (Menozzi et al., 1984). The stability of the dimeric species appears to be strongly influenced by substitution of the chromophore moiety. 4-DemethoxyDr

shows a distinctly lower tendency toward dimerization when compared with the parent drug. If self-aggregation could arise, the process of absorption would not follow the Lambert-Beer law (Chaires et al., 1982). Fortunately, under these experimental conditions, association including dimerization may be ignored, obeying Beer's law over a wide range of concentration. Therefore, the chemical shifts may be ascribed to inclusion.

The effect of addition of DM-γ-CyD on the ^{1}H-NMR spectrum of Dr and 4-demethoxyDr in aqueous solution was also examined. The assignments of the proton signals of anthracyclines were undertaken based on comparison with ^{1}H-NMR spectra of analogous compounds (Wilson et al., 1976; McLennan and Lenkinski, 1984). In Fig. 5, the effect of DM-γ-CyD on the ^{1}H chemical shifts of Dr in 0.1 M KD_2PO_4 (pH 4.0) at 30°C is shown. As shown in Fig. 5, inclusion of Dr within DM-γ-CyD leads to downfield shifts for C1-H, C2-H, C3-H, C4-OCH_3, $C8_{eq}$-H, $C8_{ax}$-H, $C10_{eq}$-H and $C10_{ax}$-H that are significantly larger than the others. In particular, the chemical shifts of the C1-H, C2-H, C3-H and C4-OCH_3 signals pointing toward the D-ring of the aglycone moiety in the Dr molecule are most prominent.

On the other hand, complexation with 4-demethoxyDr-DM-γ-CyD causes downfield shifts for $C8_{ax}$-H, $C8_{eq}$-H, $C10_{ax}$-H and $C10_{eq}$-H, which are similar to those of the Dr-DM-γ-CyD complex, whereas the C1-H, C2-H, C3-H and C4-H signals pointing toward the D-ring of the aglycone moiety of 4-demethoxyDr in the presence of DM-γ-CyD are considerably broadened and shifted (Fig. 6). This led to the suggestion that

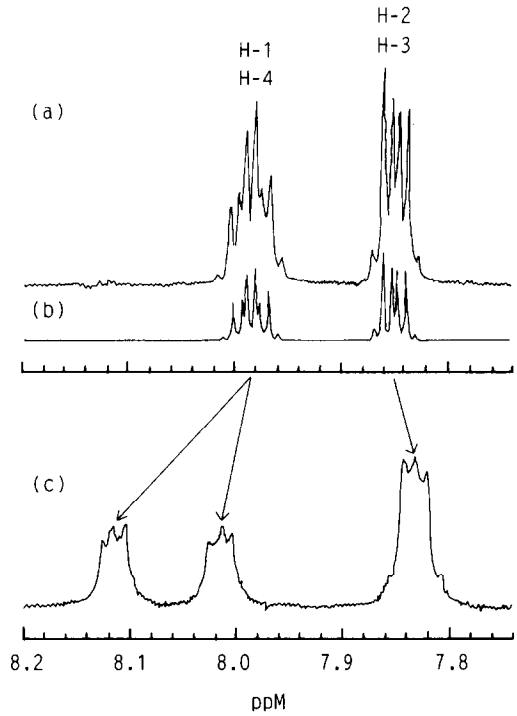


Fig. 6. ^{1}H-NMR spectra (400 MHz) of 4-demethoxydaunorubicin (4-demethoxyDr) in the presence of octakis(2,6-di-*O*-methyl)-γ-cyclodextrin (DM-γ-CyD) in 0.1 M KD_2PO_4 (pH 4.0) at 30°C; (a) 4-demethoxyDr alone (3 mM), (b) computer-simulated 4-demethoxyDr spectrum, (c) 4-demethoxyDr (3 mM)-DM-γ-CyD (3 mM).

