nize" both residues forming the bond. Moreover, when different peptides containing the same terminal bonds are compared, large differences in reactivity are observed. Thus, with LAP, Ala_4 (LDLL) is not digested under our conditions, Ala_3 (LDL) is digested in trace amounts only, and Ala_4 (LDDL) is digested at an appreciable rate. Yet all three peptides contain the N-terminal LD-type bond. Additional examples of this kind can be found in Table III. This is an indication that not only the nature of the bond hydrolyzed determines its susceptibility to cleavage, but that residues further removed also exhibit an influence on the rate of enzymatic attack. Similar findings with respect to the action of CP-A on LL bonds have been described in a preliminary communication (Schechter *et al.*, 1965). It was shown by comparing kinetic rate constants that the enzyme "recognizes" at least four residues in the substrate. This was deduced from the observation that the C-terminal bonds of the three tetrapeptides LLLL, DLLL, and LDLL are split at relative rates of about 100:10:1. Other proteolytic enzymes seem to behave in a similar manner. Papain was shown to interact simultaneously with five residues in the substrate (Schechter *et al.*, 1965). Results obtained recently in this laboratory indicate that trypsin and aspergillopeptidase B (Subramanian and Kalnitsky, 1964) recognize a sequence of five and seven residues, respectively, in a peptide chain.

Acknowledgement

The authors wish to thank Miss Gila Ashkenazi and Miss Etti Dascal for expert technical assistance.

References

Neurath, H. (1960), *Enzymes 4*, 11.
Schechter, I., Abramowitz, N., and Berger, A. (1965), *Israel J. Chem. 3, 101.*
Schechter, I., and Berger, A. (1965), *Israel J. Chem. 3*, 98.
Schechter, I., and Berger, A. (1966), *Biochemistry 5*, 3362 (this issue; preceding paper).
Smith, E. L., and Hill, R. L. (1960), *The Enzymes 4*, 37.
Subramanian, A. R., and Kalnitsky, G. (1964), *Biochemistry 3*, 1861.

The Interaction of Silver Ion with Guanosine, Guanosine Monophosphate, and Related Compounds. Determination of Possible Sites of Complexing*

Anthony T. Tu and José A. Reinosa†

ABSTRACT: Complex formation of guanosine, guanosine 3′(2′)-monophosphate, inosine, inosine 5′-monophosphate, theophylline, caffeine, uridine, uridine 3′(2′)-monophosphate, and ribose with silver ion at neutral pH was studied. It was found that guanosine, guanosine 3′(2′)-monophosphate, inosine, inosine 5′-monophosphate, and theophylline combined with silver ion in equimolar ratio. An equivalent amount of hydrogen ion was released from each compound on complexing. There was considerable hypochromicity at the wavelength of maximum ultraviolet absorption on complexing.

Little change in the ultraviolet spectra was observed for the compounds that did not combine with silver. The infrared absorption spectra indicated that the carbonyl stretching bands disappeared after complexing with the exception of theophylline. Possible sites for silver in the complexes are proposed.

The ability of nucleosides (Edelman *et al.*, 1960; Eichhorn *et al.*, 1966; Ropars and Viovy, 1965a), nucleotides (Eichhorn and Clark, 1965; Rich, 1959), and nucleic acids (Trim, 1959; Yamane and Davidson, 1962; Fuse *et al.*, 1965; Coates *et al.*, 1965) to complex with certain metals has been reported by various investigators. Occurrence of some metals in nucleic acids (Jungner, 1951; Holden and Pirie, 1955; Wacker and Vallee, 1959; Tu, 1961) and tobacco mosaic virus ribonucleic acid (TMV-RNA) (Loring *et al.*, 1958) and viruses (Hoagland *et al.*, 1941; Racker and Krimisky, 1945; Loring *et al.*, 1959) has also been reported. The significance of these trace metals present in such important biological macromolecules cannot be fully understood unless the nature of the metal binding with the nucleotide unit is clarified. Most of the metal–nucleic acid complexes are insoluble materials. Trim (1959) used this property as a means for isolating

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polynucleotides from plant tissue. However, the silver–nucleic acid complex gives relatively soluble products (Shimizu, 1957). Singer and Fraenkel-Conrat (1962) observed that a Ag–RNA complex of TMV origin showed resistance to ribonuclease digestion, and that no significant loss of infectivity was detected. Yamane and Davidson (1962) concluded that the site for silver in the Ag–DNA complex involved the deoxyribonucleic acid (DNA) bases. Reinosa and Tu (1964) also concluded that the complex formation cannot be attributed to the phosphate group in the Ag–RNA complex, and suggested that the RNA bases are involved.

Ropars and Viovy (1965b) suggested that the guanine base was involved in the Cu–DNA interaction. Yamane and Davidson (1960) were unable to propose a structure for the DNA–Hg complex; however, they suggested that the guanine base might be involved. They excluded the possibility of the phosphate group as the site of complexing.

In order to investigate this problem, guanosine and guanosine 3′(2′)-monophosphate, as well as the compounds structurally similar to these, inosine and inosine 5′-monophosphate, were studied. For further elucidation of the site of complexing, uridine and uridine 3′(2′)-monophosphate, theophylline, caffeine, and ribose were also examined.

Experimental Sections

Materials. Guanosine, GMP, and IMP were obtained from the California Foundation for Biochemical Research, and inosine from the Sigma Chemical Corp. Caffeine was purchased from Chemical Commerce, and theophylline was a product of the Nutritional Biochemicals Corp. Silver nitrate was CP grade crystals from Merck and Co. All the other chemicals were reagent grade.

