Relation of Conformation to Antitumor Activity of Platinum(II) Complexes of 1,2-Cyclohexanediamine and 2-(Aminomethyl)cyclohexylamine Isomers against Leukemia P388

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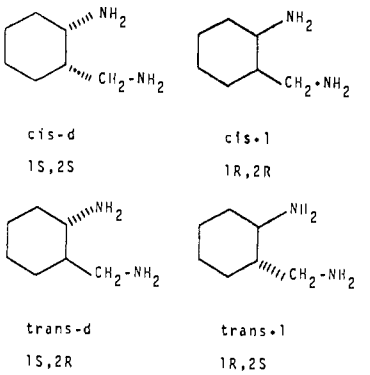
The antitumor activity of various platinum(II) complexes of 1,2-cyclohexanediamine and 2-(aminomethyl)cyclohexylamine isomers against leukemia P388 was evaluated by means of the platinum analogue study protocol recommended by the National Cancer Institute. For the former complexes, trans isomers are more efficacious than the corresponding cis isomers. For the latter complexes, cis isomers seem to be somewhat more active than trans isomers. 2-(Aminomethyl)cyclohexylamine platinum complexes exhibited higher activity than 1,2-cyclohexanediamine complexes in this tumor system. These findings encouraged us to determine the structural differences between 1,2-cyclohexanediamine and 2-(aminomethyl)cyclohexylamine complexes. Their structures of platinum complexes were elucidated from circular dichroism and ^{13}C NMR spectral analyses, and it has been concluded that the cyclohexane ring of *cis*-1,2-cyclohexanediamine is nearly perpendicular to the chelate ring, while both rings of *trans*-1,2-cyclohexanediamine and *trans*-2-(aminomethyl)cyclohexylamine complexes lie in a common plane. The structure of *cis*-2-(aminomethyl)cyclohexylamine complexes is flexible, and the cyclohexane ring is not perpendicular to the chelate ring. The coplanarity of trans isomers and the flexibility of *cis*-2-(aminomethyl)cyclohexylamine complexes allow them easy approach to the target DNA. However, the perpendicular ring of *cis*-1,2-cyclohexanediamine complexes would prevent their interactions with DNA molecules due to the steric hindrance.

Since the discovery of the antitumor activity of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) by Rosenberg et al. in 1969,[1] many platinum complexes have been synthesized and their antitumor activity has been examined. The structure–activity relationships of the platinum complexes became clear from the test of the *cis*-DDP analogues. It was soon established that the trans analogues were inactive, and the effective platinum complexes appear to be the cis analogues containing substituted amines, although the nature of the amine is critical. More detailed analyses of the structure–activity relationships were carried out elsewhere,[2,3] but few discernible trends are apparent. Recently, platinum(II) complexes of 1,2-cyclohexanediamine (dach) have been found very effective against various tumors,[2-8] but the ligand used was a mixture of geometrical and optical isomers. We prepared various platinum(II) complexes of three isomers,[9] cis, trans-*d*, trans-*l*, and tested their antitumor activity against Sarcoma 180,[10] and some differences of the activity were found among the platinum complexes of the isomers. The conformation of the dach–platinum complexes may have a relation to their antitumor activity.

Chart I



In addition to the dach–platinum complexes, we newly prepared various platinum complexes of 2-(aminomethyl)cyclohexylamine (amcha), which forms a six-membered chelate ring upon coordination. Their antitumor activity was tested against leukemia P388 and compared with activity of dach–platinum complexes. 2-(Aminomethyl)cyclohexylamine has two geometrical isomers, cis and trans forms, both of which have two optical isomers, *d* and *l* forms (Chart I).

In order to clear the relation of conformation to antitumor activity of platinum complexes, we preliminarily determined the conformation of the platinum complexes containing dach and amcha isomers by means of CD and ^{13}C NMR spectral analyses.

Results and Discussion

Circular Dichroism Spectra. 1,2-Cyclohexanediamine–Platinum(II) Complexes. It has been reported that the circular dichroism (CD) spectrum of $[Pt(NH_3)_2(trans\text{-}l\text{-dach})]Cl_2$ exhibits two positive bands at 284 and 222 nm with $\Delta\epsilon$ values of +0.40 and +0.86, respectively, indicating a λ-gauche form of the chelate ring. These two CD bands were assigned to band II ($^1A_{1g}$–^3E_g) and band IV ($^1A_{1g}$–^1E_g) from the longer wavelength side.[11]

(1) B. Rosenberg, L. VanCamp, J. E. Trosko, and V. H. Mansour, *Nature (London)*, **222**, 385 (1969).
(2) T. A. Connors, M. Jones, W. C. J. Ross, P. D. Braddock, A. R. Khokhar, and M. L. Tobe, *Chem.-Biol. Interact.*, 5, 415 (1972).
(3) M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem.*, 2, 187 (1973).
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(8) R. J. Speer, H. Ridgway, D. P. Stewart, L. M. Hall, A. Zapata, and J. M. Hill, *J. Clin. Hematol. Oncol.*, 7, 210 (1977).
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(10) Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi, and K. Kuretani, *J. Med. Chem.*, 21, 1315 (1978).
(11) H. Ito, J. Fujita, and K. Saito, *Bull. Chem. Soc. Jpn.*, **40**, 2584 (1967).

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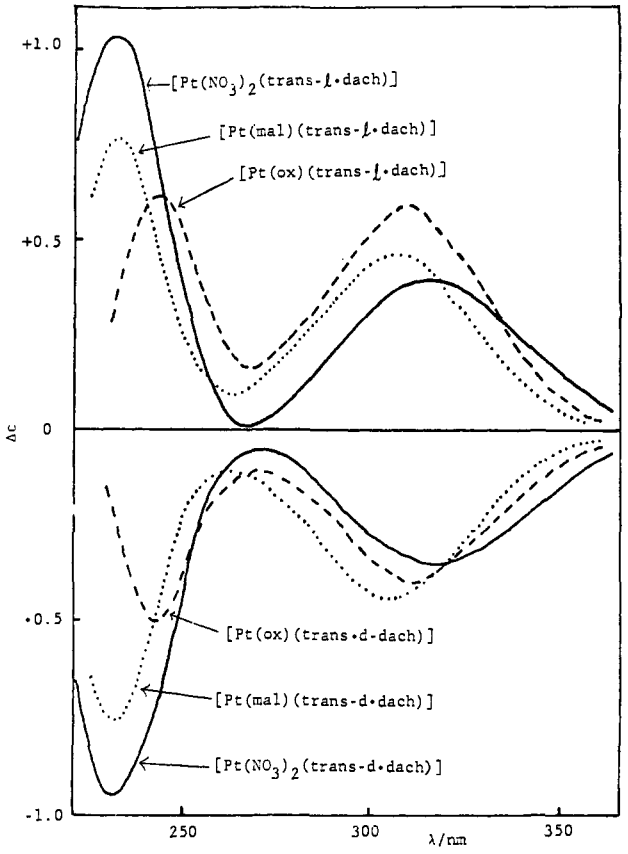

Figure 1. CD spectra of dach–platinum(II) complexes.

