sion-controlled reactions with H_3O^+. Additional work is needed before a decision can be made concerning alternate mechanisms A and B. The thermodynamic and kinetic behavior of the nickel–triglycine complexes also differ from those of the copper–triglycine complexes because of the relative instability of $NiH_{-1}L$.

Acknowledgment. Helpful discussions with Professors M. Eigen and F. A. Long are gratefully acknowledged. This investigation was supported by National Science Foundation Grant No. GP6725X, and by Public Health Service Research Grant No. GM-12152 from the National Institute of General Medical Sciences.

Chelation of Uranyl Ions by Adenine Nucleotides. IV. Nuclear Magnetic Resonance Investigations, Hydrogen-1 and Phosphorus-31, of the Uranyl–Adenosine 5′-Diphosphate and Uranyl–Adenosine 5′-Triphosphate Systems[1]

Kenneth E. Rich, Raghunath T. Agarwal,[2] and Isaac Feldman[3]

Contribution from the Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620. *Received April 4, 1970*

Abstract: Nmr spectra, ^1H (100 MHz) and ^{31}P (40 MHz), were obtained for uranyl nitrate–adenosine diphosphate (U–ADP) and –adenosine triphosphate (U–ATP) mixtures in D_2O having various stoichiometries and basicities over the pD range ≈7–11. The spectra show that from pD 7.7 to 11 an equimolar U–ATP mixture consists of 2:2 sandwich-type (ST) dimeric chelates, **2**, in which the ligands are the β and γ phosphoryl groups and the ribose hydroxyls, but from pD 6.8 to 7.3 non-ST chelates, in which the uranium is bound only to the β and γ phosphoryl groups, predominate. About 10% of the complex is in the ST form at pD 7.3 in 1:1, 0.05 *M* U–ATP solution, but none is present in a 1:2 solution with the same pD and uranium concentration. Unlike the U–ATP and U–AMP systems, the U–ADP system does not contain 2:2 ST complexes at any pD. Rather, at pD 7.7 only non-ST complexes (**4** and **5**), in which uranium is chelated by the two phosphoryl groups, exist in 1:1 and 1:2 U–ADP mixtures, but these disproportionate to 4:2 ST chelates and free ADP above pD 7.7. Unlike the behavior of non-ST U–AMP complexes, U–ADP and U–ATP non-ST complexes (adenine) ring-stack to a *greater* extent than do free nucleotides. The U–ATP complex (non-ST form) dephosphorylates completely to the non-ST U–ADP complex in near-neutral solution within 2 days at 27°, but at higher pD, 9.7, dephosphorylation of U–ATP (ST form) directly to the U–AMP ST complex seems to occur.

The structures and reactions of the complexes in uranyl ion–adenine nucleotide mixtures are of interest because of their possible involvement in uranium inhibition of sugar transport into a biological cell[4] and because of the use of uranyl compounds in tissue staining.[5]

Potentiometric titration curves of equimolar mixtures (U–ADP and U–ATP) of uranyl nitrate and the adenine 5′-nucleotides, ADP and ATP, depend on the time interval between each addition of base and the subsequent pH measurement.[6] If pH readings are taken 2 min after each addition of base, the titration curves show two inflection points (at $r \approx 1.5$, pH ~4.5, and $r \approx 4$, pH ≈10 for U–ADP; at $r = 1.0$, pH ~4.5 and $r \approx 4.2$, pH ≈9.5 for U–ATP,[7] but three inflection points are seen if pH readings are taken 24 hr after each addition of base. The new inflection is at $r = 3.0$, pH ≈7 for both systems. This effect of time is due to acid-producing dephosphorylation which, being most extensive in the $r = 2$–3 region, causes an inflection point to appear at $r = 3$ within 24 hr.

Structures of the complexes up to the first inflection point deduced from the titrations were treated earlier.[6] Nmr studies of the U–AMP system in neutral and basic solution were also discussed previously.[8,9] The present paper describes an nmr investigation of the U–ADP and U–ATP chelate structures in neutral and basic mixtures.

Experimental Section

The experimental procedures and conditions have been described earlier.[8,9] We wish to emphasize, however, that solubility con-

(1) (a) This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, and has been assigned Report No. UR-49-1235. It was partially supported by a Special Research Resource Grant, No. RR-00220-07, from the Division of Research Resources of the National Institutes of Health, and by the U. S. Public Health Service Training Grant No. 1T1 DE175. (b) Part of this paper was presented at the Second Rochester Conference on Toxicity at Rochester, N. Y., June 1969.

(2) On leave from University of Roorkee, Roorkee, India.

(3) To whom correspondence and reprint requests should be directed.

(4) L. Hurwitz, Ph.D. Thesis, University of Rochester, Rochester, N. Y., 1953.

(5) M. Beer, *Lab. Invest.*, **14**, 1020 (1965).

(6) I. Feldman, J. Jones, and R. Cross, *J. Amer. Chem. Soc.*, **89**, 49 (1967).

(7) r = moles of $(CH_3)_4NOH$ added/total moles of nucleotide.

(8) R. P. Agarwal and I. Feldman, *J. Amer. Chem. Soc.*, **90**, 6635 (1968); **91**, 2411 (1969).

(9) I. Feldman and K. E. Rich, *ibid.*, **92**, 4559 (1970).