Chemical Titrations. The method of Shimizu (1956), originally used for Ag–RNA complex studies, was used for the Ag–nucleotide and Ag–nucleoside complexes. The pH of the RNA solution was first adjusted to exactly pH 7.00 with NaOH using a Radiometer, type TTT1, automatic titrator. A measured amount of 0.01 N $AgNO_3$ varying between 10 and 50 μmoles (a) was mixed with a known amount of the sample (2–10 μmoles in 20–50 ml). The uncomplexed silver ion was removed with excess of 0.01 N NaCl varying between 10 and 50 μmoles. (b) The chloride ion was then back titrated with 0.01 N $AgNO_3$ (c) using K_2CrO_4 as an indicator. All titrations were made with a microburet, Radiometer autoburet, type ABU 1a. The amount of silver bound to the samples was calculated from the difference between total amount of silver (a and c) and the amount of chloride ion used (b).

Conductometric Titration. Complex formation between Ag^+ and GMP and IMP was also established and followed by conductometric titration. A weighed amount of nucleotide, 100–200 μmoles, was dissolved in deionized water, and the pH was adjusted to 7.0 with NaOH. The volume was brought to 50 ml. The solution was titrated with 0.01 N $AgNO_3$, 1 ml at a time at 25°. A blank using the same volume of water without nucleotide was also titrated with 0.01 N $AgNO_3$ under the same conditions. These measurements were corrected for volume change and plotted against the amount of silver ion added. A radiometer conductivity meter, type CDM2, with a double platinum plate electrode was used for these measurements.

Hydrogen Ion Release. To determine the hydrogen ion release, 100 μmoles of each sample was dissolved in water and the pH adjusted to 7.0 using the automatic pH titrator and autoburet, type ABU 1a. An agar–agar salt bridge containing 0.5 N KNO_3 between saturated KCl calomel electrode and the solution being titrated was used. The volume was brought to 50 ml, and 300 μmoles of silver nitrate was added. The mixture was allowed to stand for 20 min. The hydrogen ion released was titrated with 0.01 N NaOH to the original pH of 7.0.

Ultraviolet Absorption Spectra. The absorption spectra of the nucleosides, nucleotides, and related compounds and their silver complexes were measured in a Cary 15 recording spectrophotometer. Both phosphate and borate ions combine with silver ion to form insoluble silver salts. Therefore, neither phosphate nor borate buffer was used. Each sample was dissolved in deionized water and the pH of the solution adjusted to 7.00 with NaOH using the pH stat Radiometer titrator, type TTT1. The final concentration of each sample was adjusted to 0.04 μmole/ml. To form the silver complex, each sample was mixed with an equimolar amount of silver ion. Since the pH dropped after complexing for the absorption study, the pH was readjusted to 7.00 with NaOH using the titrator, and the volume was then adjusted to make the final concentration of sample 0.04 μmole/ml.

Infrared Absorption Spectra. Infrared absorption measurements were carried out in a Beckman Model IR-8, double-beam recording spectrophotometer, in the region from 4000 to 625 cm^{-1}. All spectra were determined using potassium bromide pellets. For the experiment with the Ag complexes, the nucleoside or nucleotide was mixed with silver ion, and the excess silver was removed by dialysis at 3°. Water was removed under reduced pressure, and the solid Ag–nucleoside or Ag–nucleotide complex was pulverized and thoroughly mixed with potassium bromide. A thin pellet was compressed for the infrared absorption studies.

A mull of sample suspended in mineral oil and smeared on a sodium chloride window was also used for obtaining infrared spectra. The results were no different from those secured with the potassium bromide disks. Therefore, KBr only was used as diluent for our study.

[1] Abbreviations used: GMP, guanosine 3′(2′)-monophosphate; IMP, inosine 5′-monophosphate; UMP, uridine 3′-(2′)-monophosphate.

Results

Stoichiometric Relation. The number of moles of silver ion bound to guanosine and GMP, as determined by the chemical method, was found to be in unimolar ratio (Table I). Inosine and IMP have similar structures

TABLE I: Mole Ratios of Silver to Guanosine, GMP, Inosine, IMP, Theophylline, Caffeine, Uridine, UMP, and Ribose Determined by Chemical Methods.

Sample	Ag/Sample (mole/mole) Mean ±95% Confidence Limit	Nearest Integer for Ag/ Sample	No. of Determinations
Guanosine	1.010 ± 0.036	1	14
GMP	0.958 ± 0.099	1	7
Inosine	0.968 ± 0.070	1	7
IMP	0.976 ± 0.014	1	7
Theophylline	0.988 ± 0.075	1	6
Caffeine	0.066 ± 0.002	0	5
Uridine	0.040 ± 0.060	0	5
UMP	0.055 ± 0.087	0	5
Ribose	0.005 ± 0.011	0	4