Table I. Numerical Data of CD for Pt(II) Complexes of 1,2-Cyclohexanediamine

complexes	band II: λ_{max}, nm ($\Delta\epsilon$)	band IV: λ_{max}, nm ($\Delta\epsilon$)
$[Pt(NO_3)_2$(*trans*-*l*-dach)]	330 (+0.40)	246 (+1.03)
$[Pt(NO_3)_2$(*trans*-*d*-dach)]	330 (−0.35)	246 (−0.95)
$[Pt(SO_4)$(*trans*-*l*-dach)]	330 (+0.35)	246 (+0.93)
$[Pt(SO_4)$(*trans*-*d*-dach)]	330 (−0.34)	246 (−0.93)
[Pt(ox)(*trans*-*l*-dach)]	325 (+0.59)	257 (+0.61)
[Pt(ox)(*trans*-*d*-dach)]	325 (−0.40)	257 (−0.50)
[Pt(mal)(*trans*-*l*-dach)]	320 (+0.46)	245 (+0.74)
[Pt(mal)(*trans*-*d*-dach)]	320 (−0.44)	245 (−0.71)

As shown in Figure 1, $[Pt(NO_3)_2$(*trans*-*l*-dach)] showed two positive CD bands at 330 and 246 nm with $\Delta\epsilon$ values of +0.40 and +1.03, respectively. $[Pt(SO_4)$(*trans*-*l*-dach)] also showed two CD bands at the same wavelengths with $\Delta\epsilon$ values of +0.35 and +0.93. These CD spectra are analogous to the CD spectrum of $[Pt(NH_3)_2$(*trans*-*l*-dach)]Cl_2. The longer wavelength shifts of the CD bands in the dinitrato– and sulfato–platinum complexes can be explained by taking account of the lower position of O,O-coordination in the spectrochemical series than that of N,N-coordination. Therefore, the two CD bands at 330 and 246 nm can be assigned to band II ($^1A_{1g}$–^3E_g) and band IV ($^1A_{1g}$–^1E_g). As shown in Table I, similar CD spectra were also observed for oxalato– and malonato–platinum complexes, whose the absolute CD strength ($|\Delta\epsilon|$) of band IV is lower than that of the sulfato– and dinitrato–platinum complexes, due to the strong absorption of the carboxyl groups below 300 nm. All of these CD data indicate that a λ-gauche conformation is predominant in *trans*-*l*-dach–platinum complexes.

On the contrary, the mirror-image CD spectra of *trans*-*d*-dach–platinum complexes imply a δ-gauche prevailing conformation.

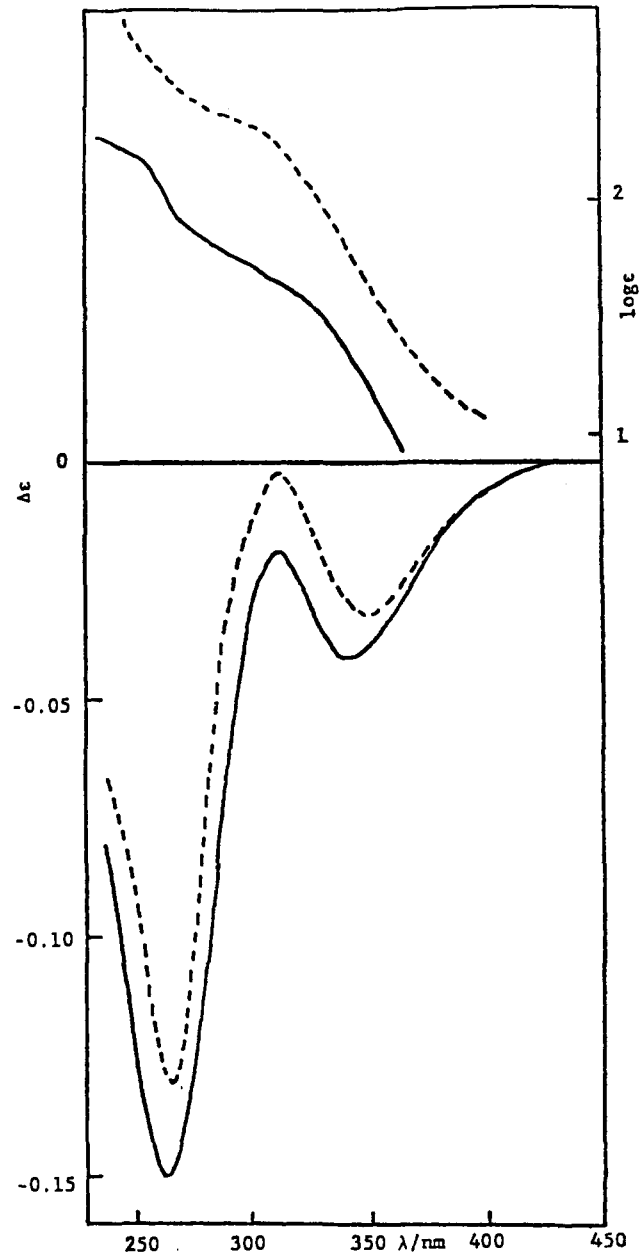

Figure 2. Absorption and CD spectra of $[Pt(SO_4)$(*cis*-*d*-amcha)] (—) and $[Pt(NO_3)_2$(*cis*-*d*-amcha)] (···) in water.

2-(Aminomethyl)cyclohexylamine–Platinum(II) Complexes. 2-(Aminomethyl)cyclohexylamine (amcha) has two geometrical isomers, cis and trans, each of which can be resolved into optical isomers. Therefore, there are four isomers: trans-*d*, trans-*l*, cis-*d*, and cis-*l*. These isomers coordinate to platinum ions forming a six-membered chelate ring, whose conformation will be discussed from their CD spectra.