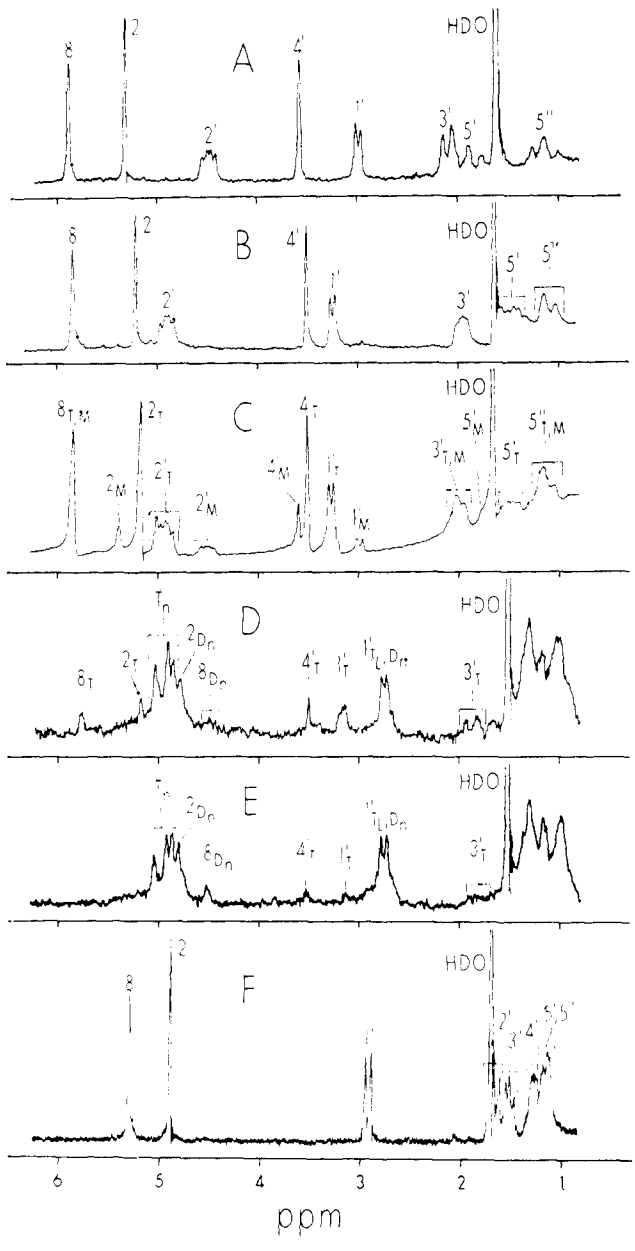


Figure 1. Pmr spectra (100 MHz) of 5′-ATP and equimolar uranyl nitrate–5′-ATP mixtures in D_2O at 27° and various pD values: (A) U–AMP, pD 11, 0.1 *M*; (B–E) U–ATP; (B) pD 8.7, $r = 4.0$; (C) pD 10.5, $r = 4.15$; (D) pD 7.3, $r = 3.0$; (E) pD 6.8, $r = 2.4$ (0.1 *M* for (B) and (C), 0.05 *M* for (D) and (E)); (F) ATP, no uranium added, pD 9.2. pD is adjusted with $(CH_3)_4NOH$ before lyophilization. $(CH_3)_4N^+$ is used as internal standard. Band frequency assignments are indicated by the numbers on the figures. All bands in (A) and (B) are ST (see text) chelate bands. All bands in (F) are free-ligand bands. In (C), (D), and (E), the subscripts M, D, and T designate AMP, ADP, and ATP bands; subscripts L and n designate free-ligand and non-ST (see text) complex ion bands; bands without subscript L or n are ST-type bands. Assignments in (C), (D), and (E) were made by comparison with (A), (B), and (F).

siderations[6,9] forced us to use systems which usually gave a low signal/noise ratio. Hence, each spectrum presented is an accumulation of a number of scans. Further, nonspinning 10-mm tubes had to be used for the phosphorus work, so that, in addition to their low S/N ratio, our phosphorus nmr spectra lack fine structure. The necessity of constantly monitoring the bridge balance and lock signal frequently made it impractical to accumulate more than 64 scans in obtaining a phosphorus spectrum. Bands in the phosphorus spectra usually had a half-width of at least 1 ppm. For this paper

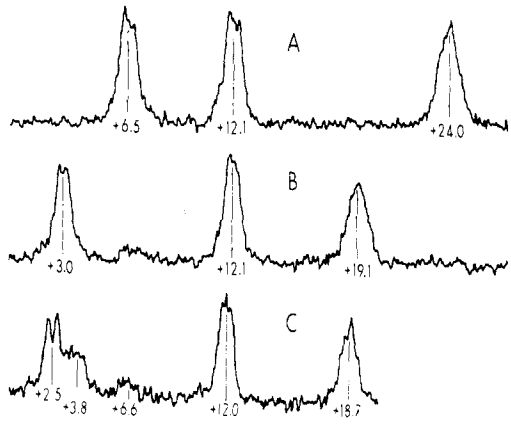


Figure 2. ^{31}P nmr spectra (40 MHz) of 5′-ATP and equimolar (0.20 *M*) mixtures of 5′-ATP and uranyl nitrate at 27°. Chemical shifts are measured upfield of the ^{31}P signal of H_3PO_4, which was used as external standard. (A) ATP in D_2O, no uranium added, pD 10; (B) U–ATP in D_2O, pD 10.4; (C) U–ATP in H_2O, pH 8.

the midpoint of a phosphorus band is considered to be its resonance frequency, with an accuracy of at least 0.4 ppm.

We were usually able to use 0.1 *M* mixtures above pH 8, but below pH 8 we had to use 0.05 *M* mixtures because of the very poor quality of 0.1 *M* spectra. However, even in 0.05 *M* solutions the increase in viscosity and the rate of dephosphorylation below pH 8 limited the number of scans we could usefully accumulate. We found that the most meaningful spectra for the purpose of elucidating the chelate structure *before* dephosphorylation were accumulations of 32 2.5-min scans, which were completed about 2.5 hr after the final lyophilization product[8] had been dissolved in D_2O.

The pD of a D_2O solution was taken to be the Beckman pH meter reading $+0.4$[10] and was usually about 0.2 unit above the pH which the original aqueous solution had before lyophilization.

We are using the convention that proton resonance frequencies are positive downfield of the reference frequency, in our case the $(CH_3)_4N^+$ signal, but that phosphorus nmr bands are at positive locations upfield of the reference, in our case 85% H_3PO_4. As in our earlier work,[9] the H_3PO_4 spectrum was recorded separately before and after each nucleotide spectrum was taken. The difference between these two H_3PO_4 peak positions was never more than 0.25 ppm for any spectrum which we considered to be acceptable for this paper.

Results

U–ATP System. ^1H and ^{31}P nmr spectra were recorded for U–ATP mixtures at various pD values in the range ≈7–11 and at several different stoichiometries. Representative spectra are shown in Figure 1 for protons and in Figure 2 for phosphorus. The pmr spectrum of a 0.1 *M*, equimolar U–ATP mixture in D_2O between pD 8 and 9 ($r = 3.9$ to 4.1) is typified by the pD 8.7 ($r = 4.0$) spectrum shown as Figure 1B. This spectrum is sufficiently similar in general appearance to the 1:1 U–AMP spectrum at pD 11 (Figure 1A), which was analyzed earlier,[8,9] to make it obvious that in Figure 1B the 5.83-ppm singlet, the 5.17-ppm singlet, the 4.92-ppm quadruplet, the 3.50-ppm singlet, and the 3.25-ppm doublet are, respectively, the H_8, H_2, $H_{2'}$, $H_{4'}$, and $H_{1'}$ signals. For the U–AMP spectrum the H_8 and H_2 signals were differentiated by noting that, because of deuterium exchange with H_8,[11] heating a U–AMP mixture at 80° for 4 hr produced a 23% decrease in the intensity of the

(10) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).
(11) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **86**, 696 (1964).