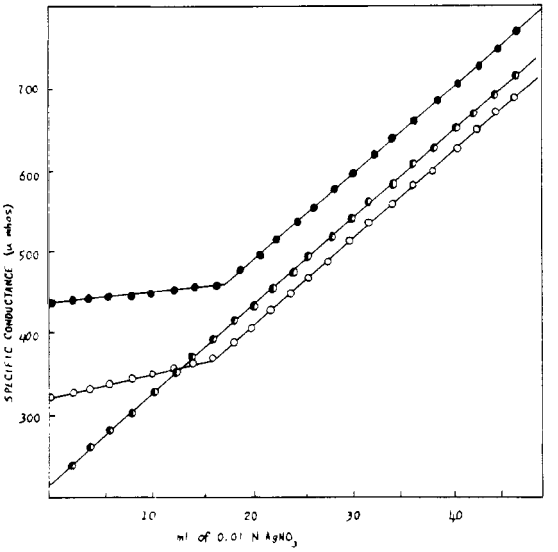


FIGURE 1: Conductometric titration of GMP and IMP with Ag^+. Each sample containing 145 μmoles of GMP and 160 μmoles of IMP in 50 ml of water at pH 7.0 was titrated with 0.01 N $AgNO_3$, 1 ml at a time. However, in this figure, corrected specific conductivities were plotted for every 2 ml. —●—●—, GMP; —○—○—, IMP; —◐—◐—, water blank at pH 7.

to those of guanosine and GMP, except there is no amino group at position C-2. Both inosine and IMP bind to 1 mole of silver ion. Theophylline also gave a unimolar ratio with silver ion. However, caffeine, uridine, UMP, and ribose did not bind with silver ion at all. The stoichiometric relation of silver ion to GMP and IMP was also determined by conductometric titration (Figure 1).

Both GMP and IMP gave a sharp deflection in the titration curves. Guanosine and inosine did not give clear deflection points, and therefore the stoichiometric relation could not be determined by the conductometric method. The results obtained for GMP and IMP by this method also confirmed the equimolar ratio of silver ion to GMP and IMP. Six conductometric titrations were made with GMP, and the number of moles of silver bound to GMP was calculated; the value was 1.15 with 95% confidence limit of 0.10. For IMP the mean with 95% confidence limit was 1.18 ± 0.20 based on five experiments. The study of hydrogen ion release after complexing also indicates a unimolar stoichiometric relationship of guanosine, GMP, inosine, IMP, and theophylline with silver ion.

For this study, 100 μmoles of each sample was mixed with silver ion, and the proton released was titrated to the original pH with standard NaOH. For guanosine and GMP, 103 and 115 μmoles of NaOH, respectively, were used. For inosine and IMP the results were also comparable and 111 and 95 μmoles of NaOH were required for the neutralization of the protons released. Theophylline also required a similar amount of NaOH, namely, 108 μmoles. However, practically no NaOH was required to bring the pH back to 7.0 in the case of caffeine, uridine, UMP, ribose, and a silver nitrate blank. It is then apparent that one hydrogen atom was replaced by a silver ion in guanosine, GMP, inosine, IMP, and theophylline.

Ultraviolet Absorption Spectra Studies. In general, hypochromicity at the wavelength of maximum absorption occurred in guanosine, GMP, inosine, IMP, and theophylline after complexing with silver ion. The change in the ultraviolet absorption spectra of guanosine, GMP, inosine, IMP, and theophylline after the addition of silver ion can be seen in Figure 2.

The compounds that did not combine with silver ion, such as caffeine, gave very little change in the absorption spectra. The results agree well with the conclusion drawn from the studies by chemical titration, conductometric titration, and hydrogen release.

Infrared Absorption Spectra Studies. Blout and Fields (1950) studied the infrared spectra of a number of purine bases, and found that guanine, hypoxanthine, xanthine, theophylline, theobromine, and caffeine showed an intense band in the region of 1680–1720 cm^{-1}. They assigned this band to the C=O stretching vibration. Other bands at 1610 and 1555 cm^{-1} were assigned to C=C and C=N stretching modes in the purine ring system.

The infrared spectra of guanosine, GMP, inosine, IMP, and theophylline were studied before and after

