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Complexes between Nucleic Acid Bases and Bivalent Metal Ions. IV.¹⁾
Syntheses and Spectroscopic Analyses of Adenine-Zinc
Chloride and -Calcium Chloride Complexes²⁾

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New 1:1 adenine-ZnCl₂ and 2:1 adenine-CaCl₂ complexes were obtained from water, ethanol, or 90% ethanol solution. The infrared and proton magnetic resonance spectra of the complexes were characterized to investigate the binding site of the metal to adenine by comparison with those of other adenine-metal complexes. On the basis of these data, it is suggested that Zn or Ca is coordinated with the N(1) site of neutral adenine base.

Keywords—adenine-metal complexes; zinc; calcium; infrared spectra; complexation-sensitive band; protonation-sensitive band; proton magnetic resonance spectra; lower-field shift

Introduction

Adenine is well known to form various complexes with metal ions.^{4,5)} In particular, many Cu (II) or Co (II) complexes containing adenine have been prepared, and the binding of the metal to the N (3) or N (9) position of adenine was indicated by infrared (IR),⁶⁾ proton magnetic resonance (PMR),^{6a,b)} and X-ray crystallographic analyses (in Fig. 1).⁷⁻¹¹⁾ On the other hand, there are fewer reports on adenine-Zn complexes than on the Cu (II) and Co (II) complexes.^{12,13)} An X-ray diffraction study indicated the binding of ZnCl₂ to the N (7) position of the adeninium ring protonated at N (1), as shown in Fig. 1,^{13c)} and also suggested

- 1) Part III: S. Shirotake, *Chem. Pharm. Bull.*, **28**, 956 (1980).
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- 4) R. Weiss and H. Venner, *Z. Physiol. Chem.*, **333**, 169 (1963); R. Weiss and H. Venner, *Monatsber. Deut. Akad. Wiss. Berlin*, **13**, 199 (1971).
- 5) R.M. Izatt, J.J. Cristensen, and J.H. Rytting, *Chem. Rev.*, **71**, 439 (1971); D.J. Hodgson, "Progress in Inorganic Chemistry," Vol. 23, ed. by S.J. Lippard, John Wiley and Sons, Inc., New York, 1977.
- 6) a) T. Sakaguchi and M. Ishino, *Nippon Kagaku Kaishi*, **1974**, 1480; b) T. Sakaguchi and M. Tan-no, *Nippon Kagaku Kaishi*, **1974**, 1637; c) T. Fujita and T. Sakaguchi, *Chem. Pharm. Bull.*, **25**, 1055 (1977); d) T. Fujita and T. Sakaguchi, *Chem. Pharm. Bull.*, **25**, 2419 (1977).
- 7) E. Sletten, *Chem. Commun.*, **1967**, 1119; E. Sletten, *Acta Crystallogr.*, **25B**, 1480 (1969).
- 8) A. Terzis, A.L. Beauchamp, and R. Rivest, *Inorg. Chem.*, **12**, 1166 (1973).
- 9) P. de Meester, D.M.L. Goodgame, K.A. Price, and A.C. Skapski, *Chem. Commun.*, **1970**, 1573; P. de Meester and A.C. Skapski, *J. Chem. Soc. Dalton Trans.*, **1972**, 2400.
- 10) P. de Meester, D.M.L. Goodgame, K.A. Price, and A.C. Skapski, *Biochem. Biophys. Res. Commun.*, **44**, 510 (1971); P. de Meester and A.C. Skapski, *J. Chem. Soc. Dalton Trans.*, **1973**, 424.
- 11) a) P. de Meester and A.C. Skapski, *J. Chem. Soc.*, (A), 2167 (1971); b) K. Tomita, T. Izuno, and T. Fujiwara, *Biochem. Biophys. Res. Commun.*, **54**, 96 (1973); c) P. de Meester and A.C. Skapski, *J. Chem. Soc. Dalton Trans.*, **1973**, 1596; d) T.J. Kistenmacher, L.G. Marzilli, and C.H. Chang, *J. Am. Chem. Soc.*, **95**, 5817 (1973); e) T.J. Kistenmacher, *Acta Crystallogr.*, **29B**, 1974 (1973).
- 12) G. Weitzel and T. Spehr, *Z. Physiol. Chem.*, **313**, 212 (1958).
- 13) a) M.J. McCall and M.R. Taylor, *Acta Crystallogr.*, **32B**, 1687 (1976); b) M.J. McCall and M.R. Taylor, *Biochim. Biophys. Acta*, **390**, 137 (1975); c) L. Srinivasan and M.R. Taylor, *Chem. Commun.*, **1970**, 1668; d) M.R. Taylor, *Acta Crystallogr.*, **29B**, 884 (1973).