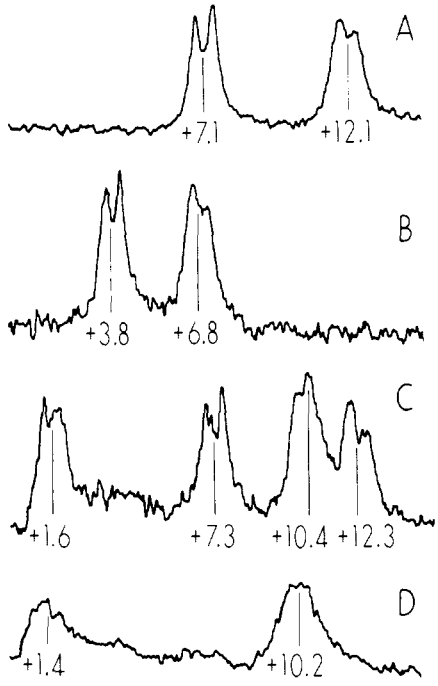


Figure 3. ^{31}P nmr spectra (40 MHz) of 5′-ADP and mixtures (0.1 *M* in ADP) of 5′-ADP and uranyl nitrate in D_2O at 27°. Chemical shifts are measured upfield of the ^{31}P signal of H_3PO_4, which was used as external standard. (A) ADP, no uranium added, pD 10; (B) 1:1 U–ADP, pD 7.7; (C) 1:1 U–ADP, pD 10.2; (D) 2:1 U–ADP, pD 10.2.

H_8 signal. The $H_{3'}$ band (1.97 ppm) in the U–ATP spectrum was identified by a spin-decoupling experiment; irradiation of either the $H_{1'}$ doublet or the $H_{3'}$ band collapsed the $H_{2'}$ quadruplet to a doublet, and irradiation of the $H_{2'}$ quadruplet collapsed both the $H_{1'}$ band and the $H_{3'}$ band to singlets. As had been observed for the U–AMP system,[8] $H_{4'}$ does not couple with other protons in U–ATP. By elimination, then, the 1.45-ppm band must be the $H_{5'}$ band.

As in the U–AMP spectrum at high pD, the H_8 and H_2 signals in the U–ATP spectrum for $r \approx 4$ are, respectively, about 0.5 and 0.25 ppm downfield of their positions in the absence of uranium. A 2:1 U–ATP spectrum at pD 8.7 is almost identical with the 1:1 spectrum. 1:2 and 1:3 U–ATP spectra for this pD are, clearly, superpositions of the 1:1 spectrum plus the (excess) ligand spectrum (Figure 1F).

The proton spectrum of a 1:1 U–ATP solution with $r > 4.1$ is a superposition of the 1:1, $r = 4$, U–ATP spectrum and a very small amount of the 1:1 U–AMP spectrum. This is evident by comparing Figure 1C with 1A and 1B. The spectral contribution from U–AMP is noticeable first at pD 9.7 ($r = 4.15$) and becomes larger as the pD is raised.

Figures 1D and 1E show the 32-scan pmr spectra of 0.05 *M*, equimolar U–ATP mixtures with $r = 3.0$ (pD 7.3) and $r = 2.44$ (pD 6.8), respectively. A comparison of Figures 1B and 1E shows that the main complex present in a U–ATP mixture with $r = 2.44$ (*i.e.*, before the second inflection) does not involve bonding of uranium to the ribose group, for the only discernible ribose proton signals downfield of HDO, which Figures 1B and 1E have in common, and which are therefore attributable to the U–ATP complex, are extremely weak (*i.e.*, $4'_T$, $1'_T$, and $3'_T$ in Figure 1E). The same type of complex (*i.e.*, with ribose not involved in the chelation) is the predominant form in the $r = 3$ mixture, but the small peaks at 5.83, 5.23, 3.55, and 3.22 ppm in Figure 1D show that this mixture also contains a small amount ($\approx 10\%$ of total ATP) of the same chelate which is the chief form near $r = 4$. Increasing r further to a value above 3 increases the relative intensities of these latter four spectral bands until at $r = 3.4$ (pD 7.7) the pmr spectrum is quite similar to the $r = 4$ spectrum.

A comparison of the pmr spectra in Figures 1D, 1E, and 4B shows that a small amount of dephosphorylation of the U–ATP complex to give some U–ADP took place in both the $r = 2.4$ (Figure 1E) and the $r = 3$ (Figure 1D) mixtures within the 2.5-hr interval required to obtain these 32-scan spectra. This is evident from the presence of the small U–ADP bands labeled D_n at 4.60 and 4.86 ppm in Figures 1D and 1E. The pmr spectrum of the U–ATP solution with $r = 2.4$ after it had aged 2 days was almost identical with Figure 4B, showing that complete dephosphorylation to U–ADP had occurred in that time. A 1:2 (0.1 *M* ATP), pD 7.3 spectrum also showed no ribose bands, other than $H_{1'}$, downfield of HDO during 32 scans, but it did indicate about 25% dephosphorylation to U–ADP within one scan (15 min after the final lyophilization product was dissolved in D_2O).

The phosphorus nmr spectra of 1:1 ($r = 4.2$) and 2:1 ($r = 6.4$) U–ATP mixtures at pD 10.4–11.4 are almost identical (Figure 2B). The γ and β bands in Figure 2B are, respectively, 3.5 and 5.0 ppm downfield of their locations, 6.5 and 24 ppm, in the spectrum of uncomplexed, deprotonated ATP (Figure 2A). The α band frequency is unaffected by the presence of uranium. Presumably because of the very low nmr sensitivity of ^{31}P, the very small amount of U–AMP hydrolysis product at pD 10.4 (shown by the pmr spectra discussed above) is not detectable in Figure 2B. However, a free-AMP signal appeared in the ^{31}P spectrum of a pD 10 mixture which had aged 2 months.

At pH 8 an aqueous 1:1 ($r = 3.9$) U–ATP solution gives a phosphorus spectrum (Figure 2C) which has five bands, three bands near the resonance frequencies of Figure 2B plus small bands at about 3.8 and 6.6 ppm. These latter bands can be identified as the two U–ADP bands by comparison with the U–ADP spectra discussed below. However, in D_2O this mixture (*i.e.*, 1:1, pD 8.2) gives only a hint of a U–ADP band (*i.e.*, a low shoulder near 3.8 ppm) during the time required for 32 scans. This agrees with the pmr spectra discussed above. An equimolar, pD 6.9 mixture, which was quite viscous and which contained some precipitate, gave a ^{31}P spectrum which had three broad ($\approx$2-ppm width) bands centered at 3.6, 11.6, and 18.6 ppm. The first band is probably a mixture of the U–ATP γ band and a U–ADP β band, and its location implies a greater contribution of U–ADP at pD 6.9 than at pD 8.2. The U–ADP α band seems to be lost in the noise.