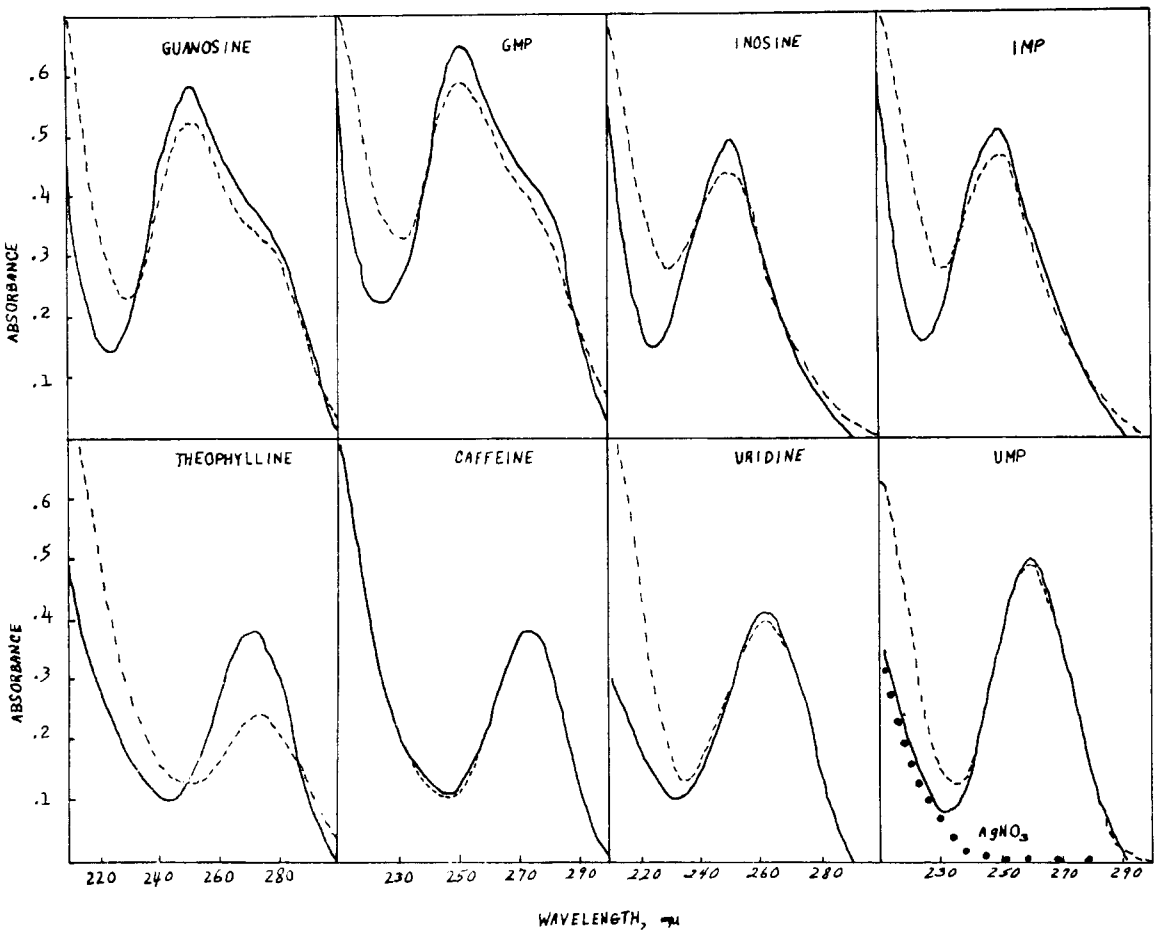


FIGURE 2: Ultraviolet absorption spectra of guanosine, GMP, inosine, IMP, theophylline, caffeine, uridine, and UMP, in the presence of and absence of $AgNO_3$, at pH 7.0. The concentration of each sample was 4×10^{-5} M. The silver concentration also was 4×10^{-5} M. ———, sample without silver; ------, sample plus silver; ••••, silver nitrate alone.

complexing with silver ion. In guanosine three major peaks at 1725 (a), 1680 (b), and 1630 cm^{-1} (c) were observed (Figure 3). The prominent band at 1590 (c) with two small shoulders at 1720 (a) and 1670 cm^{-1} (b) corresponding to the two original bands remained in the Ag-guanosine complex. The band at 1590 cm^{-1} which may correspond to C=C or C=N stretching vibrations remained unchanged. In GMP two distinct bands in the region corresponding to C=O, C=N, and C=C bond stretching were also observed. They were located at 1690 (a) and 1590 cm^{-1} (b). On complexing silver ion with GMP, the first band disappeared giving only small shoulders corresponding to the wave numbers of the original. A distinct peak at 1610 (b) was observed after complexing which showed a shift from the original position of 1590 cm^{-1} which probably is due to C=C or C=N stretching of the purine ring.

In inosine, the major peak in the region of interest was at 1690 (a) and the second distinct peak at 1590 cm^{-1} (b) (Figure 3). On complexing with silver ion, the first major peak (a) disappeared almost completely, and the prominent peak (b) at 1590 cm^{-1} became very intense. Again a band corresponding to C=O stretching (1690 cm^{-1}) disappeared, and a band due to C=C or C=N stretching of the purine ring (1590 cm^{-1}) remained the same.

Similar results were also obtained for IMP and Ag–IMP. In IMP without silver, the major peak was at 1680 (a) with a smaller peak at 1590 cm^{-1}. After silver ion was attached to IMP, the original major peak almost disappeared giving only a trace of peak at 1680 (a), and a new major peak (b) was formed at 1615 cm^{-1}.

In theophylline the change in the spectra after complexing was different from that in the compounds mentioned above. In the 1600–1800-cm^{-1} region there were two distinct peaks, one at 1720 (a), and one at 1680 cm^{-1}. These main bands shifted 30 cm^{-1}, to 1690 and 1650 cm^{-1}, while they maintained similar patterns. Unlike guanosine, GMP, inosine, and IMP, theophylline showed a considerable change in absorp-