Fig. 1

the binding of ZnCl_3^- to N (7) and N (1) of 9-methyladenine.^{13a,b)} However, no complex of neutral adenine with ZnCl_2 has been reported, and no adenine-alkaline earth metal complex is known.

In the present study, the interaction of Zn^{2+} or Ca^{2+} with adenine was examined in ethanol, water or acidic solution, and new adenine-zinc chloride and -calcium chloride complexes were obtained. To identify the binding site of Zn^{2+} or Ca^{2+} , the infrared (IR) and protonmagnetic resonance (PMR) spectra were compared with those of tri-chloro adeninium-zinc,^{13c)} adeninato-copper (II) (4/2),⁷⁾ adenine-copper (II) chloride (4/2),⁹⁾ adenine-cobalt (II) chloride (2/1),^{4,6a,11a,c)} and adenine-nickel (II) chloride (2/1) complexes,^{4,6a)} in which the binding sites are known.

Experimental

Materials

Adenine (from Wako Pure Chemical Industries, Tokyo) was recrystallized from water. Metal chloride was from Koso Chemical Co., Tokyo. Deionized water was redistilled before use.

Syntheses

Adenine-Zinc Chloride (1/1) Complex (Adenine- ZnCl_2)—Method I. Adenine (135 mg) was dissolved in EtOH (100 ml) with stirring at 65°, ZnCl_2 (1 g) was added, and the mixture was boiled under reflux for 2–3 hr, then allowed to stand in a thermostat at 40°. After 1–2 days, white micro-columnar crystals (of adenine- ZnCl_2) were obtained. *Anal.* Calcd for $(\text{C}_5\text{H}_5\text{N}_5)\text{ZnCl}_2$: C, 22.16; H, 1.86; N, 25.81; Zn, 24.09. Found: C, 22.21; H, 1.90; N, 26.16; Zn, 23.79.

Method II: Adenine (135 mg) was dissolved in water (50 mg) with stirring at 65°, ZnCl_2 (1 g) was added, and the mixture was heated at 70° for 2–3 hr, then allowed to stand in a thermostat at 40°. After 3–4 days, white prismatic crystals of the complex were obtained. *Anal.* Found: C, 21.87; H, 1.84; N, 25.92; Zn, 23.98.

Adenine-Calcium Chloride (2/1) Complex (Adenine- CaCl_2)—Adenine (135 mg) was dissolved in 90% EtOH (100 ml) with stirring at 60°, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (1 g) was added, and the mixture was boiled under reflux for 5–6 hr, then allowed to stand in a thermostat at 40°. After 2–3 days, white micro-columnar crystals (of adenine- CaCl_2) were obtained. *Anal.* Calcd for $(\text{C}_5\text{H}_5\text{N}_5)_2\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: C, 28.78; H, 3.39; N, 33.57; Ca, 9.61. Found: C, 28.60; H, 3.66; N, 33.55; Ca, 9.95.