On the basis of X-ray diffraction analysis, it has been reported that *l*-2,4-pentanediamine (ptn) forms a six-membered chelate ring with λ-skew conformation in $[Co(l\text{-ptn})_3]Cl_3$.[12] However, it was also reported that puckering takes place between λ-skew and chair conformations.[13] Concerning platinum complexes of ptn and 1,3-butanediamine (bn), Appleton and Hall[14] suggested that

(12) A. Kobayashi, F. Marumo, and Y. Saito, *Acta Crystallogr., Sect. B*, **28**, 3591 (1972); *ibid.*, **29**, 2443 (1973).
(13) M. Kojima, M. Fujita, and J. Fujita, *Bull. Chem. Soc. Jpn.*, **50**, 898 (1977).
(14) T. G. Appleton and J. R. Hall, *Inorg. Chem.*, **9**, 1807 (1970).

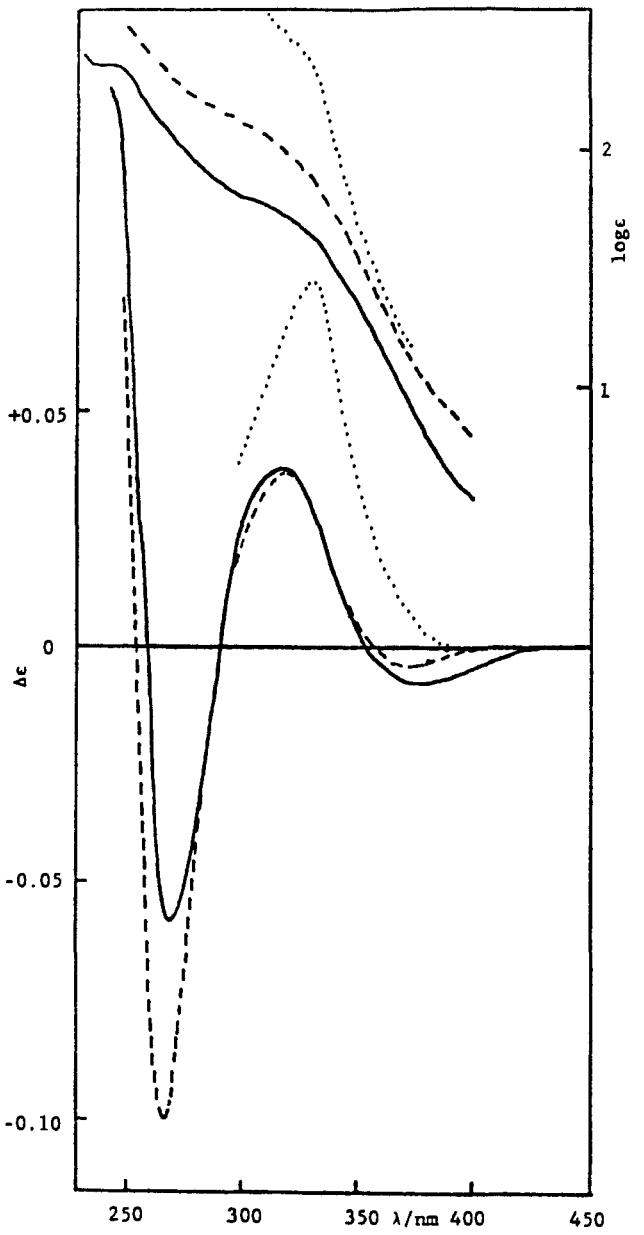

Figure 3. Absorption and CD spectra of [Pt(SO$_4$)(*trans*-*l*-amcha)] (—), [Pt(NO$_3$)$_2$(*trans*-*l*-amcha)] (···), and [Pt(ox)(*trans*-*l*-amcha)] (···) in water.

the chair conformer is preferred; this has been confirmed for [Pt(NH$_3$)$_2$(*l*-ptn)]Cl$_2$ and [Pt(*l*-ptn)$_2$]Cl$_2$ by X-ray diffraction analysis.[15]

Among the antitumor-active platinum complexes of amcha, CD spectra were obtained for platinum complexes containing nitrate, sulfate, and oxalate ions as leaving groups; the others are sparingly soluble in H$_2$O. Their absorption spectra (AB) in the ultraviolet region are illustrated in Figures 2 and 3, and there exists a distinction in their absorption maxima between *trans*-amcha– and *cis*-amcha–platinum complexes. The difference can be seen more clearly in their CD spectra, i.e., the sulfato–platinum complex of *trans*-*l*-amcha shows three CD bands at 370, 320, and 270 nm, while that of *cis*-*d*-amcha shows two CD bands at 340 and 265 nm. The CD spectrum of [Pt(SO$_4$)(*cis*-*d*-amcha)] is similar to the CD spectra of six-membered platinum complexes containing bn and ptn, i.e., [Pt(NH$_3$)$_2$(*d*-bn)]Cl$_2$ and [Pt(NH$_3$)$_2$(*d*-ptn)]Cl$_2$.[16]

(15) Y. Nakayama, S. Ooi, and H. Kuroya, *Bull. Chem. Soc. Jpn.*, 52, 914 (1979).

Table II. Absorption and CD Spectral Data in H$_2$O

complexes	AB: λ_{max}, nm (log ϵ)	CD: λ_{max}, nm ($\Delta\epsilon$)
[Pt(SO$_4$)(*trans*-*l*-amcha)]	370 sh	370 (−0.007)
	320 (1.73)	320 (+0.038)
	270 sh	270 (−0.058)
	244 (2.37)	
[Pt(NO$_3$)$_2$(*trans*-*l*-amcha)]	370 sh	370 (−0.004)
	320 sh	320 (+0.037)
	295 (2.17)	
		267 (−0.100)
[Pt(SO$_4$)(*cis*-*d*-amcha)]	320 (1.59)	340 (−0.041)
	275 sh	
	240 (2.24)	265 (−0.15)
[Pt(NO$_3$)$_2$(*cis*-*d*-amcha)]	320 sh	350 (−0.032)
	295 (2.35)	
	275 sh	
	250 sh	265 (−0.13)
[Pt(ox)(*trans*-*l*-amcha)]	315 (2.51)	327 (+0.083)
	250 (3.39)	
	210 (3.66)	

Since ammonia is higher in the spectrochemical order than sulfate and nitrate ions, CD bands of [Pt(SO$_4$)(*cis*-*d*-amcha)] shift to a longer wavelength region than those of [Pt(NH$_3$)$_2$(*d*-bn)]Cl$_2$ and [Pt(NH$_3$)$_2$(*d*-ptn)]Cl$_2$. The CD signs of *cis*-*d*-amcha–platinum complexes coincide with those of *d*-bn– and *d*-ptn–platinum complexes, suggesting that the CD strength is due to the vicinal effect of the asymmetric carbon atoms with the *S* configuration in *cis*-*d*-amcha.

