Arch. Pharm. Res. **1**(1), 1~6(1978)

On the Reaction of Nucleic Acid Derivatives with Rhodium(II) (isobutyrate)$_4$L$_2$

Byung Sul Yu and Bak-Kwang Kim

College of Pharmacy, Seoul National University, Seoul 151, Korea

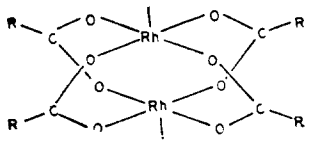
(Received 30 August 1978)

Abstract □ Rhodium (II) (isobutyrate)$_4$L$_2$, an antitumor drug, was shown to react with nucleic acid base derivatives, A, G, U and C in chloroform solution. When these derivatives were treated with one of novel metal compounds, rhodium carboxylate in chloroform solution, a fairly strong complex formation was observed by spectroscopic techniques. The characteristic of these complex was that binding occured at the two axial positions of rhodium (II) (isobutyrate)$_4$L$_2$ to the NH or NH$_2$ group of the base in the ligands.

Keyphrases □ Rhodium isobutyrate–antitumor activity–binding with the imino or amino group of the purine or pyrimidine bases of nucleic acids–the binding detected by IR, UV, and NMR spectroscopic techniques.

Novel metal compounds such as platinum and rhodium have been studied for the past several years.[1-8] These compounds are of particular interest because of their antitumor activity, inhibiting the synthesies of DNA, RNA, and proteins *in vivo*.

In 1974 Erck *et al.*[9] reported that rhodium (II) (isobutyrate)$_4$L$_2$ acted as DNA-dependent, DNA inhibitor, and that it associated with active sites of enzymes such as –NH, –SH and –OH. In 1975 Bear *et al.*[10] reported the results of a study of the effects of rhodium carboxylates on the tumor cell *in vivo*. The binding occured at the two axial positions of rhodium carboxylate to a nitrogen donor in



the ligands. Recently, we reported[11] the result of the association of 6–NH$_2$ of 9–ethyladenine with rhodium (II) (isobutyrate)$_4$L$_2$ in chloroform solution. Many investigators reported[12-23] that some anticancer drugs seemed to work by combining with DNA and thereby distorting its structure and function.

The purpose of our research is to provide a more fundamental mechanism of such drug action with biological substances on molecular level by using spectroscopic techniques such as IR, ^{1}H NMR, UV spectroscopics. We examined the interaction mechanism of one of the components of DNA with rhodium compounds in chloroform solution.

EXPERIMENTAL

Materials

Rhodium (II) (isobutyrate) $_4L_2$ was kindly provide by Dr. I. M. Chang of Natural Products Research Institute, Seoul National University. 9-Ethyladenine (A), 1-cyclohxyluracil (U) and 2′3′-benzylidine-5′-tryl-cystidine (C) were purchased from Cyclo Chemical Co., and 2′3′5′-isobutylic-carbonyl ester guanosine (G) was prepared by the reaction of guanosine with isobutylic anhydride in pyrimidine.

The mixture of anhydrous pyridine (2 ml) and guanosine (1 m Mol) added with isobutylic anhydride (1 ml) was stirred for 48 hours at room temperature. Excessive isobutylic anhydride was removed by successive evaporation. The sample was recrystalized from the chloroform solution. White crystaline powder was obtained (20 mg).

Procedure

For the measurement of IR, ^{1}H NMR and UV spectra, the samples were dissolved in chloroform-d, which was purified by passing alumina column.

Infrared spectra were measured with a Hitachi Model 225 Infrared Spectrometer. A five-millimeter fused quartz cell was used for the measurement in the 3600–3200 cm^{-1} region. The infrared spectra shown in the figures of this paper were given in the absorbance scale, which was calculated from the observed transmission assuming the solvent curve as base line.

^{1}H NMR spectra were recorded on a JEOL Model FX 100 pulse Fouries transform NMR Spectrometer operated at 100 MHz.