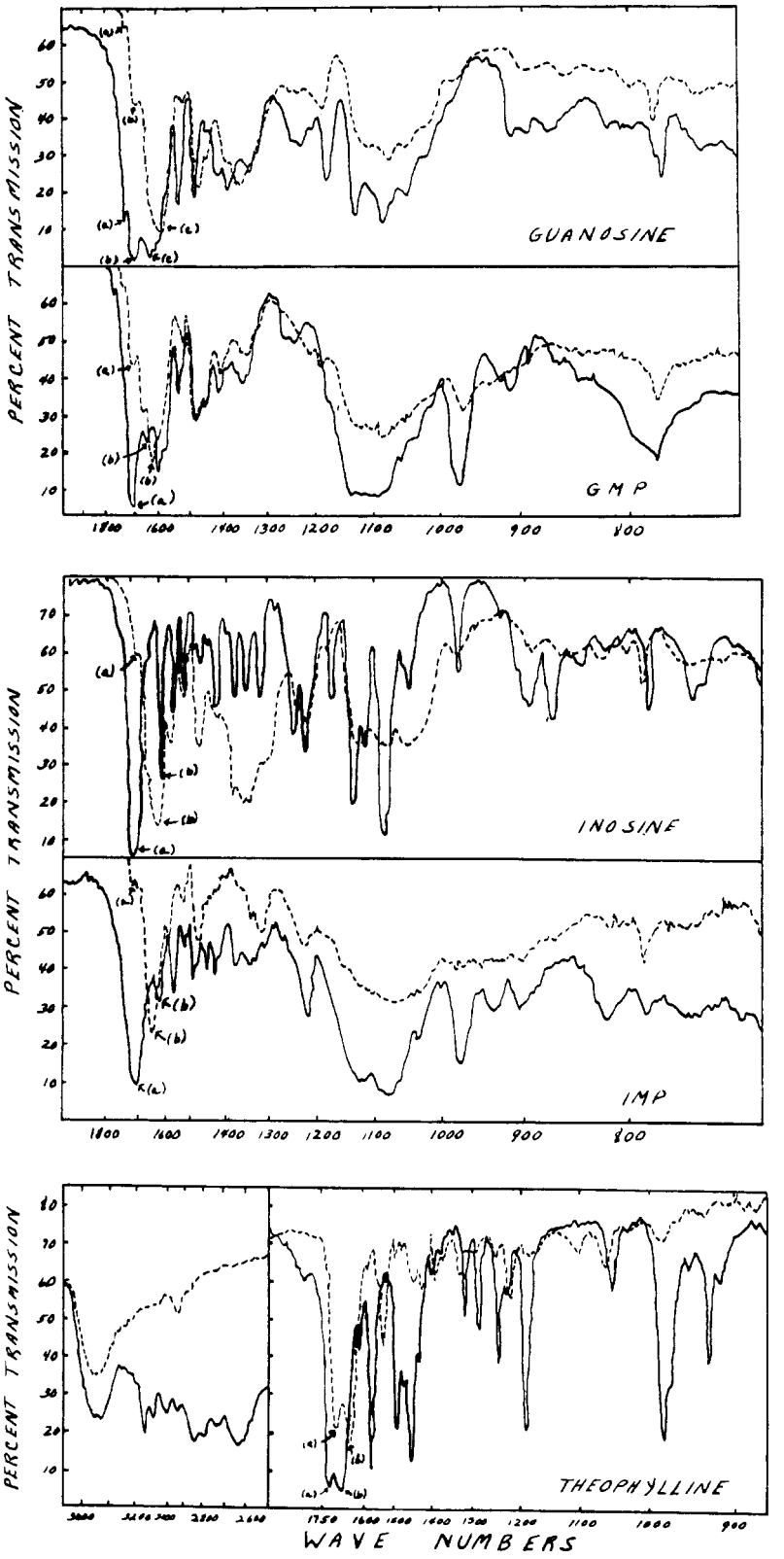
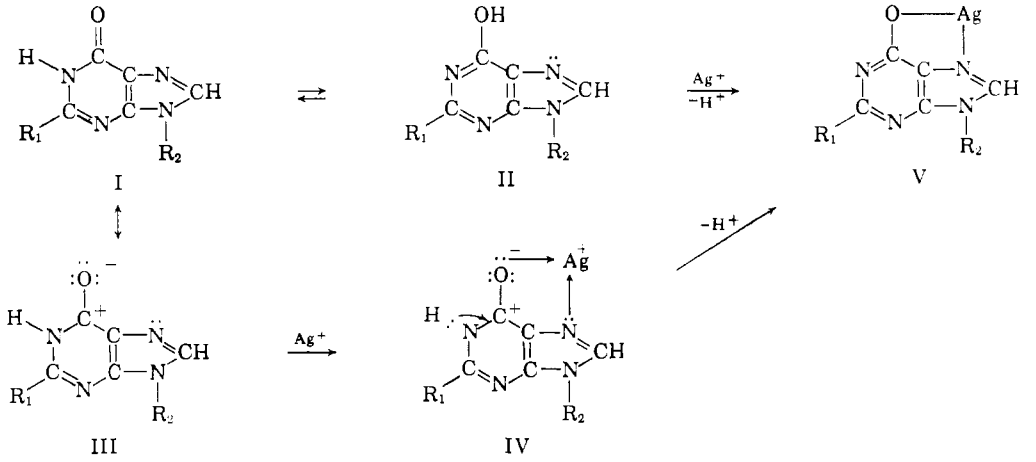


FIGURE 3: Infrared absorption spectra of guanosine, GMP, inosine, IMP, theophylline, before (———) and after (----) interacting with silver ion. Uncombined $AgNO_3$ was removed by dialysis so that no nitrate band appeared.

SCHEME I: Proposed Mechanism for the Formation of Ag–Guanosine, Ag–GMP, Ag–Inosine, and Ag–IMP Complexes[a]



[a] For guanosine, $R_1 = NH_2$, R_2 = ribosyl group; for GMP, $R_1 = NH_2$, R_2 = ribosyl 3′(2′) phosphate; for inosine, $R_1 = H$, R_2 = ribosyl group; for IMP, $R_1 = H$, R_2 = ribosyl 5′-phosphate.

tion in the region of 2500–3200 cm^{-1}. This region comprises the free NH bond stretching and the hydrogen-bonded NH stretching. On complexing with silver ion the broad band (2500–3200 cm^{-1}) in theophylline largely disappeared.

Discussion

From the unimolar stoichiometric relation of silver ion to guanosine, GMP, inosine, IMP, and theophylline, silver ion appears to attach to some functional group common to all these compounds. The unimolar relationship was further confirmed by the fact that 1 mole of hydrogen ion was released from each mole of these compounds (see Scheme I).