Adenine-Copper(II) Chloride (4/2), Adeninato-Copper(II) (4/2), Adenine-Cobalt(II) Chloride (2/1), and Adenine-Nickel (II) Chloride (2/1) Complexes (adenine- Cu(II)Cl_2 , adeninato- Cu(II) , adenine- Co(II)Cl_2 , and adenine- Ni(II)Cl_2)—These complexes were synthesized by the method of Weiss and Venner.⁴⁾

Tri-Chloro Adeninium-Zinc (1/1) Complex (adeninium- ZnCl_3)^{12,13c)}—Adenine (135 mg) was dissolved in 0.1 N HCl (30 ml) with stirring at 60°, ZnCl_2 (1 g) was added. The mixture was heated at 70° with stirring for 2–3 hr, then cooled at room temperature, and EtOH (20 ml) was added to the solution. After 1–2 days, colorless columnar crystals (of adeninium- ZnCl_3) were obtained. *Anal.* Calcd for $(\text{C}_5\text{H}_6\text{N}_5)\text{ZnCl}_3$: C, 19.50; H, 1.97; N, 22.75; Zn, 21.23. Found: C, 19.37; H, 1.97; N, 22.81; Zn, 21.16. The elemental and IR spectral data for the crystals were comparable to those of adeninium- ZnCl_3 prepared according to the method of Weitzel and Spehr.¹²⁾

Measurement of IR Spectra—The spectra of these complexes were measured on a Hitachi EPI-295 spectrophotometer, in KBr disks and in EtOD, DMSO (dimethyl sulfoxide), and DMSO- d_6 solutions. The spectra in solution were obtained by using an As_2Se_3 cell (0.1 mm).

Measurement of PMR Spectra—The sample was dissolved to 0.1 M concentration (of the ligand) in DMSO- d_6 (from Sigma Chemical Company, U.S.A.). The chemical shifts were measured on a JEOL NM4H-100 spectrometer operated at 100 MHz at 24°. TMS was used as an internal reference.

TABLE I. Relevant Infrared Absorption Bands of Adenine, Adenine-ZnCl₂*, Adenine-CaCl₂**, Adeninium Chloride,*** and Adeninium-ZnCl₃**** in KBr Disks (300—3500 cm⁻¹ region)

Adenine	Tentative assignment	Ade-ZnCl ₂ *	Ade-CaCl ₂ **	AdeHCl***	AdeH-ZnCl ₃ ****
3290 s	ν NH ₂	3380 s	3400sb	3400 s	3350 s
	ν NH ₂	3240 s	3230 s	3200 s	3240 s
3118 s	ν NH ₂	3170 s	3120 s	3150 s	3110 s
	ν NH			3020vs	3060vs
2985 s	ν CH	3000m	2988m	2960 s	2980 s
2900m	ν NH	2930 w	2830 w	2860m	2900 w
	ν NH	2850 w			
2800m	ν NH	2800vw	2800vw	2800m	2810 w
2700m	ν NH	2680vw	2690vw	2710 w	2710 w
2600m	ν NH	2600vw	2580vw	2660 w	2610 w
1667 s	δ NH ₂ scissoring	1687 s	1680 s	1710 s	1700 s
	δ NH ₂ scissoring	1680 s			1685 s
	ν C=N ⁺	1662m	1660 m	1660m	1660m
	ν C=N ⁺			1640m	1638m
	ν C=N ⁺				1624m
1602 s	ν C=N + ν C=C	1606 s	1608 s	1612 s	1600 s
	ν C=N + ν C=C			1575m	1586m
1560sh	ν C=N + ν C=C	1565m	1564m	1562 w	1562 w
1545sh	ν C=N + ν C=C	1545 w	1545 w	1545 w	1545 w
1508 w	Ring vib.	1510sh	1510 w	1500m	1503m
	Ring vib.	1498m	1480m		
1450m	Ring vib.	1440m	1445m	1460m	1438m
1420 s	Ring vib.		1422m	1413 s	1421m
	Ring vib.	1402m	1400sh	1400m	1395m
1369 s	Ring vib.				1349m
1334 s	Ring vib.	1341m	1339m	1332m	1332m
1308 s	Ring vib.	1309m	1310m	1307m	1304m
1253 s	ν C-NH ₂ + ν C-N	1273 w	1259 w	1285vw	1280vw
	ν C-N, δ C-H	1224 s	1235m	1242 s	1234 s
1156 w		1172 w	1160 w	1138 w	1174 w
1128m		1145 w	1140 w	1120 w	1120 w
	δ NH ₂ rocking	1090 w	1097 w	1110 w	
1024m		1031 w	1023 w	1018 w	1015 w
					972 w
940m	Ring vib.	940 w	944 w	946m	
923m	Ring vib.	918 w	915 w	905 w	911 w
872 w	δ NH out-of-plane	876vw	875 w	880 w	870 w
848 w		830cm	850 w	800sh	845 w
799m	δ CH + ring vib.	790m	797 m	790m	793m
				752m	770sh
723 s	Skeletal ring vib.	727m	725m	713 s	719 s
690sh	δ NH ₂ wagging	683m	684m	678m	671m
	New ring vib.				655m
646m	Ring vib.	637m	640m	639 s	636m
622m	Skeletal ring vib.	612m	614m	620m	618m
	New ring vib.	580m	585 w	570 w	570 w
		563m	566 w		551m
542m	Skeletal ring vib.	528m	543m	536 s	532m
	ν N-Zn	347 w			347 w
337m	Skeletal ring vib.	320 w	330 w	334 w	330 w
	ν Zn-Cl	294m			292m
	ν Zn-Cl	280m			