About 10 mg to 30 mg of sample were dissolved in solvents for ^{1}H NMR spectrometer. Chemical shift was measured from internal TMS.

UV spectra were measured with a Hitachi Model 124 Spectrometer. A ten-millimeter fused quartz cell was used for the measurement in the visible region.

RESULTS AND DISCUSSION

Infrared Spectra

Interaction with 9-ethyladenine. In the spectrum of the 1 X 10^{-1}M solution of rhodium, a strong band due to the carboxylate anion asymmetrical stretching vibration was observed at 1575 cm^{-1} and a broad band from the H_2O molecule which is ligand, appeared at 3300–3500 cm^{-1} as reported previously.[11]

In order to obtain further information on the structure of Rh-A complex in chloroform solution, the spectra in the 3μ region were studied in detail. As previously discussed, the 0.005 M solution of A showed two strong bands at 3500 and 3400 cm^{-1} which were assignable to the antisymmetric stretching vibrational of the amino group (Figure 1).

When the rhodium solution was mixed in to the A solution, however, the association bands became prominant. The A bands at 3500 and 3400 cm^{-1} were decreased and association bands appeared at 3485 and 3335 cm^{-1} strong. It is difficult to assign each association band of the mixture to one or both components. The spectra of various mixtures

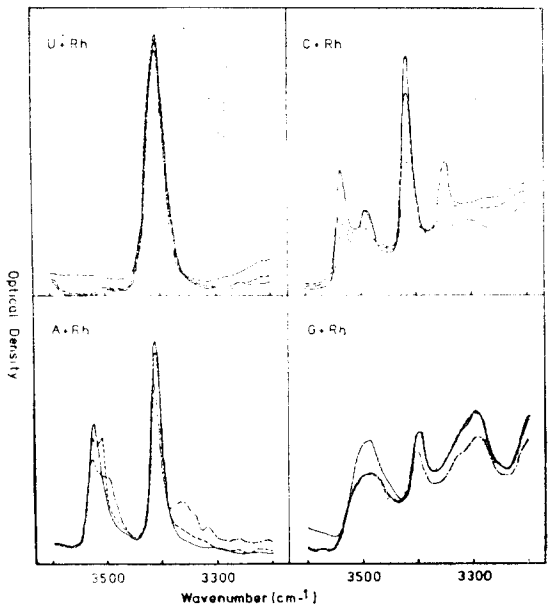


Fig. 1: Infrared spectra of the mixture solution of rhodium carboxylate and one of the A, G, U and C compounds in chloroform. —— : monomer of nucleic acid base derivatives. -·-· : addition of same mol. of Rh solution. ···· : addition of double mol of Rh solution.

of rhodium and A were given in Figure 1. The intensity of the association bands at 3485 and 3330 cm^{-1} were changed as a function of the quantity of rhodium and reached its maxmum bands at 3425 and 3335 cm^{-1} when excess rhodium was added to this mixed solution as discussed previously.[11] These results, nitrogen or hydrogen atom of NH_2 of A was associated with the rhodium carboxylate in the form of chelate.

It was reported[12,13] that the several rhodium carboxylate adducts have been prepared and that the binding occured at the two axial positions of rhodium carboxylate to a nitrogen donor in the ligands.

In 1963, Johoson *et al.*[24] reported that the anhydrous rhodium (II) acetate reacted with various donor-type ligands to form a 1:2 adduct. Rhodium is like to cobalt and behaves like metal group of oxidation number six. Infrared spectra of mixed solution of A showed that bands at 3400 and 3500 cm^{-1} did not disappear but only shifted on the addition of much rhodium solution. Thus, it is suggested that nitrogen atom at 6-NH_2 of A donates two electrons to the rhodium and that in the form of adducts.

Interaction with Other Compounds

Rhodium formed a complex with A strongly. The ligands to conform the adducts of dimeric rhodium carboxylate were various such as previously reported.[24] Nucleic acid base, adenine, guanine, uracil and cytosine, have active sites such as C-N=C, C-NH-C and C-NH_2 groups to associate with rhodium carboxylate in the form of adducts. Whether such interaction still exists with other nucleic acid base G, U and C was examined.