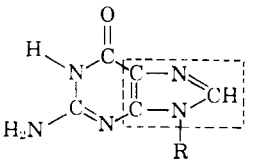
The formation of a complex was further proved by the significant changes in the ultraviolet absorption spectra of these compounds. Since the ultraviolet absorption of the nucleosides and nucleotides examined are due to the purine bases, the change in the absorption spectra strongly suggests that the site for silver in the complex is very likely on the purine base. This change in the ultraviolet spectra in the case of guanosine, GMP, inosine, IMP, and theophylline might be interpreted as the result of interaction of the silver atom with the purine ring at the site of N-7 and thus interrupting the original π-orbital system of the ring. The compounds that did not interact with silver ion showed absorption spectra with no hypochromicity at the wavelength of maximum absorption (Figure 2). Ribose as a possible site was eliminated since ribose itself did not combine with silver ion (Table I).

The phosphate moiety as a feasible site was also eliminated because guanosine and inosine which do not contain any phosphate group also formed a complex compound with silver ion. In each case where complexing occurred between silver ion with GMP and IMP, a stoichiometric amount of hydrogen was released.

The hydrogen atom cannot be derived from the primary or secondary hydrogen of the phosphate group. In our experiments the pH of the original GMP and IMP solutions was fixed to 7.0 where both the primary and secondary phosphate groups are already ionized. Moreover, guanosine, inosine, and theophylline which do not have a phosphate group also released hydrogen ion on complexing.

The structures of guanosine and inosine are very similar; they differ only in the groups at C-2 (see Scheme II). But since both of these nucleosides complex with silver, the probable site for complexing would likely be in a group other than that at C-2.

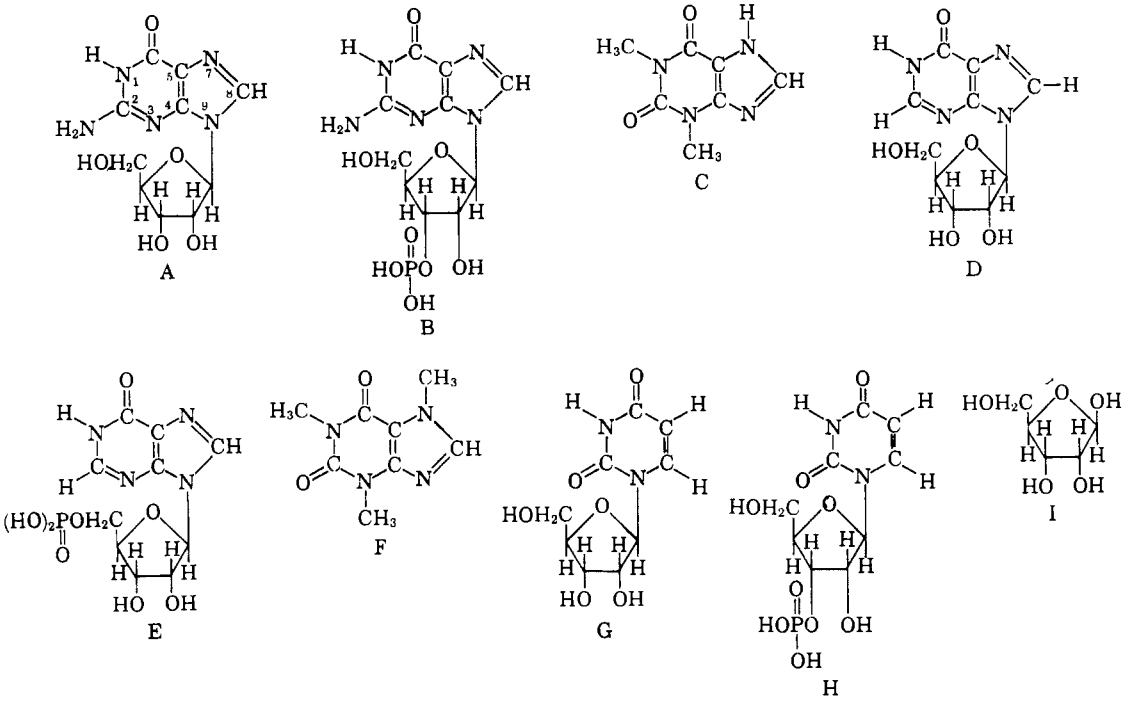
Uridine did not complex with silver ion. This suggests that the five-membered portion of the purine base (see dotted structure below) might be somehow involved in the complexing process.



The possibility of N-9 being the site for argentation was ruled out as this atom is connected to ribose in both nucleosides and nucleotides. In caffeine the positions N-1, N-3, and N-7 are bound to methyl groups. The carbonyl group at C-6, which corresponds to the carbonyl group in guanosine, GMP, inosine, IMP, and theophylline, is retained. However, caffeine did not interact with silver ion. The only difference in structure between theophylline and caffeine is the group at N-7. From these facts it is likely that the silver atom attaches to N-7 in guanosine, GMP, inosine, IMP, and theophylline.

Although guanosine, GMP, inosine, IMP, and theophylline formed a silver complex, the structure of Ag–

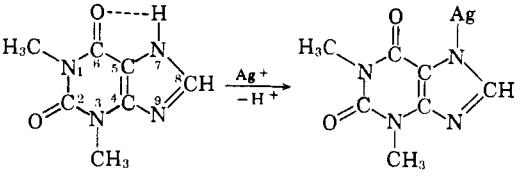
SCHEME II: Structural Formulas for the Compounds Investigated.[a]



[a] A, guanosine; B, GMP; C, theophylline; D, inosine; E, IMP; F, caffeine; G, uridine; H, UMP; I, ribose.