The CD data of amcha–platinum complexes are summarized in Table II. The CD strength of *cis*-*d*-amcha–platinum complexes is very small compared to that of *trans*-dach–platinum complexes. For example, [Pt(SO$_4$)(*cis*-*d*-amcha)] has $\Delta\epsilon$ values of −0.041 and −0.15 at 340 and 265 nm, being assigned to band II and band IV, respectively.[11] The difference in $|\Delta\epsilon|$ values can be interpreted by taking account conformational differences between these platinum complexes. In *cis*-*d*-amcha–platinum complexes, the ligand forms six-membered chelate rings, and the possible conformers are two chair forms and a δ-skew form. If the δ-skew conformer is predominant, the CD strength of *cis*-*d*-amcha–platinum complexes due to the conformational effect would expected to be similar to the CD strength observed for the *trans*-dach–platinum complexes. On the contrary, if two chair conformers are predominant, very small $|\Delta\epsilon|$ values will be expected, since the chair conformation is optically inactive and only the vicinal effect will contribute to the CD strength. In fact, compared with the $|\Delta\epsilon|$ values of 0.35 and 0.93 for [Pt(SO$_4$)(*trans*-*l*-dach)], the $|\Delta\epsilon|$ values observed for [Pt(SO$_4$)(*cis*-*d*-amcha)] were 0.041 and 0.15 for band II and band IV, respectively, indicating that the chair forms are preferred.

On the other hand, [Pt(SO$_4$)(*trans*-*l*-amcha)] exhibited a somewhat different CD spectrum, i.e., a negative band at 370 nm, a positive band at 320 nm, and a negative band at 270 nm. Concerning the signs of the CD bands for the five-membered platinum complexes of the diamines, the positive (or negative) CD bands on bands II and IV correspond to the vicinal effect due to the asymmetric carbon atoms with a *R* (or *S*) configuration. From this viewpoint, it seems that the negative and positive bands of [Pt(SO$_4$)(*trans*-*l*-amcha)] are related to the *S* and *R* configurations of the respective C(2) and C(1) asymmetric carbon atoms, and the bands were assigned to bands I, II, and III from the longer wavelength, respectively.[11] A similar CD

(16) M. Noji, K. Okamoto, and Y. Kidani, *Chem. Lett.* 741 (1979).

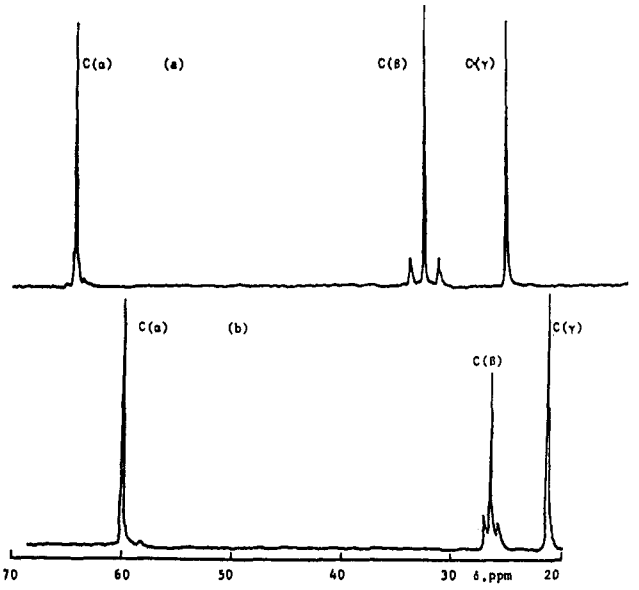

Figure 4. ^{13}C NMR spectra of $[Pt(NO_3)_2(L)]$ in D_2O: L = (a) *trans*-*l*-dach; (b) *cis*-dach.

spectrum was observed for $[Pt(NO_3)_2$(*trans*-*l*-amcha)]. Due to the strong absorption of an oxalate ion below 300 nm, [Pt(ox)(*trans*-*l*-amcha)] exhibited only one CD band at 327 nm, corresponding to band II.

On examining the molecular models of *trans*-*l*-amcha–platinum complexes, a possible conformation is either a λ-skew or a chair form, the latter of which is fixed as a *trans*-decalin ring. As shown in Table II, the observed $|\Delta\epsilon|$ values for *trans*-amcha–platinum complexes are similar than those of *trans*-dach–platinum complexes. Therefore, the low $|\Delta\epsilon|$ values can not be regarded as representing the CD band due to the conformational effect of the skew form. This observation strongly suggests that the chelate ring of *trans*-amcha–platinum complexes is in a fixed chair form.

^{13}C NMR Spectra. It had pointed out by Bagger[17] and Erickson et al.[18] that satellite peaks between ^{195}Pt and ^{13}C nuclei offer valuable information in determining conformations of platinum(II) complexes. A series of five-membered platinum complexes of diamines has been studied, and it has been reported that $^3J_{Pt-C}$ values follow a Karplus-type equation. Erickson et al. have reported a $^3J_{Pt-C}$ value of 52 Hz for an equatorial carbon atom and predicted very small values of $^3J_{Pt-C}$ for its pure axial orientation, perhaps approaching zero. Consequently, intermediate values would be expected when the puckering between axial and equatorial orientations takes place. These conclusions have been supported also by the data reported by Yano et al.[19]

As described in part CD spectra, δ- and λ-gauche forms were suggested for the five-membered chelate rings in the platinum(II) complexes of *trans*-*d*- and *trans*-*l*-dach, respectively, and the conclusion is also supported by their ^{13}C NMR spectral analyses. ^{13}C NMR spectra of $[Pt(NO_3)_2$(*trans*-*l*-dach)] and $[Pt(NO_3)_2$(*cis*-dach)] in D_2O are illustrated in Figure 4, and well-defined satellite peaks around the signal of β-carbon atoms were observed for both complexes. For the former, 65.9 Hz was obtained as $^3J_{Pt-C(\beta)}$ and 30.5 Hz for the latter. The difference between

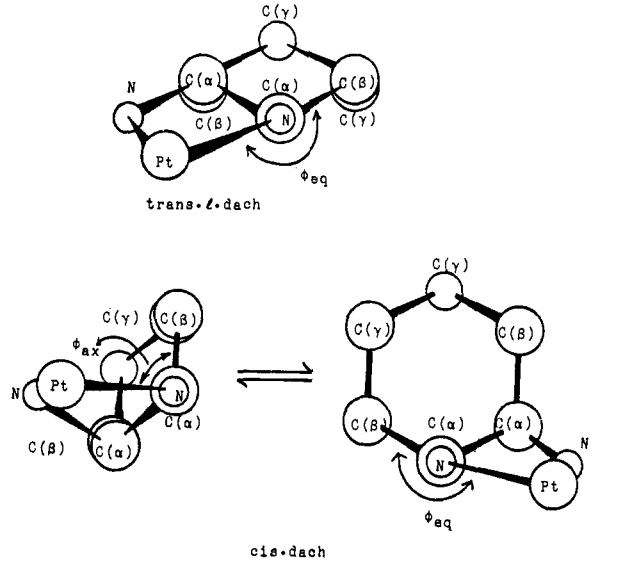

Figure 5. The chelate ring viewed down an N–C bond.

