Chem. Pharm. Bull.
19(8)1650—1655(1971)

UDC 547.963.32.03.04 : 541.486

Studies on the Metal-Nucleotides Complexes. IV.[1] Studies on the Isolated Metal-Inosinic Acid(5'-IMP) Complexes by Infrared Absorption Spectra

Michiyuki Ogawa and Takeichi Sakaguchi

Faculty of Pharmaceutical Sciences, Chiba University[2]

(Received February 6, 1971)

We could isolate metal complexes of inosinic acid (IMP) with Pb, Cd, Co (II), Zn, Cu (II), Ni and Mn as crystalline precipitates and Hg (II) was amorphous one from the solution of pH 3.0—7.0

The elementary analysis revealed that the isolated metal complexes had the composition of 1:1 of IMP to metal, except Hg-IMP whose composition is 2:3. And we discussed the infrared (IR) absorption spectra of these metal complexes and compared with thos eof IMP Na, IMP 2Na, IMP 2K, IMP Ca and IMP Ba salt. Metal complexes with Co (II), Ni, Mn, Cd, and Pb as well as the above salt compounds showed the C=O stretching absorption in the region of about 1690 cm^{-1}.

Complexation with Cu (II), Zn and Hg (II) shifted this frequency to 1710, 1708, and 1640 cm^{-1} respectively.

In the region of 1200—900 cm^{-1} absorptions due to the phosphate group are expected, the metal complexes except Cu-IMP and Zn-IMP showed the similar spectra to those of IMP salt compounds, such as IMP 2Na and IMP 2K. Cu-IMP and Zn-IMP showed a little complicated spectra also in this region.

From the above results we concluded that Cu (II), Zn and Hg (II) coordinated not only to the phosphate group but also to the hypoxanthine moiety of IMP

The coordination sites of Cu (II) and Zn on the hypoxanthine moiety were considered N-7 and that of Hg (II) was C-O from the spectral changes and elementary analysis.

Additional experiments of NMR spectra in D_2O revealed the protonation to N-7 of hypoxanthine moiety from the phsophate group of IMP free acid.

Interaction of nucleic acid and polynucleotides with certain metal ions has been observed by a number of investigators.[3]

Metal complexes of such biologically important compounds cannot be elucidated very much without fundamental knowledge regarding metal interaction with components of nucleic acid.

In the previous papers,[4] we have investigated the interaction of certain metal ions with adenosine nucleotides by isolating the metal complexes. In this investigation, we could isolate the metal complexes of IMP containing various metal ions, in which metal complexes such as Pb, Cd, Co(II), Cu(II), Zn, Ni and Mn complexes were prepared as crystalline precipitates and Hg(II) complex was obtained as amorphous one from the solution of pH 3.0—7.0.

And we investigated the infrared absorption spectra in order to get more precise informations about these metal complexes.

The interaction of IMP with metal ions such as Ag(I) and Cu(II) was investigated by Tu, *et al.*,[5] and they proposed that these metal complexes formed pentacyclic complexes involving enolic O at C-6 and N-7.

---

[1] Part III: M. Ogawa, *Yakugaku Zasshi*, **91**, 618 (1971).

[2] Location: *Yayoi-cho, Chiba-shi.*

[3] R.G. Schulman and H. Sternlich, *J. Mol. Biol.*, **13**, 952 (1952); J.H. Coates, D.O. Jordan, and V.K. Srivastava; *Biochem. Biophys. Res. Commun.*, **20**, 611 (1965); H, Venner, and Ch. Zimmer; *Biopolymers*, **4**, 321 (1966).

[4] M. Ogawa, Y. Urata, and T. Sakaguchi, *Bunseki Kagaku*, **19**, 1244 (1970); **20**, 36 (1971).

[5] Anthony T. Tu and J.A. Reinosa, *Biochemistry*, **5**, 3375 (1966); Anthony T. Tu and C.G. Griederich, *ibid.*, **7**, 4367 (1968).

But they did not isolate these metal complexes.

Result and Discussion

Isolated Metal-IMP Complexes

The elementary analysis revealed that the ratio of IMP to metal is 1: 1 in most of our isolated metal complexes and this ratio is 2: 3 in Hg-IMP alone.