vs: very strong, s: strong, m: medium, w: weak, vw: very weak, vib.: vibration.

Results and Discussion

The new adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes were isolated from ethanol or aqueous solution of adenine and the metal chloride. The IR and PMR spectra were analyzed to identify the binding site of Zn or Ca to adenine.

Infrared Spectra

In most IR studies on adenine-metal complexes, the binding site of a metal has been deduced from the vibrational shifts of the amino or imino group.^{6,14} In the present study, our attention was focused mainly upon the complexation-sensitive ring vibration bands of adenine.

The relevant infrared absorption bands in KBr disk samples are listed in Tables I and II, and the bands in EtOD solution are presented in Table III. Ring deformation and stretching bands of adenine¹⁵⁻¹⁷ were clearly observed in the spectra of all adenine-, adeninato-, and

TABLE II. Relevant Infrared Absorption Bands of Adeninato-Cu(II),* Adenine-Cu(II)Cl₂,** Adenine-Co(II)Cl₂,*** and Adenine-Ni(II)Cl₂**** in KBr Disks (300—1700 cm⁻¹ region)

Tentative assignment	Adeninato-Cu*	Adenine-Cu**	Adenine-Co***	Adenine-Ni****
δNH_2 scissoring	1665sh	1670 s	1670 s	1670 s
$\nu\text{C}=\text{N}^+$	1640m	1640m	1630m	1623m
$\nu\text{C}=\text{N}^+$	1630m			
$\nu\text{C}=\text{N} + \nu\text{C}=\text{C}$	1580sh	1590m	1594m	1595m
$\nu\text{C}=\text{N} + \nu\text{C}=\text{C}$	1560sh	1565 w	1570m	1570m
$\nu\text{C}=\text{N} + \nu\text{C}=\text{C}$	1530 w	1520m	1522 w	1522 w
Ring vib.	1487m		1487m	1488m
Ring vib.	1466m	1462m	1460m	1460m
Ring vib.	1400 s	1412m	1405m	1408m
Ring vib.	1380sh	1370 w	1366m	1363m
Ring vib.		1348 w		
Ring vib.	1340m	1332m	1334m	1330m
Ring vib.	1306m			
$\nu\text{C}-\text{NH}_2$, $\nu\text{C}-\text{N}$	1268 w	1265 w	1253 m	1257 m
$\nu\text{C}-\text{N}$, $\delta\text{C}-\text{H}$	1220m	1220m	1235m	1233m
	1150m	1180 w	1164 w	
		1118 w	1127 m	1120m
	1028 w	1030 w	1020 w	1020 w
Ring vib.	979 w	972 w	965 w	966 w
Ring vib.	940 w	937 m	928 w	930 w
$\delta\text{C}-\text{H} + \text{ring vib.}$	796m	790m	790m	790m
Skeletal ring vib.	738m	739m	730m	732m
δNH_2 wagging	680m	683m	680m	680m
New ring vib.	656m			
Skeletal ring vib.		618m	600m	600m
New ring vib.	580sh	578sh		
Skeletal ring vib.	563m	550m	543m	545m
New ring vib.	420 w	423 w	420 w	420 w
Skeletal ring vib.	328 w	330 w	330sh	330 w

s: strong, m: medium, w: weak, ring vib.: ring vibration.