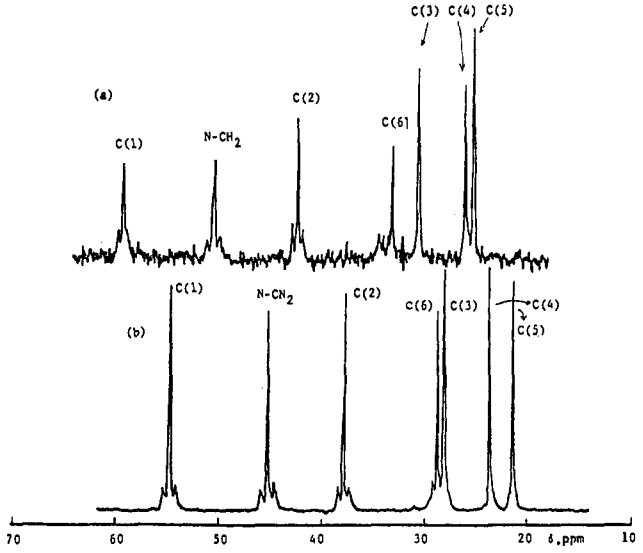

Figure 6. ^{13}C NMR spectra of $[Pt(NO_3)_2(L)]$ in D_2O: L = (a) *trans*-*l*-amcha; (b) *cis*-*d*-amcha.

these $^3J_{Pt-C(\beta)}$ values can be explained by the dihedral angles in the Pt–N–C(α)–C(β) spin system.

By examining the molecular model of $[Pt(NO_3)_2$-(*trans*-*l*-dach)], the chelate ring takes a fixed λ-gauche form, and the dihedral angle Pt–N–C(α)–C(β) is about 180° (Figure 5). By using the equation $^3J_{Pt-N-C-C} = a\cos^2\phi$ (a = a constant; ϕ = dihedral angle Pt–N–C–C), the a value is 65.9 Hz. This value is different from the values reported by Bagger[17] and Erickson et al.[18] This distinction would be explained by the nature of the ligands being trans to the diamine. Appleton et al.[20] have reported that the coupling constants $^3J_{Pt-N-C-H}$ decrease in the order H_2O > py~NH_3~en~$(CH_3)_2S$ > $P(C_6H_5)_3$, i.e., in the order of increasing trans influence. Consequently, it is possible to appropriate this consideration for the $^3J_{Pt-C(\beta)}$ value of $[Pt(NO_3)_2$(*trans*-*l*-dach)]. In $[Pt(NO_3)_2$(*cis*-dach)], averaged coupling due to rapid interconversion would give $^3J \approx 0.5a$, i.e., $^3J_{Pt-C(\beta)} = 33$ Hz. The observed $^3J_{Pt-C(\beta)}$ value was 30.5 Hz, which agrees with the expected value (Figure 5).

^{13}C NMR spectra of amcha–platinum complexes are illustrated in Figure 6, and their ^{13}C NMR data are sum-

(17) S. Bagger, *Acta Chem. Scand., Ser. A*, 28, 467 (1974).
(18) L. E. Erickson, J. E. Sarenski, and C. N. Reilley, *Inorg. Chem.*, 14, 3007 (1975).
(19) S. Yano, T. Tukada, M. Saburi, and S. Yoshikawa, *Inorg. Chem.*, 17, 2520 (1978).
(20) T. G. Appleton and J. R. Hall, *Inorg. Chem.*, 10, 1717 (1971).

Table III. Carbon-13 NMR Chemical Shifts[a] and Coupling Constants[b] in D_2O

complexes	C(α)	C(β)	C(γ)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	N·CH_2
[Pt(NO_3)$_2$(*trans·l*·dach)]	64.10 (36.6)	32.38 (65.9)	24.95							
[Pt(NO_3)$_2$(*cis*·dach)]	60.01	26.51 (30.5)	21.34							
[Pt(NO_3)$_2$(*trans·l*·amcha)]				59.28	42.25 (25.6)	30.72	26.12	25.14	33.94	50.44 (29.9)
				59.23 (20.7)		30.46	25.95		33.53 33.23 (55.5)	
[Pt(NO_3)$_2$(*cis·d*·amcha)]				55.11	38.11	28.26	23.93	21.64	28.97 (22.0)	45.47 (31.7)
				54.99 (29.3) 54.82	38.01 (27.5)					

[a] Carbon-13 shifts in parts per million from external Me_4Si. [b] The values in parentheses are the coupling constants (^{195}Pt-^{13}C).

Table IV. Changes in Activity on Leaving Groups in dach-Pt(II) Complexes against Leukemia P388

dach	leaving group	toxic dose, mg/kg	optimal dose		MED		TI
			mg/kg	% T/C	mg/kg	% T/C	
cis·DDP		12.5	3.12	230	0.78	134	4
cis	Cl_2	12.5	6.25	206	1.56	127	4
trans·d	Cl_2	12.5	6.25	228	0.78	130	8
trans·l	Cl_2	12.5	3.12	228	0.78	123	4
cis	Br_2	50	25	190	1.56	126	16
trans·d	Br_2	50	12.5	217	1.56	130	8
trans·l	Br_2	100	25	238	≤1.56	144	≥16
cis	I_2	≥200	25	141	6.25	129	4
trans·d	I_2	≥200	≥100	149	12.5	121	≥8
trans·l	I_2	≥200	≥100	182	6.25	125	≥16
cis	oxalato	50	25	200	1.56	120	16
trans·d	oxalato	40	20	198	1.56	121	13
trans·l	oxalato	25	12.5	231	1.56	125	8
cis	SO_4	25	6.25	202	0.78	122	8
trans·d	SO_4	25	6.25	200	0.39	124	16
trans·l	SO_4	12.5	6.25	212	1.56	140	4
cis	$(NO_3)_2$	≥50	12.5	180	≤1.56	144	≥8
trans·d	$(NO_3)_2$	50	12.5	198	≤1.56	151	≥8
trans·l	$(NO_3)_2$	25	6.25	187	≤1.56	147	>4
cis	D-glucuronato	50	25	208	1.56	139	16
trans·d	D-glucuronato	50	25	188	1.56	120	16
trans·l	D-glucuronato	50	25	203	0.39	124	32