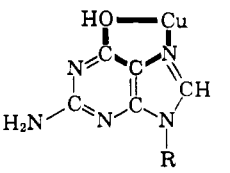
theophylline is probably different from that of the others. In the case of guanosine, GMP, inosine, and IMP, the hydrogen atom replaced was probably derived from the enolic hydroxyl at C-6 or from the hydrogen at N-1 (Scheme I). The silver atom may bridge between the oxygen atom at C-6 and N-7. This could be explained from the results of the infrared studies. It is well known that most of the C=O stretching appears in the region of 1600–1800 cm^{-1}. According to Blout and Fields (1950) and our results, the carbonyl stretching in guanosine, hypoxanthine, xanthine, caffeine, and theophylline showed distinct bands at 1680–1720 cm^{-1}. Distinct absorptions at 1685 cm^{-1} for ITP and IDP, and 1680 cm^{-1} for IMP, were attributed to C=O stretching by Epp *et al.* (1958). In the case of guanosine, GMP, inosine, and IMP, a band corresponding to the carbonyl stretching disappeared or shifted on complexing with silver. Although theophylline combined with silver, no such diminution effect was observed for this band. This indicates that the carbonyl group in theophylline was relatively intact after interaction. The hydrogen atom replaced could not be derived from the enol group at C-6 as there is no tautomeric form for theophylline due to the methyl group occupying N-10. Therefore, the proton released was very likely the hydrogen atom at N-7. Therefore, the mechanism below is proposed.

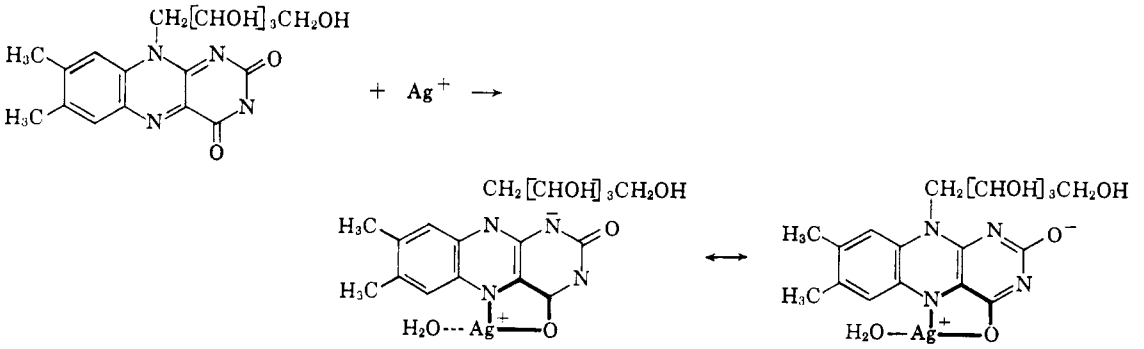
This view can be further supported by evidence also obtained in the infrared study (Figure 3). In free theophylline the oxygen atom at C-6 was believed to be hydrogen bonded to the imino hydrogen at N-7.



On combining with silver, the hydrogen bond is broken as shown in the above structure. The change in the infrared spectra after addition of silver ion to theophylline in the region of 2300–3000 cm^{-1} seems to reflect this mechanism. In contrast to theophylline, there were no such changes in the region of 2300–3000 cm^{-1} for guanosine, GMP, inosine, and IMP after the addition of silver ion. From the evidence presented above, it appears logical to propose the location of the silver atom in the guanosine, GMP, inosine, and IMP silver complexes as shown in Scheme I (V).

It is of interest that the metal complex tends to form a five-membered ring. This is the case with guanosine, GMP, inosine, and IMP. This type of pentacyclic structure involving a metal atom is not uncommon. For instance Frieden and Alles (1957) and Ropars and Viovy (1965b) proposed the structure



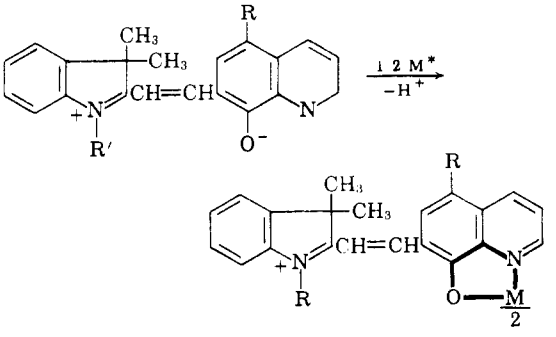


for Cu–guanosine and Cu–GMP complexes. A five-membered-ring containing metal, oxygen, nitrogen, and two carbon atoms is represented by heavy lines in the structure. In the work of these investigators hydrogen ion release was not studied. This probably is the reason they include the enolic hydrogen in the proposed structure. The importance of position N-7 in cupric ion complex formation has also been noted by Giri and Rao (1946).

Riboflavin complexes with silver at pH 7 (Hemmerich and Bamberg, 1961), and the reaction shown above was proposed to account for this.

Spence and Peterson (1962) agreed to this proposed structure from evidence obtained by infrared spectral studies. They observed a severe diminution of the 1645-cm^{-1} absorption. This was explained as resulting from the disappearance of the 4-carbonyl group in riboflavin, on complexing with silver. Again the relationship of oxygen, nitrogen, and silver atoms is quite similar to our system.

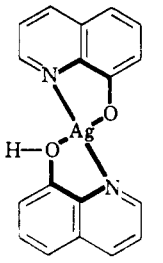
Another similar case was also reported by Phillips (1965) who proposed the structure of a metal–spiropyran complex. The metals used were not specified in their



report. Again the relative position of the nitrogen, oxygen, and metal atoms is the same as in our system.