U–ADP System. Representative phosphorus nmr spectra for the U-ADP systems are presented in Figure 3. Representative pmr spectra are given in Figure 4. Both the phosphorus nmr spectrum (Figure 3B) of the 1:1 ($r = 3$) U–ADP mixture and that of the 1:2 ($r = 2$) mixture at pD 7.7 contain two bands. In the 1:1 spec-

trum one band is at 3.8 ppm, 3.3 ppm downfield of the β signal of uncomplexed, deprotonated ADP (Figure 3A), and the second is at 6.8 ppm, 5.3 ppm downfield of the α band of ADP. In the 1:2 spectrum the two bands are at 3.4 and 6.4 ppm, respectively. The differences between the two spectra are within our experimental error. Bands near these locations, specifically 4.0 and 6.5 ppm, also occur in the 2:1 spectrum at this pD, and, in addition, there is a very broad band from -6.5 to 1.0 ppm, but it should be pointed out that this 2:1 mixture was very viscous and contained precipitate.

The pD-10.2, 1:1 U–ADP phosphorus spectrum (Figure 3C) contains four distinct bands, all of equal area, at 1.6, 7.2, 10.5, and 12.3 ppm. In addition, there is a low broad band from 2.5 to 5.5 ppm and a barely perceptible shoulder on the left side of the 7.3-ppm band. The four distinct bands of this 1:1 spectrum are present also in the 1:2 phosphorus spectrum at this pD. In the latter spectrum, however, the 1.4- and 10.4-ppm bands have equal areas and the 7.2- and 12.3-ppm bands have equal areas, but the former pair is about thrice the size of the latter pair. In the 1:2 spectrum there may be a hint of a low broad band between 2.5 and 4.5 ppm, but no shoulder is detectable on the left side of the 7.2-ppm band. A 2:1 phosphorus spectrum (Figure 3D) at the same pD consists of two large bands centered at 1.4 and 10.2 ppm, plus two small bands near the locations of the two bands in the pD 7.7 spectrum, *i.e.*, the small band centered at 3.8 ppm and the low broad band between 5 and 8 ppm. There may also be a small shoulder on the right side of the 10.3-ppm band due to free ADP. Coupling these data with the fact that the free-ADP spectrum at pD 10 (Figure 3A) has bands at 7.1 and 12.1 ppm makes it seem evident that the spectra of 1:1 and 1:2 U–ADP mixtures with pD 10.2 are compositites of the free-ADP spectrum and the pD-10, 2:1 spectrum, primarily. That is, Figure 3C is primarily a superposition of Figures 3A and 3D with little contribution from 3B.

The pmr spectrum of a 1:1 ($r = 3$) U–ADP mixture in D_2O at pD 7.7 is shown as Figure 4B. A 1:2 solution at this pD has almost the same proton spectrum as the 1:1 mixture, the only difference being that the 1:2 spectrum also has small free-ligand bands with areas less than 10% as large as the 1:1 spectral bands. One should note the surprising fact that at this pD uranyl ion causes a reversal in the order of the adenine H_8 and H_2 signals[12] compared to their positions in the spectrum of free ADP (Figure 4A). This reversal was not observed in the spectra of the U–AMP system. We are uncertain about it in the U–ATP system. Thus, uranyl ion causes the H_8 and H_2 signals of ADP at pD 7.7 to move upfield 0.8 ppm and 0.2 ppm, respectively. The pD-7.7 spectrum also shows a 0.2-ppm shift *upfield* for $H_{1'}$, whereas uranyl ion causes deshielding of $H_{1'}$ of ATP and hardly affects $H_{1'}$ of AMP. In the 1:1, pD-7.7 U–ADP spectrum, no ribose proton resonance occurs downfield of the HDO peak except the $H_{1'}$ resonance, which, however, is downfield of HDO normally (*i.e.*, in absence of uranyl ion).

(12) This conclusion was confirmed by recording a pmr spectrum after the U–ADP mixture was heated for 1 hr at 85° and then cooled back to room temperature. The heat treatment caused the H_8 signal to decrease in intensity about 40% relative to the H_2 intensity because of replacement of the H_8 by deuterium.[11]