Although association is recognizable from the other spectroscopic data in this paper, the IR spectrum of Rh-U showed almost the same spectrum as the U monomer itself.

Its NH stretching vibrational band at 3390 cm^{-1} decreased negrigibly in intensity on the addition of rhodium to the solution but the association band of mixture appeared at 3330 cm^{-1} (Figure 1 Rh+U).

The remarkable change, however, was observed in the spectra of the mixture with G and C. The symmetric and antisymmetric stretching vibrational bands of the free amino groups of G and C almost changed on the addition rhodium solution. Except for the uracil

derivative, the observed spectra were virtually identical with those of the bases (solid line).

Erck *et al.*[9] have studied the binding of rhodium (II) acetate to several macromolecules such as polyriboadenylate (poly A), polyriboguanylate (poly G) and polyribocytidylate (poly C) using equilibrium dialysis. From the paper[9], rhodium (II) acetate appeared to bind only to poly A and they tentatively concluded that the adenine preferentially interacted with rhodium (II) acetate. In fact, spectroscopic studies of rhodium complexes suggest strongly that the nitrogen atom in imide acts as a donor in the ligands[10,23,24].

The nucleic acid bases such as adenine, guanine, uracil and cytosine have donor group which can act as a ligand to complex formation with rhodium carboxylate, in their heterocyclic rings. Thus, it can be considered that nucleic acid bases such as A, G, U and C are able to bind to rhodium in chloroform solution.

From the data, the selective binding found for the several macromolecules[9] has not been observed in the association of the case of this paper. Although rhodium was found to be able to form strong complex with several base derivatives, the association of rhodium is probably different from each other.

NMR Spectra

Downfield region of ^1H NMR spectra of nucleic acid base derivatives, A, G, U, C and their mixtures of rhodium solutions are shown in Figure 2. As shown in the figure, amides NH and amines NH_2 signals (the assignments of the ^1H proteons are confirmed by previous paper[25] of A, G and U were observed in the

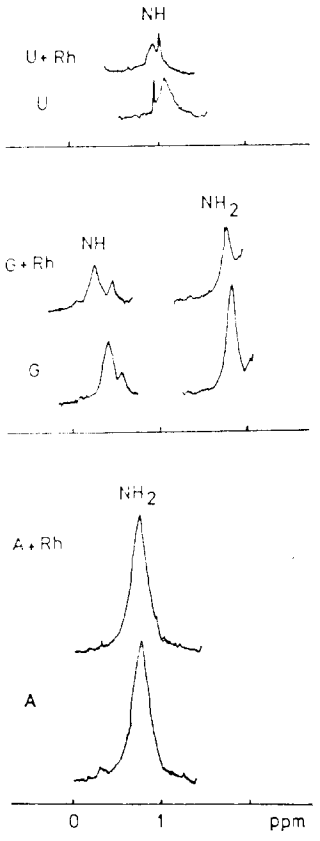


Fig. 2: Magnetic resonance spectra of the NH and NH_2 signals of A, G and U(lower) and mixed with equimolar Rh(higher) in chloroform. Chemical shifts are relative to internal TMS.

spectra as expected. When the small amount of rhodium solution was added to the base derivatives solution, a drastic resonance shift was observed in the spectra.

All of NH and NH_2 signals moved to downfield. These data suggest that the signal shift may be caused by the binding with rhodium so that it has an effect on the proton signals of NH or NH_2 groups in the nucleic acid bases.

Namely, these NMR data supported the results of the IR examination.