TABLE I. Metal Contents of IMP Metal Complexes

Metatal complexes	Formula	Calculated (%)	Found (%)
Zn-IMP	$(C_{10}H_{11}O_8N_4P)Zn(H_2O)$	15.2	15.2
Cu-IMP	$(C_{10}H_{11}O_8N_4P)Cu(H_2O)$	14.9	15.2
Mn-IMP	$(C_{10}H_{11}O_8N_4P)MN(H_2O)_2$	12.6	12.2
Cd-IMP	$(C_{10}H_{11}O_8N_4P)Cd(H_2O)_2$	22.8	23.1
Ni-IMP	$(C_{10}H_{11}O_8N_4P)Ni(H_2O)_7$	11.1	11.9
Co-IMP	$(C_{10}H_{11}O_8N_4P)Co(H_2O)_5$	11.9	11.9
Pb-IMP	$(C_{10}N_{11}O_8N_4P)Pb(H_2O)$	36.1	35.9
Hg-IMP	$(C_{10}H_{11}O_8N_4P)_2Hg_3$	46.5	45.6

Infrared Absorption Spectra of IMP2Na IMP Na and IMP Free Acid

Before illustraing the spectral changes of the infrared absorption upon complexation with metal ions, we studied the spectral changes of IMP itself by dissociation of phosphate group and also protonation on the particular site of hypoxanthine moiety of free acid IMP.

In IMP 2Na and IMP Na the strong absorption band appearing near 1680 cm^{-1} will be assigned to C=O.⁶⁾

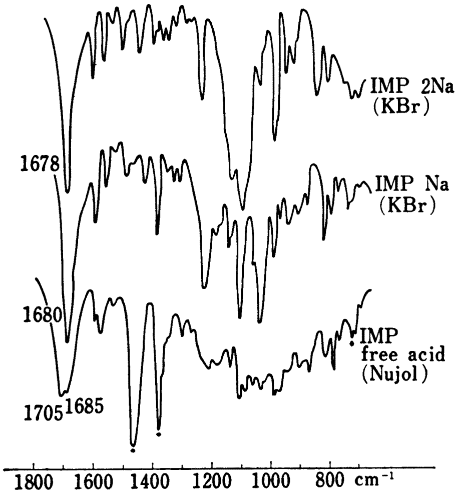

Fig. 1. Infrared Absorption Spectra of IMP, Free Acid, IMP Na and IMP 2Na

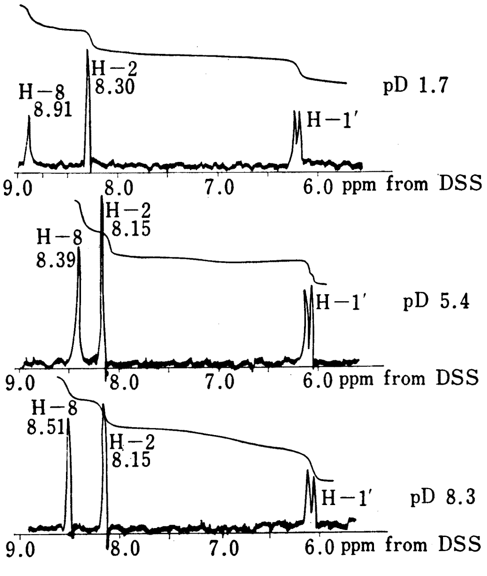

Fig. 2. NMR Spectra of IMP (Free Acid), IMP Na and IMP 2Na (*ca.* 0.18 M D_2O Solution)

---

6) E.R. Blout and M. Fields, *J. Am. Chem. Soc.*, **72**, 479 (1950).

The spectrum of free acid IMP showed the strong but a little broad absorption at 1705—1685 cm^{-1}, which will be assigned to the C=O and C=N$^+$ of aromatic ring.

To reveal this phenomenon of protonation at C=N$^+$ more clearly, we measured the nuclear magnetic resonace spectra of IMP in the form of free acid, IMP Na and IMP 2Na in D$_2$O. The phosphate group was found to have a specific deshielding and broadening effect on the H-8 proton of IMP. At the region of pD 1.7 to pD 5.4 (free acid to mono anion form of phosphate group), there is the specific upfield shift of the H-8 proton which is most likely due to the removal of the deshielding effect of the positive charge of the hypoxanthine base (0.52 ppm).