- 14) T. Fujita and T. Sakaguchi, *Chem. Pharm. Bull.*, **25**, 2953 (1977).
- 15) a) C.H. Willits, J.C. Decius, K.L. Dille, and B.E. Christensen, *J. Am. Chem. Soc.*, **77**, 2569 (1955); b) C.L. Angell, *J. Chem. Soc.*, **1961**, 504.
- 16) T. Shimanouchi, M. Tsuboi, and Y. Kyogoku, *Adv. Chem. Phys.*, **7**, 435 (1964).
- 17) M. Tsuboi and Y. Kyogoku, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, eds. W.W. Zorbach and R.S. Tipson, John Wiley and Sons, Inc., New York, 1973; M. Tsuboi, S. Takahashi, and I. Harada, "Physico-Chemical Properties of Nucleic Acids," ed. by J. Duechesence, Academic Press, New York, 1973; M. Tsuboi, "Basic Principles in Nucleic Acid Chemistry," Vol. 1, ed. by P.O.P. Ts'o, Academic Press, New York, 1974.

adeninium- metal complexes studied in this work, indicating that the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes involve the adenine skeleton. The characteristic bands on complexation are discussed below.

N-H Stretching and Deformation Modes

The absorption bands due to imidazole N-H,¹⁸⁾ which is characteristic of purine,¹⁵⁻¹⁷⁾ at 870 ($\delta\text{N-H}$ out-of-plane) and 2600–2900 cm^{-1} ($\nu\text{N-H}$)⁶⁾ in adenine disappeared on replacement of the proton by Cu^{2+} in the adeninato- Cu (II) complex,⁷⁾ while these bands were observed in the adenine- Cu (II), $-\text{Co}$ (II), and $-\text{Ni}$ (II) complexes and also in adeninium chloride and adeninium- ZnCl_3 . In the adenine-metal complexes, the intensity of the $\nu\text{N-H}$ band was very weak in comparison with that of adenine due to the intermolecular $\text{NH}\cdots\text{Cl}$ hydrogen bonding,^{6c,d,8,11)} as shown in Fig. 2. In the spectra of the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, the intensity of the $\nu\text{N-H}$ band was very weak in the solid state (in Fig. 2), but was comparable with that of adenine in $\text{DMSO}-d_6$ solution (in Fig. 3). These results suggest that the imidazole proton is not replaced in the new complexes, and also that the hydrogen participates in intermolecular hydrogen bonding in the solid state.¹⁹⁾

Fig. 2. Infrared Absorption Spectra in the Region of 2500–3500 cm^{-1} in KBr Disks

A, adenine- $\text{Cu}(\text{II})\text{Cl}_2$; B, adenine- CaCl_2 ; C, adenine- ZnCl_2 ; D, adeninium- ZnCl_3 ; E, adeninium chloride; F, adenine.

Fig. 3. Infrared Absorption Spectra in the Region of 2500–4500 cm^{-1} in $\text{DMSO}-d_6$ Solution

A, adeninium- ZnCl_3 ; B, adenine- CaCl_2 ; C, adenine- ZnCl_2 ; D, adenine.

On the other hand, in adeninium chloride²⁰⁾ and the adeninium- Zn^{13}C and $-\text{Cu}$ (II)^{6d)} complexes, a new absorption of strong intensity appeared near 3050 cm^{-1} on protonation at the N (1) position of the pyrimidine ring (in Fig. 2). Since the strong band is not present in adenine- and adeninato- metal complexes, it is assignable to the N (1)-H stretching mode. No N (1)-H band is present in the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, suggesting that the complexes retain a neutral adenine ring.