marized in Table III. In the case of amcha–platinum complexes, a clear distinction can be found in $^3J_{Pt-C(6)}$ values between [Pt(NO_3)$_2$(*trans·l*-amcha)] and [Pt·(NO_3)$_2$(*cis·d*·amcha)]; in the latter complex, the carbon·6 atom has nearly equal probability to be either in an axial or an equatorial position with the averaged coupling constant of 22.0 Hz, being about half of the value (55.5 Hz) obtained for the former, the 6-carbon atom of which has preferential equatorial orientation. But it is well-known that the angle at the metal for the 1,3-diamine chelate ring is larger than that for 1,2·diamine.[21] Therefore, it is expected that these chelates differ from each other in the dihedral angle for Pt–N–C–C fragments in platinum complexes. Consequently, it is not appropriate to use the $^3J_{Pt-N-C-C}$ value reported for 1,2-diamines for conformational analysis of the 1,3-diamine chelate ring.

From the CD spectral data, the chelate rings of amcha–platinum complexes are chair forms. In [Pt(NO_3)$_2$·(*trans·l*·amcha)] the dihedral angle is about 180° (Figure 7); therefore, the a value in the equation $^3J_{Pt-N-C-C} = a\cos^2\phi$ seems to be 55.5 Hz. [Pt(NO_3)$_2$(*cis·d*·amcha)] may take two chair forms, an angle of 180° may be expected for one chair form and about 60° for the other chair form. When two chair forms interconvert each other rapidly in a NMR time scale, an averaged coupling constant due to a rapid ring interconversion would give $^3J_{Pt-C(6)} = 0.5\ (a\cos^2 180° + a\cos^2 60°)$ (a = 55.5 Hz); that is, $^3J_{Pt-C(6)}$ = 34.7 Hz. The experimental value of $^3J_{Pt-C(6)}$ for [Pt(NO_3)$_2$(*cis·d*·amcha)] was 22.0 Hz, which is smaller than the calculated value. Thus, the abundance ratio of the equatorial and axial C–C(6) chair forms may be 0.2:0.8 in an aqueous solution (Figure 7).

Antitumor Activity. We previously reported[22,23] that dach–platinum complexes were highly active against leukemia L1210 and P388 in CDF_1 mice based on the life span (ILS, %). In this work the dose range has been expanded in order to select the most potent analogue and to clear the relation of conformation to antitumor activity, and, judging from the T/C (%) and therapeutic index (TI), was carried out with P388 mouse leukemia according to the protocol for the study of platinum analogues recommended by the National Cancer Institute. The antitumor activity of the platinum(II) complexes of dach isomers on P388 are

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Table V. Changes in Activity on Leaving Groups in amcha-Pt(II) Complexes against Leukemia P388

amcha	leaving group	toxic dose, mg/kg	optimal dose		MED		TI
			mg/kg	% T/C	mg/kg	% T/C	
cis·DDP		12.5	3.12	230	0.78	134	4
cis-d	Cl_2	≥25	6.25	242	≤0.39	135	≥16
cis·l	Cl_2	≥25	6.25	240	0.78	138	8
trans·d	Cl_2	25	12.5	226	0.39	123	32
trans-l	Cl_2	25	12.5	197	0.78	140	16
cis-d	Br_2	100	25	196	0.78	125	32
cis·l	Br_2	100	25	181	≤0.78	135	≥32
trans·d	Br_2	100	50	194	≤0.78	133	≥64
trans-l	Br_2	100	50	186	≤0.78	130	≥64
cis·d	I_2	50	25	160	≤6.25	141	≥4
cis-l	I_2	50	12.5	148			
trans-d	I_2	≥200	≥100	160	≤6.25	128	16
trans·l	I_2	100	25	158	≤6.25	128	4
cis·d	oxalato	≥50	12.5	184	≤0.78	128	16
cis-l	oxalato	50	25	193	≤1.56	131	≥16
trans·d	oxalato	≥50	≥25	171	3.12	131	≥8
trans·l	oxalato	25	12.5	245	≤0.78	128	16
cis-d	SO_4	25	6.25	189	≤0.39	159	≥16
cis-l	SO_4	25	6.25	189	≤0.78	133	≥8
trans-d	SO_4	≥100	≥50	215	≤0.78	129	64
trans·l	SO_4	25	12.5	181	0.78	146	16
cis·d	D·glucuronato	≥100	≥25	180	≤0.78	127	32
cis-l	D·glucuronato	≥50	≥25	208	≤0.78	128	32
trans-d	D·glucuronato	≥100	≥25	170	≤1.56	132	≥16
trans·l	D·glucuronato	≥100	≥25	200	≤1.56	138	≥16

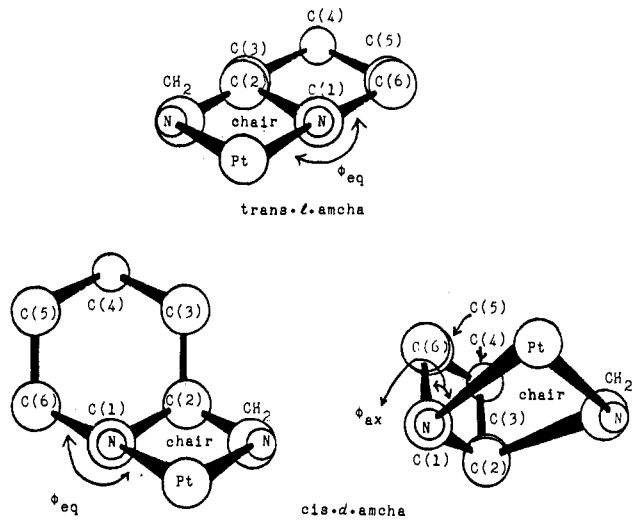

Figure 7. The chelate ring viewed down an N–C bond.

listed in Table IV, in comparison with that of *cis*·DDP. The toxicity of dach complexes is almost lower than that of *cis*·DDP, and the therapeutic indexes of the former are larger than that of the latter. As shown in Table IV, the effective dose ranges of the dihalogeno–platinum complexes were high without a significant decrease of T/C values at the optimal dose if the chlorine atoms of the dichloro complexes were replaced by bromine atoms. In the case of the dibromo–platinum(II) complex of *trans*·*l*-dach, the T/C value and therapeutic index were 238% and 16, respectively. This dibromo–platinum complex of *trans-l*-dach showed higher activity against leukemia P388 than the dichloro complex, and seemed to be one of the effective platinum complexes, though its solubility in water was poor.