UV Spectra

The spectra of 5 X 10^{-4} M solution of rho-

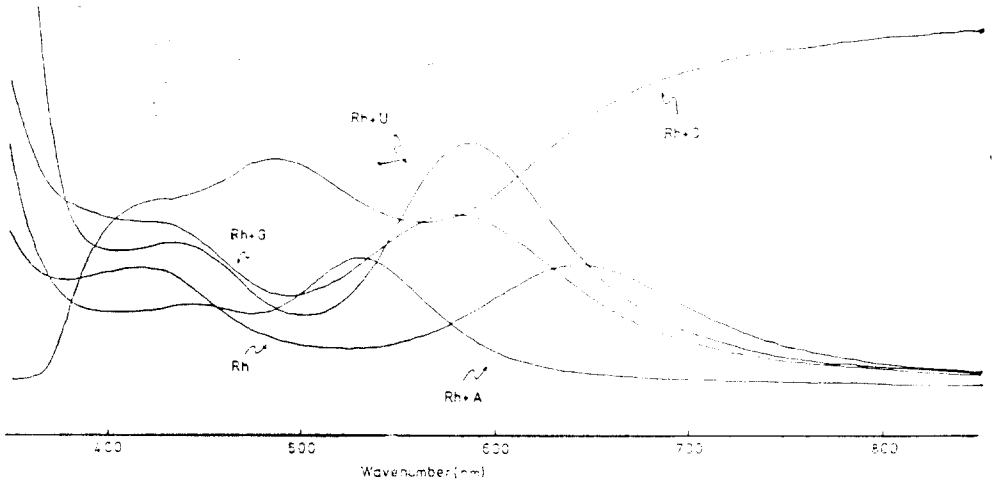


Fig. 3: Ultraviolet and visible spectra of Rh and its mixtures of nucleic acid base derivatives A,G, U and C in chloroform solution. The concentration of the solution is 5×10^{-4} M and the path length is 1 cm.

dium and its mixtures of A, G, U and C in chloroform solution are given in Figure III. Spectra in the same region were recorded for all of the mixture solutions as in case of rhodium solution. The previous paper[24] reported that the rhodium (II) acetate adducts exhibited a wide variation in colors depending on the nature of the ligand. In the spectrum of the rhodium solution, two maximum absorption bands at 420 and 640 nm were observed. When the base derivatives solutions were added to the each solution, a drastic change of absorption spectra occurred and the colors were observed as expected. From the data, however, it cannot be concluded which group is used for the complex formation in the rhodium complexes with nucleic acid bases.

For the mode of complex formation between rhodium and base derivatives, A, G, U and C, the data of these spectroscopic examinations showed that the rhodium carboxylate associated with NH or NH_2 group of bases (from IR and NMR data) and futhermore, the two axial positions of rhodium carboxylate to a nitrogen donor in the ligands (from UV data). Rhodium complex formation with nucleic bases, therfore, might inhibit the synthesis of DNA in tumor cell. These speculations are probably of no physiological importance.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Dr. I. M. Chang, Natural Products Research Institute, Seoul National University for his supplying rhodium(II) (isobutyrate)$_4$ L_2. This work was partly supported by a grant from the Ministry of Education of Korea.

LITERATURE CITED

1) Connors, T. A., Jones, M., Ross, W. C. J., Braddock, B. D., Khokhar, A. R. and Tobe, M. D., New platinum complexes with anti-tumour activity. *Chem-Biol. Interactions* **5**, 415 (1973).
2) Robins, A. B., The reaction of $_{24}$C-labelled plati-

num ethylenediamine dichloride with nucleic acid constituents. *ibid.* 6, 35(1973).

3) Robins, A. B., The reaction of C-labelled platinum ethylenediamine dichloride with adenine compounds and DNA. *ibid.* **7**, 11(1973).

4) Drobmfik, J. and HerAcek, P., Specific biological activity of platinum complexes contribution to the theory of molecular mechanism. *ibid.* **7**, 223(1973).

5) Morris, C. R. and Gale, G. R., Interactions of an antitumor platirum compound with deoxyribonucleic acid, histones, 1-amino acids, poly-1-amino acids, nucleosides and nucleotides. *ibid.* **7**, 305(1973).