Hála (1965) recently studying 8-hydroxyquiniline with silver ion suggested structure VI.

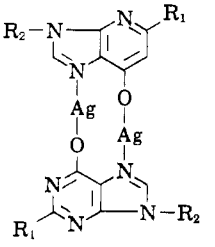
From the evidence presented above, it appears logical to propose the location of the silver atom in guanosine, GMP, inosine, and IMP silver complexes as shown in Scheme I (V). The mechanism for the complex formation takes place either through exchange of a hydrogen



VI

atom of the enol form with a silver atom, or by the mechanism illustrated in Scheme I (III and V).

Although it is rather logical to propose the pentacyclic structure involving silver ion as mentioned before, the evidence for chelation can be equally interpreted by assuming that silver atoms are bound to the oxygen atoms at C-6 and N-7 of another molecule.



for guanosine, $R_1 = NH_2$, R_2 = ribosyl group
for GMP, $R_1 = NH_2$; R_2 = ribosyl 3′(2′) phosphate
for inosine, $R_1 = H$, R_2 = ribosyl group
for IMP, $R_1 = H_1$, R_2 = ribosyl 5′-phosphate

In this alternative structure, two silver atoms between two nucleosides or nucleotides would still give the unimolar ratio. It is also interesting to note that the coordination bonds of silver would be collinear in this structure. For the theophylline–Ag complex, the proposed structure is with the silver atom attached at N-7 as shown previously.

Acknowledgment

We wish to thank Dr. R. Redington for a critical review and suggestions on the infrared spectral studies. Our thanks are also extended to Dr. J. T. Spence for

reading the manuscript. One author (J. A. R.) is grateful to the Pan-American Union for financial aid.

References

Blout, E. R., and Fields, M. (1950) *J. Am. Chem. Soc. 72*, 479.
Coates, J. H., Jordan, D. O., and Srivastava, V. K. (1965), *Biochem. Biophys. Res. Commun. 20*, 611.
Edelman, I. S., Ts'o, P. O. P., and Vinograd, J. (1960), *Biochim. Biophys. Acta 43*, 393.
Eichhorn, G., and Clark, P. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 586.
Eichhorn, G., Clark, P., and Becker, E. D. (1966), *Biochemistry 5*, 245.
Epp, A., Ramasarma, T., and Wetter, L. R. (1958), *J. Am. Chem. Soc. 80*, 724.
Frieden, E., and Alles, J. (1957), *J. Biol. Chem. 230*, 797.
Fuse, Y., Miyaki, K., and Tsuboi, M. (1965), *Bull. Chem. Soc. Japan 38* (in press).
Hála, J. (1965), *J. Inorg. Nucl. Chem. 27*, 2659.
Hemmerich, P., and Bamberg, P. (1961), *Helv. Chim. Acta 44*, 1001.
Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. (1941), *J. Exptl. Med. 74*, 69.
Holden, M., and Pirie, N. W. (1955), *Biochem. J. 60*, 46.
Jungner, G. (1951), *Science 113*, 378.
Loring, H. S., Al-Rawi, S., and Fujimoto, Y. (1958), *J. Biol. Chem. 233*, 1415.
Loring, H. S., Fujimoto, Y., and Eng, L. F. (1959), *Proc. Natl. Acad. Sci. U. S. 45*, 287.
Phillips, I. P., Müller, A., and Przystal, F. (1965), *J. Am. Chem. Soc. 87*, 17.
Racker, E., and Krimsky, I. (1945), *J. Exptl. Med. 85*, 715.
Reinosa, J. A., and Tu, A. T. (1964), *6th Intern. Congr. Biochem.*, Abstr. No. 164.
Rich, A. (1959), *Rev. Mod. Phys. 31*, 191.
Ropars, C., and Viovy, R. (1965a), *J. Chim. Phys. 62*, 408.
Ropars, C., and Viovy, R. (1965b), *Compt. Rend. 261*, 1129.
Shimizu, R. (1956), *J. Pharm. Soc. Japan 76*, 158.
Shimizu, R. (1957), *J. Pharm. Soc. Japan 77*, 676.
Singer, B., and Fraenkel-Conrat, H. (1962), *Biochemistry 1*, 852.
Spence, J. T., and Peterson, E. R. (1962), *J. Inorg. Nucl. Chem. 24*, 601.
Trim, A. R. (1959), *Biochem. J. 73*, 298.
Tu, A. T. (1961), Ph.D. Dissertation, Stanford University, Stanford, Calif.
Wacker, E. C., and Vallee, B. L. (1959), *J. Biol. Chem. 234*, 3257.
Yamane, T., and Davidson, N. (1960), *J. Am. Chem. Soc. 83*, 2599.
Yamane, T., and Davidson, N. (1962), *Biochim. Biophys. Acta 55*, 609.

CORRECTION

In the paper "Antibiotics as Tools for Metabolic Studies. V. Effect of Nonactin, Monactin, Dinactin, and Trinactin on Oxidative Phosphorylation and Adenosine Triphosphatase Induction," by Stanley N. Graven, Henry A. Lardy, Diane Johnson, and Anthony Rutter, Volume 5, June 1966, p 1729, the following corrections should be made: in Figure 2, the concentration of pyruvate is 20 μmoles/3 ml; in Figure 3, the concentrations are all millimolar and not millimoles; in Table I, the concentrations are all micromoles and not millimoles; in Table II, the concentrations are all millimolar and not millimoles.