The solubility of these platinum complexes in water is very important from the therapeutic viewpoint. Contrary to expectation, the oxalato–platinum complexes of dach isomers were not readily soluble in water. The oxalato–platinum complex of *trans*·*l*-dach exhibited a high T/C value of 231%, though the TI value was not large because of the toxicity.

In order to obtain more water·soluble complexes by modifying the leaving groups, sulfato– and dinitrato–platinum complexes of the dach isomers were prepared. Recently, Gale et al.[24] reported that $[Pt(NO_3)_2(dach)]$ had high antitumor activity against leukemia L1210, and its solubility was 3 mg/mL in 5% glucose solution. The solubility of $[Pt(NO_3)_2(dach)]$ was greater than that of $PtCl_2(NH_3)_2$ (1 mg/mL in 5% glucose solution). Among the platinum complexes we tested, the solubility of $[Pt(SO_4)(dach)]$ seems to be comparable to that of $[Pt(NO_3)_2(dach)]$. However, sulfato– and dinitrato–platinum complexes of the three dach isomers were not especially effective against leukemia P388.

D-Glucuronato complexes of the dach isomers were synthesized in the hope of obtaining reduced toxicity in the kidneys, owing to their high solubility in water. In fact, they are hygroscopic, and their solubilities are greater than that of $[Pt(NO_3)_2(dach)]$.[25] Among D-glucuronato–platinum complexes of the dach isomers, there was little difference in T/C values between cis and trans-*l*, as shown in Table IV, but the TI value of *trans*·*l*·dach was superior to that of *cis*·dach. The TI of the former was 64, while that of the latter was 16. As far as dach isomers are concerned, *trans*·*l*·dach–platinum complexes seem to be the most potent so far tested. An excellent result was especially obtained for [Pt(D-glucuronato)(*trans*-*l*-dach)], which can be expected to be one of the potent antitumor agents because of its high therapeutic index and its good solubility in water.

The antitumor activity of the platinum complexes of amcha isomers against leukemia P388 are listed in Table V. The therapeutic indexes of dihalogeno–platinum–amcha complexes are larger than those of dach and *cis*·DDP, and the toxicity of the former complexes are lower than that of two latter complexes. Among the dichloro–amcha complexes, cis isomers seem to be generally superior to trans isomers; the dichloro–platinum complexes of *cis-d*· and *cis*·*l*·amcha had greater T/C values (242 and 240%,

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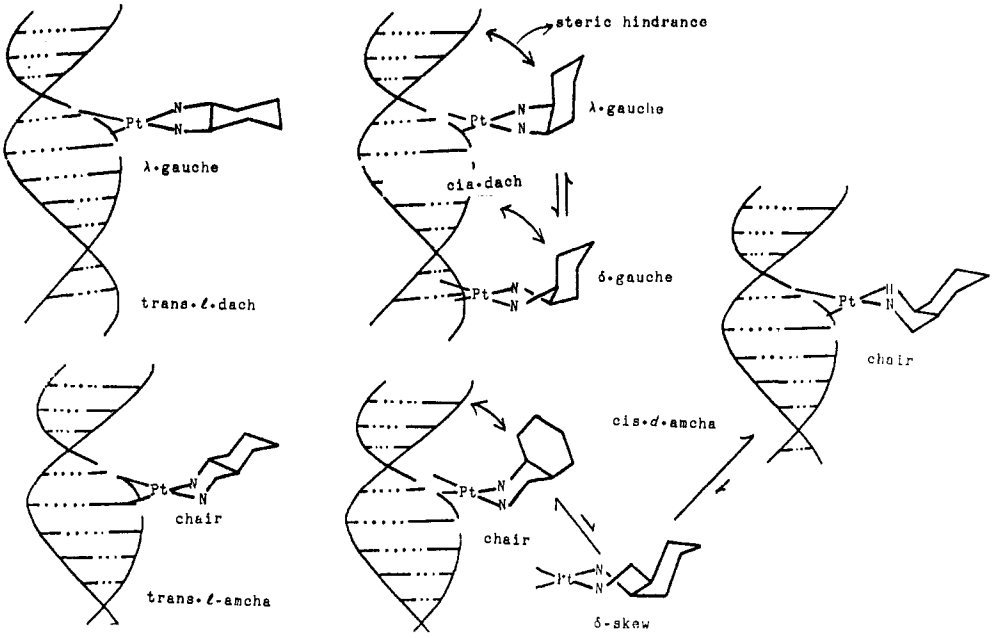

Figure 8. The relation of conformation to DNA molecules of platinum(II) complexes.

respectively), and *trans*-*d*- and *trans*-*l*-amcha had T/C values of 226 and 197%, respectively, but the therapeutic indexes of cis isomers are not larger than those of trans isomers, because of the toxicity.

In the case of dibromo complexes, the toxic doses of amcha complexes were 2 times greater, and their indexes about 2 to 8 times greater, than those of dach complexes, but there was little difference in T/C values among four amcha isomers.

The oxalato–platinum complex of *trans*-*l*-amcha exhibited a maximum T/C value of 245%, though the TI value and the toxic dose were not large, and it was readily soluble in water.

The sulfato–platinum complex of *trans*-*d*-amcha can be expected to be one of the potent antitumor agents because of its high therapeutic index and its low toxicity.

In order to obtain more water-soluble complexes with their high therapeutic indexes and their low toxicity, D-glucuronato–platinum complexes of amcha isomers were synthesized. As shown in Table V, the therapeutic indexes and T/C values of amcha complexes are similar to those of dach complexes, but the toxic doses of the former were 2 times greater than those of the latter. Although there was little difference in T/C values among the D-glucuronato complexes of the four amcha isomers, cis isomers seemed to be superior to trans isomers from the TI values.

In this work, [Pt(D-glucuronato)(*trans*-*l*-dach)] and [Pt(D-glucuronato)(*cis*-amcha)] are considered to be good candidates because of their low toxicity, high therapeutic indexes, and good solubility. Among the amcha isomers, we consider that cis-*l* isomers are one of the most active complexes based on their antitumor activity against leukemia L1210,[26] the data of which will be published elsewhere.