18) M. Dreyfus, G. Dodin, O. Bensaude, and J.E. Dubois, *J. Am. Chem. Soc.*, **97**, 2369 (1975).

19) L.J. Bellamy (ed.), "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, 1966.

20) W. Cockran, *Acta Crystallog.*, **4**, 81 (1951); J.M. Broomhead, *Acta Crystallog.*, **4**, 92 (1951).

Ring Stretching and Deformation Modes

In some reports, the C (6)-NH₂ stretching mode of adenine was assigned to the absorption band near 1310 cm⁻¹.^{6c,6d,14} However, the frequency of the band in question is hardly affected by N-deuteration,^{15b} protonation at N (1), and the binding of Cu²⁺ to the adeninato anion in the adeninato-Cu (II) complex, as shown in Fig. 4 and in Tables I and II. Moreover, an absorption band near 1310 cm⁻¹ was also observed in the spectra of 9-methylpurine,^{15b} theophylline (1312 cm⁻¹), theobromine (1302 cm⁻¹), and xanthine (1310 cm⁻¹), but was not present in pyrimidine derivatives.²¹ Thus, the band of adenine is considered to be predominantly due to imidazole ring vibration rather than C-NH₂ stretching. On the other hand, the absorption band at 1253 cm⁻¹ in adenine was split into two bands on protonation at N (1) and on metal coordination with ring nitrogen in all the adenine-metal complexes listed in Tables I and II. The splitting also occurred on metal coordination with N (3) of cytosine.²²

Fig. 4. Infrared Absorption Spectra in the Region of 1200–1400 cm⁻¹ in KBr Disks

A, adeninium-ZnCl₃; B, adeninium chloride; C, adenine; D, adenine-ZnCl₂; E, adenine-Co(II)Cl₂; F, adeninato-Cu(II); G, adenine-Cu(II)Cl₂.

21) L.M. Short and H.W. Thompson, *J. Chem. Soc.*, **1952**, 168.

22) S. Shirotake and T. Sakaguchi, *Chem. Pharm. Bull.*, **26**, 2941 (1978).

In the case of adenine, the absorption band at 1253 cm^{-1} was assigned to an external C-NH₂ stretching mode coupled with an internal C-N stretching mode, as mentioned by Tsuboi *et al.* in their IR studies on adenine derivatives.¹⁷⁾ In the adenine-metal complexes, the band around 1220 cm^{-1} is assignable to the C-N:→metal stretching mode reported by Nakamoto²³⁾ and the C-H deformation mode.¹⁷⁾

In the $900\text{--}1000\text{ cm}^{-1}$ region, adenine shows two bands at 923 and 940 cm^{-1} assignable to the ring vibrations. These bands were also observed near 930 and 970 cm^{-1} in the adeninato-Cu (II) and the adenine-Ni (II), -Co (II), and -Cu (II) complexes. On the other hand, in adeninium chloride, the band at 923 cm^{-1} of adenine was shifted to a lower-frequency region by protonation at the N (1) site, whereas the band at 940 cm^{-1} of adenine remained.

Fig. 5. Infrared Absorption Spectra in the Region of $300\text{--}700\text{ cm}^{-1}$ in KBr Disks

A, adenine-Cu(II)Cl₂; B, adeninato-Cu(II); C, adeninium-ZnCl₃; D, adeninium chloride; E, adenine; F, adenine-Ni(II)Cl₂; G, adenine-Co(II)Cl₂; H, adenine-CaCl₂; I, adenine-ZnCl₂.

- 23) K. Nakamoto (ed.), "Infrared Spectra of Inorganic and Coordination Compounds," John Wiley and Sons, Inc., 1963; K. Nakamoto, "Coordination Chemistry," ed. by A.E. Martell, Van Nostrand-Reinhold Co., New York, 1973; K. Nakamoto (ed.), "Infrared and Raman Spectra of Inorganic and Coordination Compounds," John Wiley and Sons, Inc., New York, 1978.