There is a little down field shift of the H-8 proton at the region of pD 5.4 to 8.3 (mono anion form to dianion form of phosphate group, 0.12 ppm). This shift is probably due to the effect of the ionization of the secondary phosphate on the stacking of IMP (Fig. 2).

We considered from the above results that the protonation from the phosphate group was formed on the N-7 of the hypoxanthine moiety of IMP.

Infrared Absorption Spectra of IMP 2K, IMP Ba and IMP Ca Salt

To confirm the coordination of the metal ions to the hypoxanthine moiety, we investigated the infrared absorption spectra of IMP 2K, IMP Ba and IMP Ca salt, and these alkaline and alkaline earth metals were proposed to be bound to the phosphate group alone IMP (Fig. 3).

In these compounds, C=O stretching vibration appeared in the region around 1680 cm^{-1}. It was considered from these results that if the binding site of the metal ions of IMP-metal complex were limited to the phosphate group, the wave number of the stretching vibration of C=O in the hypoxanthine moiety would be retained near 1680 cm^{-1}.

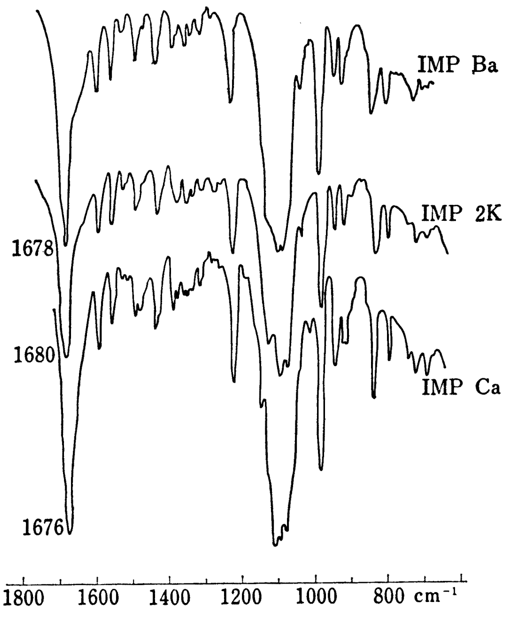

Fig. 3. Infrared Absorption Spectra of IMP Ba, IMP Ba, IMP 2K and IMP Ca (KBr)

From our expriments upon complexation with metal ions the shifts of the absorption band at this wave number (1680 cm^{-1}) was seen clearly, therefore these results will be attributed to the existance of any interaction between metal ion and hypoxanthine moiety of IMP.

Infrared Absorption Spectra of the Isolated Metal Complexes

Infrared absorption spectra of the isolated metal complexes were illustrated in Fig. 4—7. Strong band appeared in the region of 1710 cm^{-1} and 1708 cm^{-1} upon complexation with Cu(II) and Zn ions respectively.

Cu-IMP and Zn-IMP showed a little more complicated spectra than those of IMP 2Na and IMP 2K in the region of 1200—1900 cm^{-1} (Fig. 4, 5).

In these two metal complexes, the strong band was seen at 998 cm^{-1} and 1004 cm, which are assigned to the PO$^{2-}{}_3$ group of Cu-IMP and Zn-IMP respectively. And it was concluded from the elementary analysis that these complexes had no NO$_3^-$. So we ascertained the Cu(II) and Zn ions were bound to the phosphate groups of IMP.

The question arises whether the hypoxanthine moiety participates in the coordination. The C=O group at C-6 and nitrogen atom at N-7 are the feasible coordination sites.

But the former alternative is eliminated by the evidence described below.

If the C=O group of hypoxanthine moiety participated in the complex formation, the band at 1678 cm^{-1} in IMP 2Na would shift to low frequency.[7]

This is illustrated by the fact of the low frequency shift in the case of Hg-IMP (Fig. 6).

In the case of Cu-IMP and Zn-IMP, however, this carbonyl band appeared at high frequency compared with those of IMP 2Na.

We considered that the coordination sites of these compounds on the hypoxanthine moiety were the neighboring atoms of C=O group, namely N-7 or N-1 of IMP.

IMP free acid, which takes the structure of N-7 protonation form, shows the C=O and C=N$^+$ stretching vibration band in almost the same region as these two metal complexes.