6) Giraldi, T., Zassinovich, G. and Mestroni, G., Antitumor action of planar, organometallic rhodium(I) complexes. *ibid.* **9**, 389(1974).

7) Gellert, R. W. and Baw, R., The structure of the [platinum (ethylenediamine) (guanosine)$_2$] cation. *JACS*, **97**, 7379(1975).

8) Onaka, M., The bonding-site of antitumor drug, *cis*-(Pt (en) Cl_2). *Kagakutokogho* **29**, 948(1975).

9) Erck, A., Rainen, L., Whileyman, J., Chang, I. M., Kimball, A. P. and Bear, J., Studies of rhodium(II) carboxylates as potential antitumor. *Proc. Soc. Exptl. Biol. Med.* **145**, 1278(1974).

10) Bear, J. L., Gray, H. B. Jr., Rainen, L., Chang, I. M., Howard, R., Serio, G. and Kimball, A. P., Interaction of rhodium(II)carboxylates with molecules of biologic importance. *Cancer Chemother. Rep.* **59**, 611(1974).

11) Yu, B. S., Choo, S. Y. and Chang, I. M., The association of rhodium(II) (isobutyrate)$_4$ L_2 to an adenine compound. *Yakhak Hoej i* **19**, 215(1975).

12) Ward, D. C., Reich, E. and Goldberger, P. H., Base specificity in the interaction of polynucleotides with antibiotic drugs. *Science* **149**, 1259(1965).

13) Waring, M. J., Drugs which affect the structure and function of DNA. *Nature* **219**, 1320(1969).

14) Waring, M. J. and Makoff, A., Breakdown of pulse-labeled ribonucleic acid and polysomes in *Bacillus megaterium*: Actions of streptolydigin, echinomycin and triostins. *Mol. Pharmacol.* **10**, 214(1974).

15) Waring, M. J. and Wakelin, L. P., Echinomycin: a bifunctional interacalating antibiotic. *Nature* **252**, 633(1974).

16) Sobell, H. M., Jain, S. C., Sakore, T. D. and Nordman, C. E., Stereochemistry of actinomycin-DNA binding. *Nature* **231**, 200(1971).

17) Jain, S. C. and Sobell, H. M., Stereochemistry of actinomycin binding to DNA: 1, Refinement and further structral details of the actinomycin-deoxyguanosine crystalline complex. *J. Mol. Biol.* **68**, 1(1972).

18) Patel, D. J., Peptide antibiotic-dinucleotide interactions. Neclear magnetic resonance investigations of complex formation between actinomycin D and d-pGpC in aqueous solution. *Biochemistry* **13**, 2388(1974).

19) Krugh, T. R. and Neely, J. W., Actinomycin D-deoxydinucleotide interactions as a model for binding of the drug to deoxyribonucleic acid. Proton magnetic resonance results. *ibid.* **12**, 4418(1973)

20) Patel, D. J., Peptide antibiotic-nucleotide interactions. Nuclear magnetic resonance investigations of complex formation between actinomycin D and deoxyguanosine 5'-monophosphate in aqueous solution. *ibid.* **13**, 1476(1974).

21) Patel, D, J., Peptide antibiotic-oligonucleotide interactions. Nuclear magnetic resonance investigations of complex formation between actinomycin D and d-Ap Tp Gp Cp ApT in aqueous solution. *ibid.* **13**, 2396(1974).

22) Krugh, T. R. and Chen, Y. C., Actinomycin D-deoxynucleotide complexes as models for the actinomycin D-DNA complex. The use of nuclear magnetic resonance to determine the stoichiometry and the geometry of the complexes. *ibid.* **14**, 4912 (1975).

23) Kitchens, J. and Bear, J. D., A study of some rhodium(II) acetate adducts. *Inorg. Nucl. Chem.* **31**, 2415(1969).

24) Johonson, S. M., Hunt, H. R. and Neumann, H M., Preparation and properties of anhydrous rhodium(II) acetate and some adducts there of. *Inorg. Chem.* **2**, 960(1963).