have performed unrestricted Hartree–Fock (INDO method) MO calculations[11] for the ethylene–DNMO (dimethyl nitroxide) bimolecular system with various geometries. Calculated spin densities on the carbon 2s AO for the σ- and π-type interactions were positive and negative, respectively, in agreement with the observed trend.[12]

The interaction with DTBN radical at the cross-linked carbon can be further characterized from a study of ^{13}C spin-lattice relaxation time (T_1) for solutions involving the DTBN radical. We have therefore measured T_1 for ^{13}C in pyrene on a JEOL PFT-100 spectrometer (at 25.1 MHz) in a Fourier transform mode using the 180°–τ–90° pulse method. T_1's for pyrene in CS_2 solution (15 mol %) were 7.1 (C_1), 8.7 (C_2), 9.1 (C_4), 47.2 (C_{11}), and 60.3 (C_{15}) sec.[13] T_1's for C_{15} and C_{11}, the cross-linked carbons, are most strikingly affected by the addition of DTBN radical (3.0×10^{-5} M)(27.2 (C_{15}), 25.5 sec (C_{11})), while the T_1's for the other C–H carbons changed only slightly.[14] These results appear to be in accord with a direct π-type interaction between DTBN and the cross-linked carbons in pyrene.

From the present study we conclude that measurement of ^{13}C contact shifts and relaxation times are promising for the elucidation of weak interactions involving free radicals.

Acknowledgment. The authors are thankful to K. Yoshikawa and K. Matsushita for help in the experiment of pulse and Fourier transform ^{13}C nmr.

(11) J. A. Pople, D. L. Beveridge, and P. A. Dobosh, *J. Chem. Phys.*, 47, 2026 (1967); *J. Amer. Chem. Soc.*, 90, 4201 (1968).

(12) MO theoretical calculations for the σ-type interaction have been well studied (see ref 2). The C–H bond perpendicular to the oxygen or nitrogen π orbital of DMNO radical senses negative and positive spin densities on the H 1s and C 2s AO's. The four-centered π-stacking interaction induced +0.015 and +0.010 spin densities on the 2s AO of the ethyene carbons close to the O and N atoms of DMNO, respectively, for an intermolecular distance of 2.0 Å.

(13) The experimental error is ±1.0 sec.

(14) The study of ^{13}C relaxation time in the paramagnetic solution is now under way.

Isao Morishima,* Kiyoshi Kawakami, Teijiro Yonezawa
Department of Hydrocarbon Chemistry, Faculty of Engineering Kyoto University, Kyoto, Japan

Kojitsu Goto, Makoto Imanari
JOEL. Ltd., Akishima, Tokyo, Japan

Received May 23, 1972

Evidence for a Guanosine–Calcium(II) Complex. A Specific Nucleoside–Metal Interaction

Sir:

There is a wealth of information published on nucleic acid–metal ion interactions. A closer examination, however, indicates that most interactions are due to metal–phosphate complexation.[1] Recently, some base–metal interactions have been reported with Ag^+,[2a] Co^{2+}, Hg^{2+}, and Zn^{2+}.[2b]

We would like to report evidence concerning the existence of a 1:1 Ca^{2+}–guanosine complex, to our knowledge the first example of an alkaline earth metal–guanine base interaction.

(1) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, 71, 439 (1971).

(2) (a) K. Gillen, R. Jensen, and N. Davidson, *J. Amer. Chem. Soc.*, 86, 2792 (1964); (b) L. S. Kan and N. C. Li, *ibid.*, 92, 4823 (1970).

Comparison of the proton magnetic resonance spectrum (taken on a Varian A-60) of guanosine in DMSO-d_6 with similar solutions to which varying amounts of $CaCl_2$ have been added indicates a gradual downfield shift only in the N(1)–H and N(2)–H_2 resonances of guanosine (almost identical shifts for both resonances) with increasing $CaCl_2$ concentration. The C(8)–H resonance and the ribose resonances are all unaffected in their chemical shifts by the addition of $CaCl_2$ implying complexation to the pyrimidine ring. That the base portion of the nucleoside is affected by complexation is further proven by the fact that the *O*-2′,3′,5′-triacetylguanosine exhibits identical behavior as does 8-bromoguanosine. The latter result indicates that the nucleoside conformation is most likely not responsible for the complex.[3]

A similar study found *no effect* of $CaCl_2$ on all observable resonances in adenosine, cytidine, or inosine (observation of the N(1)–H resonance of inosine even in DMSO-d_6 is very difficult); thus the complex appears to be *highly specific* for *guanine bases*. $MgCl_2$ was also employed with all above molecules and showed *no such* behavior.

The equilibrium constant (37°) for the complex was determined by monitoring the N(2)–H resonance at constant guanosine concentration varying the $CaCl_2$ concentration as well as by varying the guanosine concentration at a given $CaCl_2$ concentration.