In adeninium- ZnCl_3 ,¹³⁾ the former band of adenine was shifted to a lower-frequency region on protonation, while the latter was shifted to a higher-frequency region by metal coordination with the N (7) site (Table I). In the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, the lower-frequency shift of the former implies metal coordination with the N (1) site or protonation.

In the spectra of the adeninato-Cu (II) complex, the adenine-Ni (II), $-\text{Co}$ (II), and $-\text{Cu}$ (II) complexes, and the adeninium-Cu (II) complex,^{6d,10)} the complexation-sensitive band appeared at $730\text{--}740\text{ cm}^{-1}$ on metal coordination with the N (9) position of adenine, as shown in Table II. This is comparable to the skeletal ring vibration (ring breathing)¹⁷⁾ of adenine. However, the band of adenine remained in the adeninium- ZnCl_3 and the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes (Table I).

In adeninium chloride and the adeninium- $\text{Zn}^{13\text{c})}$ and $-\text{Cu}$ (II)^{6d,10)} complexes, a new band appeared at 570 cm^{-1} on protonation at the N (1) position of adenine, and it is noteworthy that the frequency value was unaffected not only by the kind of metal but also by the binding site. Thus, the protonation-sensitive bands (νN (1)-H and ring deformation bands) are useful to distinguish protonation at the N (1) site from metal coordination with the N (1). In the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, no protonation-sensitive band was present, while a new band appeared near 580 cm^{-1} on metal coordination, as shown in Fig. 5. However, the band (580 cm^{-1}) does not effectively distinguish between metal coordination at the N (1) and N (3) sites.

Double Bond Stretching and NH_2 Scissoring Modes

In all the adenine-metal complexes the double bond stretching bands appeared in a higher-frequency region on the binding of metal or proton²⁴⁾ to nitrogen of the adenine ring (in Tables I, II, and III). Since the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes are formed by neutral adenine, the higher-frequency shift of the double bond stretching mode suggests metal coordination with nitrogen of the ring.²⁴⁾

In adenine- ZnCl_2 , the δNH_2 scissoring band was split into two, as in the case of adeninium- ZnCl_3 , in which the amino proton takes part in hydrogen bonding to chlorine.^{13c)} The NH_2 scissoring frequency of adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes was higher than those of adenine and the adeninato- and adenine-metal complexes, as shown in Tables I and II. The δNH_2 band of adenine was shifted to a higher-frequency region by the binding of a metal^{6d)} or proton¹⁵⁾ to the N (1) site. In the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, the higher-frequency shift of the NH_2 scissoring mode is suggested to be caused by the metal coordination.

Proton Magnetic Resonance Spectra

In PMR studies on diamagnetic metal complexation with adenine, adenosine, and AMP,²⁵⁻²⁷⁾ Wang and Li found initially that addition of ZnCl_2 to adenosine (0.1 M) in DMSO resulted in lower-field shifts of the NH_2 , C (2)-H, and C (8)-H signals, suggesting the binding of Zn^{2+} to C (6)- NH_2 and N (7) of the neutral adenine base. However, the NH_2 signal of nucleic acid base was reported to shift towards lower-field on interaction of the amino proton with a chloro anion.²⁸⁾ The binding site of Zn^{2+} or Ca^{2+} to adenine will next be discussed on the basis of a comparison of the present data with the data²⁵⁻²⁷⁾ obtained by the addition of metal ions.

PMR spectra of adenine and the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes in $\text{DMSO}-d_6$ are shown in Fig. 6, and those of adeninium chloride and adeninium- ZnCl_3 in Fig. 7. In the adenine- ZnCl_2 and $-\text{CaCl}_2$ complexes, the C (2)-H and C (8)-H signals coalesced into a single peak,

24) M. Tsuboi, Y. Kyogoku, and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962).

25) S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **88**, 4592 (1966); S.M. Wang and N.C. Li, *J. Am. Chem. Soc.*, **90**, 5069 (1968).