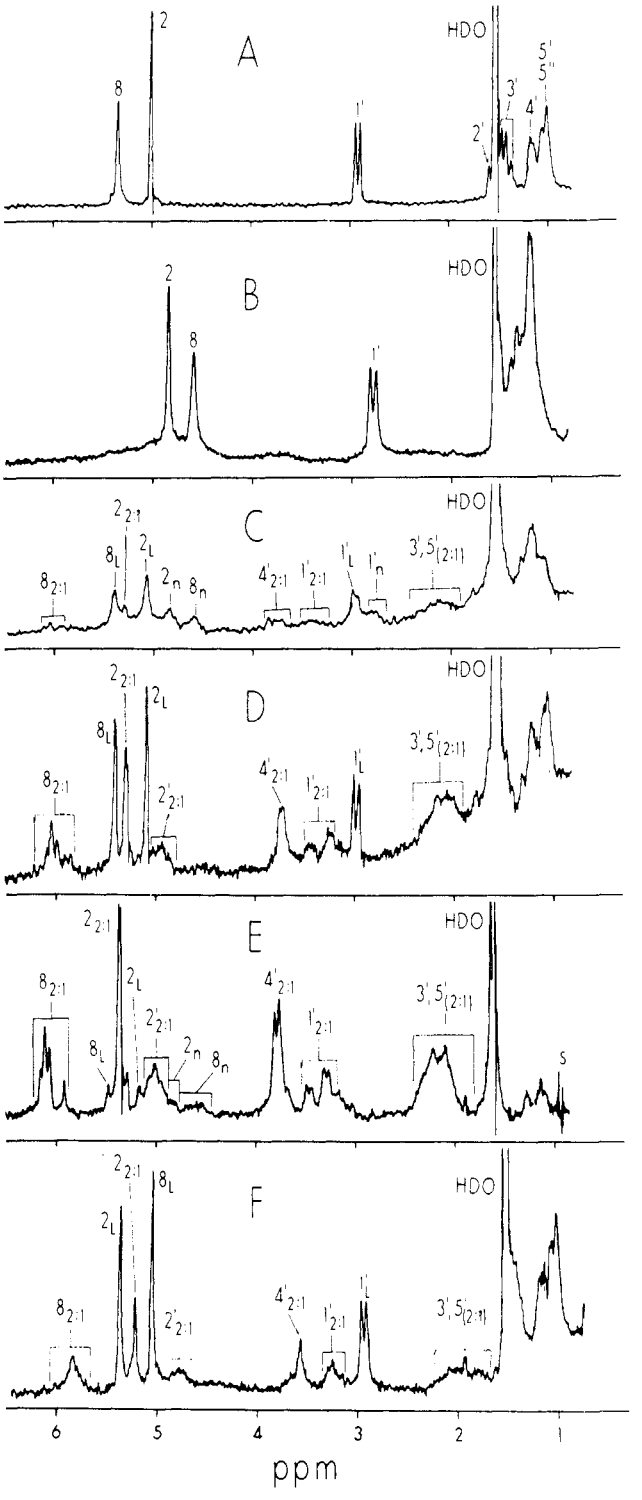


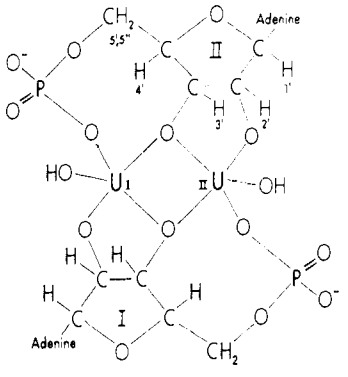
Figure 4. Pmr spectra (100 MHz) of 5′-ADP and uranyl nitrate–5′-ADP mixtures (0.1 *M* in ADP) in D_2O at 27°, $(CH_3)_4N^+$ used as internal standard: (A) ADP, no uranium added, pD 7.7; (B) 1:1 U–ADP, pD 7.7; (C) 1:1 U–ADP, pD 9; (D) 1:1 U–ADP, pD 11; (E) 2:1 U–ADP, pD 11; (F) 1:2 U–ADP, pD 11. ADP proton assignments in (A) are made by comparison with ATP assignments in Figure 1F. See text for assignments in (B) and (E). Assignments in (C), (D), and (F) are made by comparison with (A), (B), and (E). Subscripts L, n, and 2:1 refer, respectively, to free-ADP bands, non-ST bands, and 2:1 ST bands.

In agreement with the ^{31}P spectra discussed above, as the pD of an equimolar U–ADP mixture is gradually

raised from 7.7 to 11 one can see a gradual transition of the pmr spectrum to one (Figure 4D) which is apparently a superposition of a free-ADP spectrum (Figure 4A) and a 2:1 spectrum (Figure 4E) in equal proportion. The pmr spectra of solutions in the buffer region between $r = 3$ and 4 for the 1:1 U–ADP system are represented by Figure 4C, the pD-9 ($r = 3.7$) spectrum, which appears to be a composite of the pD-7.7 and -11 spectra. As in the phosphorus study, even the 1:2 spectrum at pD 11 (Figure 4F) seems to be a superposition of free-ADP and 2:1 spectra, but a small contribution of the pD-7.7 bands to the latter is absent from the 1:2 spectrum.

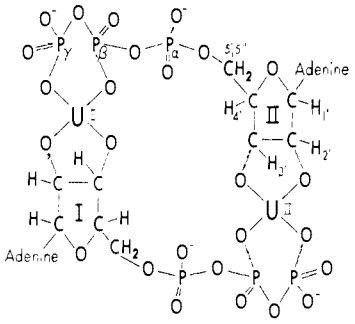
Discussion

U–ATP System. The phosphorus nmr spectra in Figure 2 show that in near-neutral and in basic solution (*i.e.*, pD 6.8–11) the uranyl ion is chelated by the β and γ phosphate groups of ATP, since uranyl ion causes a significant downfield shift of both the β and γ bands but affects the α band to a relatively insignificant extent. On this point, it seems relevant that complexation of the uranyl ion by AMP caused the ^{31}P band to move downfield to about the same extent[9] as the β and γ bands in the U–ATP spectra.



U REPRESENTS UO_2^{+2} ION ⊥ PLANE OF PAPER

1

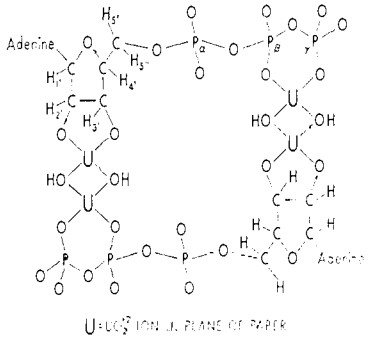


U = UO_2^{+2} ION ⊥ PLANE OF PAPER

2

The similarities in the general appearance of the pmr spectrum of the 1:1 U–ATP mixture between pD 8 and 9 (*i.e.*, $r \approx 4$), typified by Figure 1B, and of the 1:1 U–AMP spectrum at pD $\approx$11, Figure 1A, suggest that there must be great similarity in the structures responsible for these spectra. In our previous papers[8,9] we postulated a dimeric sandwich-type (ST)[13] structure, **1**, for the predominant complex in an equimolar U–AMP mixture at high pH and presented rough calculations[8] to show the compatibility of this structure with the pmr spectrum, Figure 1A. A somewhat similar 2:2 ST structure, **2**, which is compatible, at least qualitatively, with both the proton and phosphorus spectra can be given for the complex in a 1:1 U–ATP solution near $r = 4$.[14] Consistent with the nmr spectra, structure **2** involves chelation by both the β and γ phosphate groups of ATP and by the ribose hydroxyl-oxygen atoms. In this structure ribose group I is much further from uranyl group II, and ribose II is much further from uranyl I, than in **1**. As a result, the diamagnetic anisotropy effects of the uranyl groups I and II on the protons of the ribose groups II and I, respectively, should be significantly less in **2** than in **1**. In addition, a molecular model of **1**, constructed from Corey–Pauling–Koltun (CPK) space-filling atomic models, shows that the fact that $O_{3'}$ is bonded to both uranium atoms causes **1** to have an absolutely rigid nature. This suggests that in **1** there might be some ring strain which affects the U–O bond strengths and, as a consequence, the electron-withdrawing effect of the uranyl ion in the $H_{2'}$ and $H_{3'}$ resonance frequencies. Further, the anisotropy effects of the P–O bonds, especially on $H_{5'}$ and $H_{5''}$, are probably different in **1** and **2**. To attempt to reconcile quantitatively the relative positions of the ribose proton resonance frequencies would involve differences between two necessarily crude calculations and seems pointless. However, it is certain that differences, such as those observed, are to be expected.

The nearly identical appearance of the spectra, both ^1H and ^{31}P, of 1:1 ($r = 4.2$) and 2:1 ($r = 6.4$) U–ATP solutions near pD 10 leads us to postulate structure **3**



U = UO_2^{+2} ION ⊥ PLANE OF PAPER

3

for the 2:1 complex at $r = 6$. The uranium atoms are probably hydroxylated at higher r.