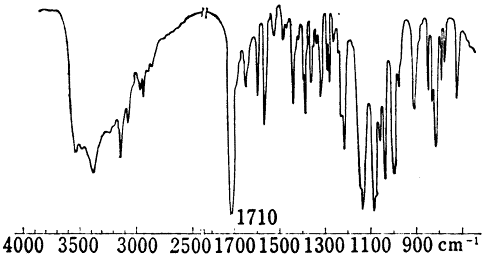

Fig. 4. Infrared Absorption Spectrum of Cu(II)-IMP (KBr)

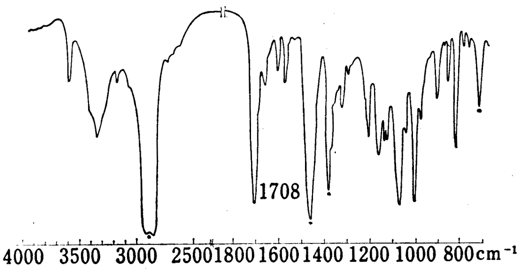

Fig. 5. Infrared Absorption Spectrum of Zn-IMP (Nujol Mull)

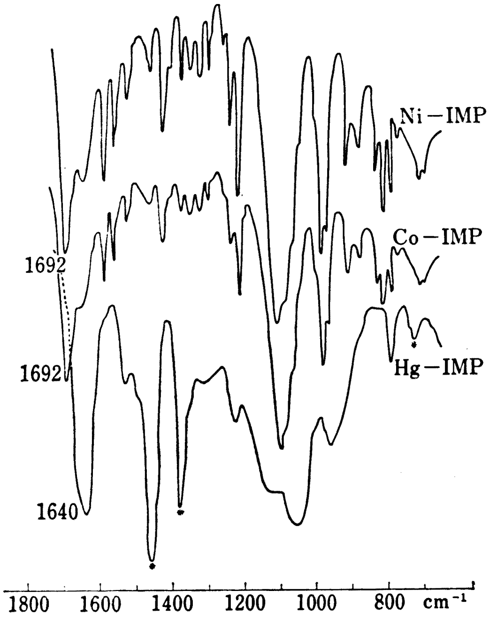

Fig. 6. Infrared Absorption Spectra of Ni-IMP, Co-IMP and Hg-IMP (KBr)

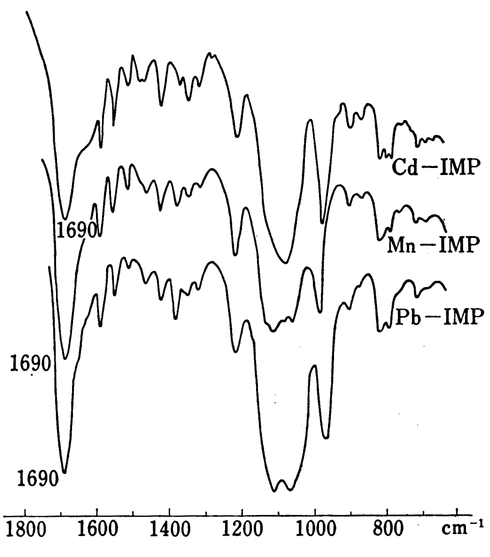

Fig. 7. Infrared Absorption Spectra of Cd-IMP, Mn-IMP and Pb-IMP (KBr)

So it can be reasonably assumed that Cu(II) and Zn ions had any interaction with hypoxanthine moiety on N-7, in the same way as protonation of IMP free acid. Proposed structure of Cu-IMP and Zn-IMP were illustrated on Chart 1.

---

[7] K. Nakamoto, "Kinzoku Chelate II (Metal ChelateII)," ed. by T. Sakaguchi and K. Ueno, Nankodo, Tokyo, 1966, pp. 41—51.