26) S. Shimokawa, H. Fukui, J. Sohma, and K. Hotta, *J. Am. Chem. Soc.*, **90**, 5069 (1968).

27) J. Granot and D. Fiat, *J. Am. Chem. Soc.*, **99**, 70 (1977).

28) C.H. Chang and L.G. Marzilli, *J. Am. Chem. Soc.*, **96**, 3656 (1974).

TABLE III. Double Bond Stretching Vibrations of Adenine, Adenine-ZnCl₂, and Adenine-CaCl₂ in EtOD Solution (1500—1700 cm⁻¹ region)

Adenine	Tentative assignment	Adenine-ZnCl ₂	Adenine-CaCl ₂
	C=N ⁺	1670m	1665m
1620 s	C=N + C=C	1623 s	1623 s
1576m	C=N + C=C	1605m	1600m
	C=N + C=C	1570m	1573m
1560sh	C=N + C=C	1560sh	1563sh
1512m	C=N + C=C	1520 w	1515 w
	C=N + C=C	1507 w	1508 w

Fig. 6. Proton Magnetic Resonance Spectra in DMSO-*d*₆ Fig. 7. Proton Magnetic Resonance Spectra in DMSO-*d*₆

showing that the extent of lower-field shift of the C (2)-H is greater than that of the C (8)-H. On the basis of PMR spectral studies on purine derivatives,²⁹⁾ the greater lower-field shift of the C (2)-H compared to the C (8)-H indicates that the pyrimidine ring of adenine was more positively charged than the imidazole ring in the complexes. From a comparison of Fig. 6 with Fig. 7, it is suggested that the lower-field ring proton shift in the adenine-ZnCl₂ and -CaCl₂ complexes was not caused by protonation at the N (1) site, but was caused by metal coordination with the N (1) or N (3) site.²⁹⁻³¹⁾ The binding of the metal to N (1) of adenine is a quite consistent with the interpretation of the IR spectra.

- 29) C.D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960); C.D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 229 (1960); M.P. Schweizer, S.I. Chan, G.K. Helmkamp, and P.O.P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964); W.C. Corburn, M.C. Thorpe, J.A. Montgomery, and K. Hewson, *J. Org. Chem.*, **30**, 1110 (1965); F.J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1965); J.M. Read and J.H. Goldstein, *J. Am. Chem. Soc.*, **87**, 3440 (1965).
- 30) B.H. Lynch, B.C. Macdonald, and J.G.K. Webb, *Tetrahedron*, **24**, 3595 (1964); J.J. Batterham (ed.), "NMR Spectra of Simple Heterocycles," John Wiley and Sons, Inc., New York, 1973.
- 31) G. Fraenkel, R.E. Carter, A. McLachlan, and J.H. Richards, *J. Am. Chem. Soc.*, **82**, 5846 (1960); C. Maclean and E.L. Mackor, *Mol. Phys.*, **4**, 241 (1961); T. Schaefer and W.G. Schneider, *Can. J. Chem.*, **51**, 966 (1963).

As shown in Fig. 7, the NH_2 resonance of adeninium chloride appeared at lowerfield by 215 Hz compared to that of adenine on protonation at the N (1) site, and the NH_2 signal of adeninium- ZnCl_3 was shifted to higherfield by 30 Hz compared to that of adeninium chloride on the binding of ZnCl_3^- anion to the N (7) site of the adeninium ring. Therefore, the lower-field shift of the NH_2 signal compared to that of adenine in adenine-metal complexes is caused not only by a charge-reversed interaction²⁸⁾ of the amino proton with Cl^- but also by the binding of a metal to nitrogen of the ring.

In this work, the coalescence of the C (2)-H and C (8)-H peaks is an interesting finding, because it is the first report that the binding of metal to the pyrimidine ring of adenine leads to the coalescence of the C (2)-H and C (8)-H peaks. Taking into account the PMR data of Li et al. and our examination, it may be concluded that Zn has affinity for the N (1) and N (7) sites of neutral adenine base.^{13a,b)}

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