As was also the case for the U–AMP spectra at pD 11, the relative areas of the free-ligand peaks and the peaks

(13) To differentiate between sandwich-type, or ST, complexes (such as **1** and **2**) and complexes, such as **4** and **5**, in which the ribose group is not involved in the chelation, we will refer to the latter as non-sandwich-type, or non-ST, complexes.

(14) There is no inconsistency in our depicting the uranium atoms of **2** as being unhydroxylated while considering them to be hydroxylated in **1**. The structures we have presented are those prevailing at the pH's at which they are most stable. For **1** this pH is $\approx$11 where $r = 5$, but for **2** this pH is $\approx$8.5 where $r = 4$. The uranium atoms of **2** undoubtedly do hydroxylate at higher pH (higher r), but then dephosphorylation occurs.

due to complexes in the spectra of 1:2 and 1:3 U–ATP mixtures at pD 9 showed that these systems contain primarily the 2:2 ST complex plus excess ligand.

From the smallness of the ribose proton peaks, except $H_{1'}$, downfield of HDO in Figure 1E, it is evident that non-ST complexes predominate at pD 6.8, $r = 2.4$, in equimolar (0.05 *M*) U–ATP solution. Figure 1D shows that by pD 7.3 about 10% of the U–ATP has become ST form. By pD 7.7, all the complex is ST type. However, no evidence was found for the ST complex at pD 7.3 in a 1:2 solution (0.05 *M* U, 0.1 *M* ATP). Further, the proton frequencies are the same in the 1:2, pD-7.3 spectrum as in the 1:1, pD-6.8 spectrum, showing that the uranium-to-nucleotide bonding is similar in the two solutions. These non-ST complexes will be discussed further in the next section where U–ADP non-ST complexes are treated.

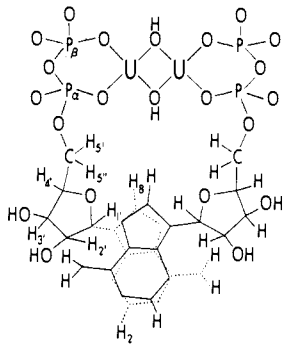
U–ADP System. The results obtained for the U–ADP system are surprising, since one might expect the U–ADP system to fit a description somewhat intermediate between the U–AMP and the U–ATP systems.

The fact that a 1:1 ($r = 3$) U–ADP solution at pD 7.7 is devoid of precipitate and gives a ^{31}P spectrum which contains no free-ligand band is conclusive evidence that this solution contains only a complex with 1:1 stoichiometry. In a 1:2 U–ADP ($r = 2$) solution having the same pD, over 80% of the ADP is complexed as 1:2 species, the remainder (<20%) being equally distributed as free ADP and 1:1 complex. This composition is evident from the pmr spectrum of the 1:2, pD-7.7 solution, since it contains small free-ligand bands with areas less than 10% as large as the complexed-ADP bands.

As was noted above for the U–ATP system at pD 6.8, the absence of any ribose proton band other than $H_{1'}$ downfield of the HDO peak in either the 1:1 or the 1:2 U–ADP spectrum shows that the uranyl ion is *not* bound to ribose in either the 1:1 or the 1:2 complex. The similar appearance of the ^{31}P spectra of 1:1 and 1:2 U–ADP mixtures at pD 7.7 (*i.e.*, consisting of two bands displaced downfield of the free-ligand bands to about the same extent in the two mixtures) indicates that there is complexation of *all* the phosphate in these mixtures and that the uranium-to-phosphate bonding is similar in both mixtures. Since it is also physically impossible for one uranium atom to be attached simultaneously to the four phosphate groups and the two adenine groups of a 1:2 complex, it seems certain that in both the 1:1 and the 1:2 U–ADP complexes at pD 7.7 the uranium is bound *only* to the phosphate groups.

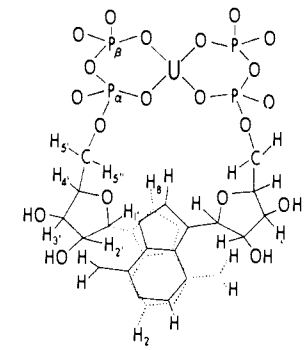
The *upfield* movement of both adenine protons suggests that complexation of uranyl ions by ADP near pD 7.7 *increases* the stacking of the adenine groups.[15] The much larger change for H_8 (0.8 ppm) compared to H_2 (0.2 ppm), which causes the reversal in their relative order, suggests further that the adenine groups are oriented to place H_8 close to the phosphate and the uranyl groups so that H_8 experiences a significant diamagnetic anisotropy effect from these groups in addition to the ring-current effects of stacking.

The non-ST structures **4** and **5** are in accord with all the bonding and geometric features attributed in the foregoing discussion to the 1:1 ($r = 3$) and 1:2 ($r = 2$) U–ADP non-ST complexes at pD 7.7. The non-ST U–ATP structures should be similar.



U: UO_2^{+2} ION ~ 30° FROM ⊥ TO PLANE OF PAPER

4



U: UO_2^{+2} ION ~ 30° FROM ⊥ TO PLANE OF PAPER

5

Adenine ring stacking was not detected in the U–AMP system. The T_n peaks in Figures 1D and 1E upfield of the H_8 position in Figure 1F indicate at least some enhancement of ring stacking by complexation in the non-ST U–ATP species. Molecular models of U–AMP non-ST chelates indicate that ring stacking could occur in the U–AMP system *if* the uranyl ion were bound by the phosphate in a monodentate fashion, but not if it were bound in a bidentate manner in each of neighboring chelates. Though bidentate chelation of a uranyl ion by phosphate has not yet been shown, it was suggested earlier,[9,16] and it would seem to be possible, since there is evidence that uranyl ion is bound bidentately by COO^-,[17] CO_3^{2-},[18] and NO_3^-[19] ions in crystals and by COO^-,[20,21] NO_3^-,[16] and $(RO)_2PO_2^-$[2,16] in aqueous solution. In the U–ATP system it seems likely that the mutual repulsion of the negatively charged α phosphoryl groups would decrease ring stacking relative to that in the U–ADP system, even at pD <7.7,

(15) The theory of the effect of stacking of the adenine groups on the chemical shifts of H_8 and H_2 has been discussed in A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **89**, 3612 (1967), and in M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *ibid.*, **90**, 1042 (1968).

(16) A. E. Comyns, *Chem. Rev.*, **60**, 115 (1960).