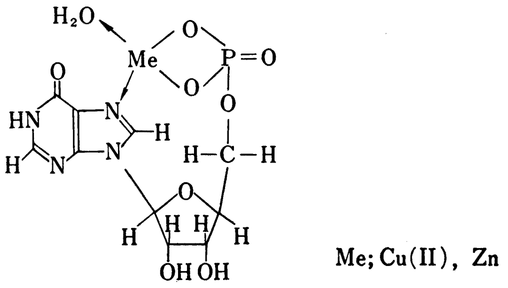

Chart 1. Proposed Structure of Cu-IMP and Zn-IMP

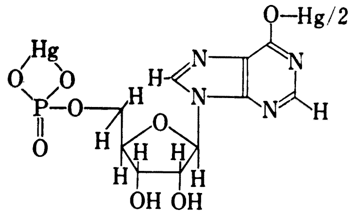

Chart 2. Proposed Structure of Hg-IMP

From the facts that the C=O stretching vibration of Hg-IMP appeared at the low frequency than that of IMP 2Na (1640 cm^{-1}), and the spectra of the phosphate group of Hg-IMP were similar to the IMP 2Na, it is considered that Hg(II) ion is bound both to enolic C-O at 6 and phosphate group. Proposed structure is illustrated as below.

Other metal complexes, such as Mn-IMP, Cd-IMP, Ni-IMP, Co-IMP, and Pb-IMP showed the C=O frequency near that for IMP 2Na (1690 cm^{-1}), and showed the similar spectra of phosphate group to those of IMP 2Na.

Therefore, it was confirmed that these metal ions are bound to the phosphate group, but it was not still clear if these metal ions were coordinated to the hypoxanthine moiety or not.

We are now studying to try to make clear these points by further experiments isolating other metal complexes with IMP analog, such as guanylic acid.

Experimental

Apparatus——Infrared Absorption Spectra: Hitachi Infrared Spectrometer; type EPI G3 and type 215.

Nuclear Magmetic Resonance Spectra: Japan Electron Optics. Lab., type JNM-4H-100

pH: Hitachi-Horiba type M-5 with 6028-10T combination glass electrode.

Reagents——Inosinic acid disodium salt (IMP 2Na) was purchased from Kohjin Co., Ltd. and Yamasa Co., Ltd.

Other reagents used are JIS special grade reagents without further purification.

Inosinic Acid Cu-Complex (Cu-IMP): IMP 2 Na (0.4 g) was dissolved in H_2O of 30 ml and the pH of the solution was adjusted to 4.5 with dil. HNO_3. A solution of Cu $(NO_3)_2 3H_2O$ (0.24 g) in 15 ml of H_2O was added to the above solution. Heated at 60° for 20 min. light blue crystals precipitated. The precipitate was collected by filtration and washed with H_2O, EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Cu(H_2O)$: C, 27.95; H, 3.04; N, 13.10. Found: C, 27.74; H, 3.11; N, 13.07.

Inosinic Acid Zn-Comple (Zn-IMP): This compound was prepared by the same procedure as mentioned above. Instead of Cu $(NO_3)_2$, Zn $(NO_3)_2$ was used. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Zn(H_2O)$: C, 27.90; H, 3.02; N, 13.00. Found: C, 27.78; H, 3.05; N, 12.65.

Inosinic Acid Mn-Complex (Mn-IMP): IMP 2Na in 30 ml of H_2O was added to a solution of Mn $(NO_3)_2$ $4H_2O$ (0.25 g) in 15 ml of H_2O with stirring. The mixed solution was allowed to stand for overnight after heated at 60° for 15 min. The crystalline precipitate was collected by filtration and washed with H_2O, EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)$ Mn $(H_2O)_2$: C, 27.45; H, 3.43; N, 12.81. Found: C, 27.49; H, 2.96; N, 12.74.

Inosinic Acid Cd-Complex(Cd-IMP), Inosinic Acid Ni-Complex(Ni-IMP) and Inosinic Acid Co-Complex (Co-IMP): These compounds were prepared by the same procedure as Mn-IMP, using $Cd(No_3)_2$, $Ni(NO_3)_2$ and $Co(NO_3)_2$. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Cd(H_2O)_2$: C, 24.30; H, 3.04; N, 11.33. Found: C, 24.64; H, 2.88; N, 11.51. Calcd. for $(C_{10}H_{11}O_8N_4P)$ $Ni(H_2O)_7$: C, 22.55; H, 4.71; N, 10.55. Found: C, 22.51; H, 4.71; N, 10.42. Calcd. for $(C_{10}H_{11}O_8N_4P)Co(H_2O)_5$: C, 24.25; H, 4.23; N, 11.35. Found: C, 24.38; H, 4.31; N, 11.57.