The antitumor activity of the platinum complexes might be explained by speculating on their stereochemical structures. The cyclohexane ring of *cis*-dach complexes is nearly perpendicular to the chelate ring, while both rings of *trans*-dach complexes lie in a common plane as illustrated in Figure 5. The same relationship was observed in the *trans*-amcha complexes (Figure 7), but the chelate ring of *cis*-amcha complexes is more flexible than that of *cis*-dach complexes; therefore, the cyclohexane ring of *cis*-amcha complexes is not perpendicular to the chelate ring (Figure 7). At the present time, the antitumor activity of platinum complexes is considered to be due to their interactions with the final target DNA molecules, and from this viewpoint the coplanarity of *trans*-dach and *trans*-amcha allows them easy approach along the large grooves in DNA molecules to interact with DNA bases (Figure 8). On the other hand, the platinum complexes of *cis*-dach have perpendicular cyclohexane rings and, hence, they would prevent their interactions with DNA molecules due to steric hindrance, but the flexible structure of *cis*-amcha complexes may make the conformational change to avoid the steric hindrance and may allow more easy approach to the target DNA than trans isomers (Figure 8).

Experimental Section

1,2-Cyclohexanediamine (dach) was commercially obtained from Tokyo Kasei Co. Ltd. Separation of dach isomers was made according to the reported method.[9]

The *cis*- and *trans*-2-(aminomethyl)cyclohexylamines (amcha) were prepared from the corresponding *cis*- and *trans*-1,2-cyclohexanedicarboxylic acids according to the method reported by Armarego et al.[27] Resolution of amcha isomers was made according to the reported method.[28]

Platinum(II) complexes of dach and amcha isomers were synthesized according to the methods described in literatures.[2-5]

Antitumor Activity. One million P388 cells were transplanted intraperitoneally into (BALB/C × DBA/2)F_1 (CDF_1) mice on day 0. Treatment was given intraperitoneally twice on days 1 and 5. The treated and control groups contained 6 and 10 mice, respectively. Several platinum complexes are not soluble in water, and in these cases suspensions were prepared in physiological saline with the addition of a few drops of Tween 80. The median survival days of both treated (T) and control (C) groups were calculated. The antitumor activity was expressed as the percent T/C. The lowest dose where the value of percent T/C exceeded 120 was evaluated as the minimum effective dose (MED). The therapeutic index (TI) was expressed as the ratio of optimal dose to MED, by means of the platinum analogues study protocol

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recommended by the National Cancer Institute.

Measurement. FT ^{13}C NMR spectra were obtained at 25 MHz with broad-band proton decoupling on a JEOL JNM-FX-100 spectrometer employing the solvent deuterium signal as an internal lock. A total of 20 200–25 800 FID's (8192 points) was averaged to provide the desired signal to noise ratio in the 2.5-kHz frequency spectra. Pulse angles of 45° were employed with no pulse delay. The ambient temperature was room temperature. Tetramethylsilane sealed in a capillary was used as an external reference. All NMR spectra were measured in D_2O solutions. Absorption spectra (AB) were measured in H_2O with a Shimadzu UV 200 recording spectrometer. Circular dichroism (CD) spectra were measured in H_2O with a JASCO J-40 spectropolarimeter. All measurements were performed at room temperature.

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Showdomycin Analogues: Synthesis and Antitumor Evaluation

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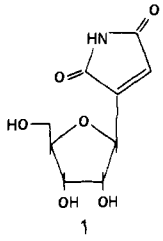
The synthesis of *N*-β-D-ribofuranosyl derivatives of maleimide, 3-methylmaleimide, and 3-chloromaleimide was accomplished in three steps from ribosylamine. The synthetic ribosides can be considered N-nucleoside analogues of showdomycin, which is an antitumor antibiotic of the C-nucleoside type. Although the three analogues were cytotoxic to cultured L1210 cells, no in vivo antitumor activity was found with the murine P388 leukemia test system. Drug transport studies were done in an attempt to trace the biological fate of the analogues.

Showdomycin (1) is an antitumor antibiotic of the C-



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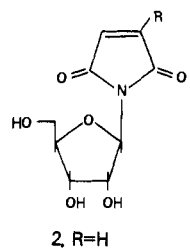
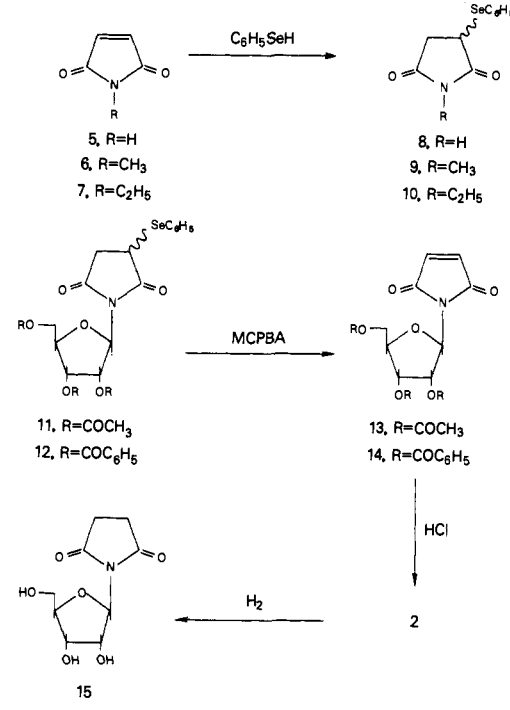


2, R=H
3, R=CH_3
4, R=Cl

nucleoside series that structurally can be considered a ring-contracted analogue of pseudouridine or an analogue of the sulfhydryl reagent *N*-ethylmaleimide (7). Although 1 and 7 inhibit cellular growth in a similar manner, the former is not simply an indiscriminate alkylating agent as is the latter: nucleosides prevent the inhibitory effects of showdomycin but not those of *N*-ethylmaleimide.[3] Apparently, the ribose moiety of 1 contributes to a facilitated entry into cells. Once taken up by the cells, the showdomycin exerts its alkylating capability on intracellular sulfhydryl groups.

Although the therapeutic range of showdomycin is too narrow for use in the treatment of clinical cancer, the specificity which the ribosyl substituent confers on the transport of maleimide across the cell membrane contributes to the continued interest in this antibiotic.[3,4] Also worthy of note is that showdomycin produced a marked radiosensitizing effect on *Escherichia coli*[5] which was enchanced when experiments were done under anoxic conditions.[6] These studies prompted a clinical trial[7] in which

Scheme I



it was shown that showdomycin was effective as a radiosensitizer in the treatment of malignant brain tumors with small dose radiotherapy.

The novel nucleoside structure and biological activity of 1 focused our efforts on the synthesis of analogues of 1 belonging to the naturally occurring N-nucleoside series in which a range of chemical reactivities in the maleimide double bond could be achieved by appropriately selected substituents. In this paper, we report the synthesis and biological evaluation of the *N*-ribosylmaleimides 2–4.

Chemistry. The initial strategy for synthesizing the *N*-ribosyl analogues called for protection of a requisite maleimide by the addition of benzeneselenol (C_6H_5SeH) across the double bond prior to condensation with a ribose

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