(17) W. H. Zachariasen and H. A. Plettinger, *Acta Crystallogr.*, **12**, 526 (1959).

(18) C. L. Christ, J. R. Clark, and H. T. Evans, *Science*, **121**, 472 (1955).

(19) J. L. Hoard and J. D. Stroupe in "Spectroscopic Properties of Uranium Compounds," NNES, Div. III, G. H. Dieke and A. B. F. Duncan, Ed., McGraw-Hill, New York, N. Y., 1949, p 13.

(20) I. Feldman and L. Koval, *Inorg. Chem.*, **2**, 145 (1963).

(21) V. A. Golovnya, and L. K. Shubochkin, *Zh. Neorg. Khim.*, **8**, 1116 (1963); *Nucl. Sci. Abstr.*, **17**, 4796 (1963).

where non-ST chelates exist. The non-ST U–ATP chelates may still, however, polymerize to some extent as in the U–ADP system. Dephosphorylation in the U–ATP system precludes the use of pH titrations in studying polymerization in this system.

The results above pD 7.7 (*i.e.*, $r > 3$) suggest that the 2:2 non-ST U–ADP complex disproportionates to a large extent above this pD to free ADP and 2:1 complex, this reaction being nearly complete at $r \approx 4$. This conclusion seems evident from the fact that as the pD of a 1:1 U–ADP mixture is continually raised (starting at pD 7.7), both the proton spectrum and the phosphorus spectrum undergo gradual transition to a spectrum which appears to be a composite of free-ligand and 2:1 spectra primarily.

It seems probable that the 2:1 U–ADP complex at high pD has a 4:2 ST structure similar to the 4:2 U–ATP structure, **3**, except of course that in the former the phosphate groups which chelate the uranium are α and β. This opinion follows from the fact that in the high-pD, 2:1 pmr spectrum (Figure 4E) there are bands downfield of the HDO signal which are clearly ribose proton bands, and which disappear from the 2:1 spectrum as the pD is lowered as far as pD ~8. The bands we are referring to here are the two-proton bands centered at 2.15 ppm (which by analogy with the U–AMP spectral assignments[8] we attribute to the $H_{3'}$ and $H_{5'}$ bands merged together) and the one-proton bands at 3.77 and 5.0, which are probably the $H_{4'}$ and $H_{2'}$ bands, respectively. The area from 3.0 to 3.55 ppm, which contains the two small doublets at 3.28 and 3.47 ppm, represents only one proton, which is most likely $H_{1'}$. These assignments seem obvious from comparison with the U–AMP and U–ATP spectra.

Unlike the high-pD spectra of the U–AMP and U–ATP systems, the U–ADP spectra show splitting of the adenine proton bands, of $H_{4'}$, and of the $H_{1'}$ doublet into several doublets.

This splitting is most evident in the 2:1 spectrum, Figure 4E. The fact that the spectral area between 3.0 and 3.5 ppm, including the two doublets at 3.28 and 3.47 ppm, is due to only one proton makes it most likely that this splitting is not due to spin–spin coupling, but rather to either the unsymmetrical nature of the 4:2 ST complex or to the presence of unsymmetrical mixed complexes. We prefer the latter explanation since there is evidence for some of the non-ST form and also for free ADP in the 2:1 mixture even at high pD, and since we had previously[9] noted such splitting in the U–AMP system between pD 7.9 and 8.9 where mixed complexes and free ligand were present. Further, we can see no reason why the 4:2 ST structure should be unsymmetrical in the U–ADP system but not in the U–ATP or U–AMP system at high pD.

Relative Stabilities of Sandwich-Type Uranyl–Adenine Nucleotide Chelates. It is evident from the above discussion and from our previous report on the U–AMP system[9] that the relative order of the stabilities of the 2:2 ST chelates in the three systems with respect to dissociation and/or disproportionation is U–ATP $\gg$ U–AMP $\gg$ U–ADP. The order of the second inequality is unexpected. However, a study of the chelate models constructed from the CPK atomic models suggests the following as a possible explanation of this series.

Models show that in either structure **2** or **3** rotations about the $C_{4'}$–$C_{5'}$ and $C_{5'}$–O axes allow either monomeric unit to twist about $\pm 135°$ out of the plane of the other monomeric unit. Hence, the U–ATP system gains very little, if any, vibrational entropy by disproportionation of **2** to **3**. In the U–AMP system, also, there is no significant increase in vibrational entropy upon disproportionation, but in this case the reason is that both the 2:2 and 4:2 ST chelate models are perfectly rigid structures,[9] in which $O_{2'}$, $O_{3'}$, and the uranium atoms are coplanar. However, as we have suggested above, the rigidity of the U–AMP chelates implies that there is probably some ring strain which could contribute to the instability which the U–AMP ST chelates show[9] below pH 11. On the other hand, the CPK models of U–ADP 2:2 and 4:2 ST chelates indicate that the latter chelate is considerably more flexible than the former. Either monomeric unit of the 4:2 ST U–ADP model can rotate through 180° relative to the second monomeric unit (primarily by rotation about the $C_{4'}$–$C_{5'}$ and $C_{5'}$–O bonds), but only about 45° rotation can be performed on the 2:2 ST model because of hindrance between an $H_{3'}$ atom with an O_α atom in one direction and between two uranyl oxygen atoms in the other direction. Thus, we suggest that the order of stabilities of the three 2:2 ST chelates might be due to ring strain in **1**, which is absent in **2**, and to the greater flexibility of the 4:2 ST U–ADP structure relative to that in the 2:2 ST U–ADP structure.

Comparison of UO_2^{2+}–ATP and Monatomic Cation–ATP Chelate Structures. To date, nmr has furnished the following structural information on M^{2+}–ATP complexes. In equimolar, aqueous M^{2+}–ATP mixtures with pH in the range 4.5–9 and ATP concentration ≈ 0.1 *M*, the diamagnetic ions Ca^{2+}, Mg^{2+}, and Zn^{2+} bind to the β and γ phosphate groups of ATP.[22] Mg^{2+} and Ca^{2+} ions do not significantly affect the pmr spectrum of ATP,[22,23] but at pH 7.2 the H_8 signal in a Zn^{2+}–ATP spectrum lies 0.25 ppm downfield of its position in the ATP spectrum.[22] Cohn and Hughes concluded therefrom that Zn^{2+} forms a complex with the adenine ring of ATP. The ^{15}N nmr work of Happe and Morales[24] seems to support this conclusion in that Zn^{2+} causes a 3.0-ppm deshielding effect on the N_7 and the NH_2-nitrogen resonances of ATP. However, the recent studies of Schweizer, *et al.*,[15] have shown that the small Zn^{2+}-induced deshielding effects on the H_8 resonance observed by Cohn and Hughes might be due simply to decreased stacking of the adenine bases. Though data are lacking, it seems that destacking may possibly be responsible for the results of Happe and Morales also, especially since their studies involved relatively high ATP concentrations, 0.5–0.9 *M*, at which there is considerable ring stacking in the absence of metal ion.