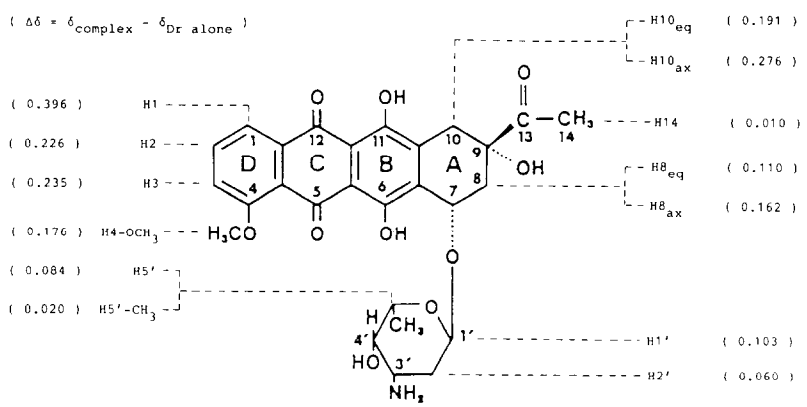
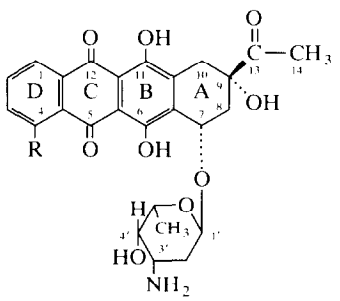


Fig. 5. Influence of octakis(2,6-di-*O*-methyl)-γ-cyclodextrin on the ^{1}H chemical shifts of daunorubicin in 0.1 M KD_2PO_4 (pH 4.0) at 30°C.

TABLE 5

Effect of octakis(2,6-di-O-methyl)-γ-cyclodextrin (DM-γ-CyD) on ^1H chemical shifts of daunorubicin (Dr) and 4-demethoxydaunorubicin (4-demethoxyDr)



Dr: R=OCH_3
4-demethoxyDr: R=H

Proton	Δδ (ppm)			
	Dr		4-DemethoxyDr	
	With DM-γ-CyD	50% MeOD	With DM-γ-CyD	50% MeOD
1	0.217	0.294	[b]	0.194
2	0.116	0.137	[b]	0.046
3	0.125	0.138	[b]	0.046
4	–	–	[b]	0.194
4-OCH_3	0.091	0.085	–	–
7	0.182	0.155	0.297	0.168
8_{eq}	0.071	0.029	0.097	0.023
8_{ax}	0.085	0.049	0.107	0.023
10_{eq}	0.103	0.068	0.138	0.061
10_{ax}	0.192	0.139	0.250	0.156
14	−0.005	−0.035	−0.009	−0.057
1′	0.053	−0.012	0.100	−0.022
2′	0.053	−0.014	0.079	−0.020
3′	[a]	−0.058	[a]	−0.055
4′	[a]	−0.063	[a]	−0.066
5′	0.055	0.005	0.064	−0.007
5′-CH_3	0.012	−0.010	0.017	−0.021

Positive values denote downfield shifts.
[a] Not determined due to overlapping CyD signal.
[b] Not determined due to broadening peak.

the D-ring of the aglycone function in anthracyclines is involved in the process of inclusion into the DM-γ-CyD cavity.

As shown in Table 5, the chemical shifts of only the aglycone regions except aminosugar moieties in the anthracycline-DM-γ-CyD complexes are similar to those of the anthracyclines dissolved in $CD_3OD:D_2O$ (1:1). This result supports the hypothesis that the aglycone region of anthracycline is involved in the lipophilic environment within the DM-γ-CyD cavity.

Moreover, it should be noted that on complex formation, C7-H of 4-demethoxyDr is more distinctly shifted downward when compared with the parent drug. This can be explained by the fact that during formation of the 4-demethoxyDr-DM-γ-CyD complex, the D-ring of the aglycone moiety in 4-demethoxyDr is more deeply included in the DM-γ-CyD cavity and, as a result, a marked change in conformation is induced. Namely, the result will be a decrease in electrostatic repulsion between the protonated amino function of the sugar moiety and the oxygen of the C6-H position located in the B-ring of the aglycone moiety and subsequently an increase in the rate of reaction of glycosidic bond cleavage.

The above findings imply that the hydrophobic nature of the guest molecule and the steric factors between the host and guest molecules are responsible for these interactions (Uekama et al., 1982).

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