Inosinic Acid Pb-Complex(Pb-IMP): IMP 2Na (0.4 g) was dissoved in H_2O of 30 ml and a solution of $Pb(NO_3)_2$ (0.33 g) in 15 ml of H_2O was added with stirring. After continued stirring for 15 min, the crystalline precipitate was collected by filtration and washed with H_2O, EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Pb(H_2O)$: C, 21.00; H, 2.10; N, 9.82. Found: C, 20.34; H, 2.22; N, 9.95.

Inosinic Acid Hg-Complex(Hg-IMP): IMP 2·Na was dissolved in 2% HAc and a solution of 20% $HgAc_2$ (in 2% HAc) was added. After heated at 60° for 20 min, amorphous precipitate was collected by filtration and washed with 2% HAc, EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)_2Hg_3$: C, 18.16; H, 1.70; N, 8.70. Found: C, 18.82; H, 1.84; N, 8.78.

Inosinic Acid Mono Sodium Salt (IMP Na): IMP 2Na (0.2 g) was dissolved in 20 ml of H_2O and the pH of the solution was adjusted to 4.5 with dil·HCl. IMP Na was precipitated by adding EtOH (30 ml) and ether (15 ml) to the above solution. The precipitate was collected by filtration and washed with EtOH and ether. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)$ Na (H_2O): C, 30.95; H, 3.36; N, 10.42. Found: C, 30.55; H, 2.55; N, 10.33.

Inosinic Acid Barium Salt (IMP Ba): IMP 2Na (0.4 g) was dissolved in 30 ml of H_2O and a solution of $BaCl_2$ $2H_2O$ (0.24 g) in 15 ml of H_2O was added. After stirred for 20 min, crystalline precipitate was collected by filtration and washed with H_2O, EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Ba(H_2O)_3$: C, 22.31; H, 3.16; N, 10.42. Found: C, 21.92; H, 2.55; N, 10.33.

Inosinic Acid (IMP free acid)[8]: IMP 2 Na (1 g) was dissolved in 50 ml of H_2O and passed through the Amberlite IRA 122 (H^+form, 100 ml) column and washed with H_2O. The eluate and the washed water was concentrated under diminished pressure below 45°. The residue was dissolved in MeOH (40 ml) and precipitated by adding ether (20 ml).

The amorphous precipitate was collected by filtration and washed with MeOH and ether successively. Recrystallized from 80% MeOH, needles. *Anal.* Calcd. for $(C_{10}H_{13}O_8N_4P)$ (CH_3OH): C, 34.86; H, 4.47; N, 14.74. Found: C, 34.62; H, 4.66; N, 14.73. NMR (in D_2O) ppm from DSS: 3.35 (3H, s, CH_3-OH), 4.23 (2H, m, $J=15$ cps, H_2-C-5), 4.47 (2H m, $J=20$ cps, ribose-H), 4.78 (HDO), 6.20 (1H, d, $J=4$ cps, C'_1-H), 8.91 (1H, s, brod, H-C-8).

Inosinic Acid Dipotassium Salt (IMP 2K): IMP free acid (0.2 g) was dissolved in 15 ml of H_2O, and the pH of the solution was adjusted to7.5 with dil. KOH solution of H_2O. IMP 2K was precipitated by adding 40 ml of EtOH and allowed to stand in refrigerator for overnight.

The crystalline precipitate was collected by filtration and washed with EtOH and ether successively. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)K_2(H_2O)_3$: C, 25.14; H, 3.77; N, 11.72. Found: C, 25.59; H, 3.88; N, 11.33.

Inosinic Acid Calcium Salt (IMP Ca): This compound was prepared by the same precedure as applied in the preparation of IMP Ba, but $CaCl_2$ was used instead of $BaCl_2$. *Anal.* Calcd. for $(C_{10}H_{11}O_8N_4P)Ca(H_2O)_2$: C, 28.42; H, 3.55; N, 13.26. Found: C, 28.28; H, 3.36; N, 13.07.

Acknowledgement The authors are grateful to Mr. H. Mori, director of pharmaceutical development division of Kowa Co., Ltd., for his permission to publish this work. Thanks are also due to Mr. A. Nara and his staffs of Kowa Co., Ltd., Tokyo Lab. for elementary analysis and also to Miss F. Kuriyagawa, for her measurements of NMR spectra.