Until recently, nmr investigations of the binding of paramagnetic metal ions to ATP have been carried out with huge excess of ATP, *e.g.*, $[Mn^{2+}] \leqq 10^{-3}$ *M* and [ATP] ≈ 0.1 *M* in the Mn^{2+}–ATP work.[22,25a] In such solutions, it was found that at pH 7.2–9 Cu^{2+} ions bind to the β and γ phosphates of ATP, but that Mn^{2+}, Co^{2+},

(22) M. Cohn and T. R. Hughes, Jr., *J. Biol. Chem.*, 237, 176 (1962).
(23) G. G. Hammes, G. E. Maciel, and J. S. Waugh, *J. Amer. Chem. Soc.*, 83, 2394 (1961).
(24) J. A. Happe and M. Morales, *ibid.*, 88, 2077 (1966).
(25) (a) H. Sternlicht, R. G. Shulman, and E. W. Anderson, *J. Chem. Phys.*, 43, 3123 (1965); (b) *ibid.*, 43, 3133 (1965).

and Ni^{2+} interact simultaneously with all three phosphate groups.[22,25a] The latter two ions were studied only at pH 8.5–9. At pH ~7 all four paramagnetic ions bind to the adenine ring of ATP also,[22,25b] the latter three all the time they are bound to the phosphate.[25b]

To resolve apparent disagreement between these conclusions and previous ultraviolet[26] and temperature-jump[27] studies which involved solutions containing only 10^{-4} *M* ATP, Sternlicht, *et al.*,[28] performed nmr experiments on Mn^{2+}–ATP solutions with only $\approx 5 \times 10^{-4}$ *M* ATP. These authors claim to have shown that at low ATP concentration, $\approx 5 \times 10^{-4}$ *M*, primarily 1:1 complexes are present, but that 1:2 complexes predominate at high ATP concentration, ≈ 0.1 *M*, *i.e.*, with only $\approx 10^{-4}$ *M* Mn^{2+} in each case. Sternlicht, *et al.*, believe that in the 1:2 complex the Mn^{2+} binds simultaneously to the phosphate of one nucleotide and to the adenine of the second nucleotide, but that the metal is bound only to the phosphate in the 1:1 complex.

(26) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).
(27) G. G. Hammes, and S. A. Levison, *Biochemistry*, **3**, 1504 (1964).
(28) H. Sternlicht, D. E. Jones, and K. Kustin, *J. Amer. Chem. Soc.*, **90**, 7110 (1968).

Except for the existence of multiple equilibria which depend strongly on solution conditions, there is little reason to expect much similarity between UO_2^{2+}–ATP complexes and monatomic cation–ATP complexes in which all three phosphate groups are involved, since ligands attached to the uranyl ion are restricted to positions close to its equatorial plane. On the other hand, one might look for relationships between UO_2^{2+}–ATP chelates and those monatomic cation–ATP complexes, like Cu^{2+}–ATP, in which only two phosphate groups are involved, but it is apparent that much more extensive and systematic studies, *e.g.*, over wider pH and concentration ranges, are needed for systems containing monatomic cations than are now available. It is of interest to this point that Brintzinger[29] in 1961 claimed that his pH titrations showed involvement of the ribose hydroxyl group in the complexation of Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cu^{2+} by ATP in basic solution. However, he believed that, unlike the binding in our suggested UO_2^{2+}–ATP structures, there was an intervening water molecule attached by its oxygen atom to the metal and by hydrogen bridges to the two ribose hydroxyl oxygens.

(29) H. Brintzinger, *Helv. Chim. Acta*, **44**, 1199 (1961).

Triarylboron Anion Radicals and the Reductive Cleavage of Boron Compounds

J. E. Leffler,* G. B. Watts, T. Tanigaki, E. Dolan, and D. S. Miller

Contribution from the Department of Chemistry, Florida State University, Tallahassee, Florida 32306. *Received April 9, 1970*

Abstract: The boron nuclear spin coupling constant of the triphenylboron anion radical is positive. The assignment of coupling constants to the ortho, meta, and para protons has been confirmed by a study of the tris(*p*-deuteriophenyl)- and tris(3,5-dideuteriophenyl)boron esr spectra. The lines corresponding to $m_B = \pm 3/2$ are selectively broadened either by incomplete averaging of the anisotropic hyperfine coupling or by an unidentified reaction that modulates the boron coupling constant. Prolonged reaction of triphenylboron anion radical with alkali metals in DME degrades it to biphenyl anion radical, *both halves of which come from the same triphenylboron*. The behavior of tris(*p*-chlorophenyl)boron, tris(*p*-methoxyphenyl)boron, tris(*p*-dimethylaminophenyl)boron, tris(*p*-tolyl)boron, several trialkylborons, diphenylboron chloride, and dimesitylboron fluoride with alkali metals is also described.

Most triarylborons react with alkali metals in DME[1] or other ethers to give anion radicals whose electron spin resonance spectra show quartet splittings due to the predominant ^{11}B isotope.[2] These spectra are of theoretical interest because the triarylboron anion radicals are isoelectronic with the corresponding triarylmethyl neutral radicals and triarylaminium cation radicals. One of the properties of interest is the sign of the isotropic coupling constant of the central ^{11}B, ^{13}C, or ^{14}N. The coupling constant is known to be *positive* for ^{13}C and ^{14}N in the triphenyl compounds,[3,4] but has not been determined with more bulky aryl groups that might be twisted further out of the plane of the central bonds.

At one time the coupling constant for trimesitylboron anion radical was believed to be negative,[2a] but the data have since been reinterpreted.[2d]

* Address correspondence to this author.
(1) 1,2-Dimethoxyethane.
(2) (a) H. van Willigen, Dissertation, Amsterdam, 1965; (b) S. I. Weissman and H. van Willigen, *J. Amer. Chem. Soc.*, **87**, 2285 (1965); (c) J. E. Leffler, E. Dolan, and T. Tanigaki, *ibid.*, **87**, 927 (1965); (d) H. van Willigen, private communication.
(3) (a) H. van Willigen and S. I. Weissman, *J. Chem. Phys.*, **44**, 420 (1966); (b) F. C. Adam and S. I. Weissman, *J. Amer. Chem. Soc.*, **80**, 2057 (1958).
(4) H. van Willigen, *ibid.*, **89**, 2229 (1967).