Following Kan and Li[2b] for a 1:1 Ca^{2+}–guanosine complex

$$G + Ca \overset{K}{\rightleftharpoons} G\text{–}Ca \tag{1}$$

$$K = \frac{[G\text{–}Ca]}{[G][Ca]} = \frac{x}{([G]_0 - x)([Ca]_0 - x)} \tag{2}$$

where x is the concentration of complex and $[G]_0$ and $[Ca]_0$ are initial concentrations of guanosine and calcium, respectively. The observed chemical shift for the N(2)–H resonance ν_0

$$\nu_0 = \frac{x}{[G]_0}\nu_{G\text{–}Ca} + \frac{([G]_0 - x)}{[G]_0}\nu_G \tag{3}$$

where $\nu_{G\text{–}Ca}$ and ν_G are the complexed and uncomplexed N(2)–H resonances (for the same guanosine concentration), respectively. Equation 3 can be rewritten in terms of $\Delta_0 = (\nu_0 - \nu_G)$ and $\Delta_t = (\nu_{G\text{–}Ca} - \nu_G)$ as

$$\Delta_0 = (x/[G]_0)\Delta_t \tag{4}$$

Combining with eq 1 and inverting finally leads to

$$\frac{[Ca]_0}{\Delta_0} = \frac{1}{K\Delta_t} + \frac{1}{\Delta_t}([Ca]_0 + [G]_0 - x) \tag{5}$$

Plots of $[Ca]_0/\Delta_0$ *vs.* $([Ca]_0 - x)$ or $([G]_0 - x)$ lead to Figure 1 where x is varied in an iterated manner to give the best straight line. For a Δ_t of 46 ± 2 Hz (60 MHz) slope one obtains a $K = 17 \pm 1$ l./mol as the mean of the two results. Data used for Figure 1 are given in Table I.

Some other pertinent observations defining properties of the complex are as follows. (1) The complex exhibits great stability at elevated temperatures.

(3) D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inkeep, and H. Eyring, *ibid.*, 93, 1600 (1971).

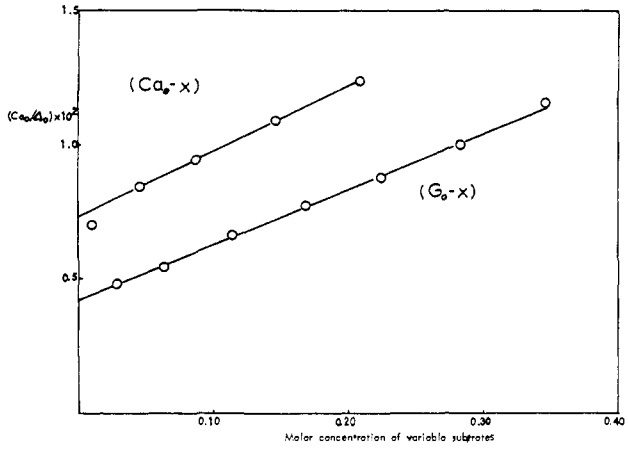


Figure 1. Plot used in determining K.

Table I. Chemical Shifts of Guanosine–Calcium(II) Complex[a]

$[CaCl_2]$,[d] M	[Guanosine],[b] M	ν_G(N(2)–H$_2$),[c] Hz	ν_0(N(2)–H$_2$), Hz	Δ_0
0.0			389	0
0.084			401	12
0.168			409	20
0.253			416	27
0.338			420	31
0.422			423	34
	0.070	385	414	29
	0.141	387	413	26
	0.211	389	410	21
	0.282	389	407	18
	0.352	390	406	16
	0.423	392	406	14
	0.493	393	405	12

[a] Measured at 37°, 60 MHz on Varian A-60, chemical shifts with respect to TMS, terms defined in text. [b] [G] = 0.282 M, for varying $CaCl_2$. [c] ν_G(N(2)–H) = 389 Hz. [d] $[CaCl_2]$ = 0.141 M for varying guanosine.

Preliminary data at 122° give a K of 10 ± 2. (2) The complex is shown not to be an artifact of the solvent DMSO alone since addition of $CDCl_3$ does not destroy it. (3) The complex has also been found to exist in partially aqueous solution. Following the procedure of Yates and Welch[4] we titrated guanosine and guanosine + $CaCl_2$ (1:4 molar ratio) at 25° in 70% by wt DMSO–H_2O (1:2 mole ratio) with a glass electrode (Radiometer pHm 26). The pK_a for loss of N(1)–H is 11.25 without and 9.6 with the $CaCl_2$ present in this mixture,[5] implying that the presence of Ca^{2+} enhances acidity. A titration in pure H_2O gave very small changes for a 1:1 molar ratio (near 0.1 pK lowering by Ca^{2+}) in the same direction as was found in a DMSO–H_2O mixture. (4) The resonances attributed to N(1)–H and N(2)–H show marked broadening upon Ca^{2+} addition implying an intermediate exchange rate for the process. The observed peak areas are apparently unaffected by the exchange process within nmr integration limits. (5) The C(8)–H resonance shows sharpening upon complexation perhaps due to enhanced lactim–lactam tautomerization as suggested recently.[6]

(4) K. Yates and G. Welch, *Can. J. Chem.*, 50, 474 (1972).
(5) The 2-pK unit shift of guanosine from the value in aqueous solution is similar to the shifts observed in ref 4.
(6) G. C. Y. Lee and S. I. Chan, *J. Amer. Chem. Soc.*, 94, 3218 (1972).

We do not have any immediate suggestions concerning the biochemical significance of such a highly specific complex between the pyrimidine ring of guanosine and Ca^{2+}. One may note the Ca^{2+}-mediated cyclic GMP effect on cyclic AMP function,[7] however, as well as the known binding of Ca^{2+} by RNA.[8]

We are continuing the characterization of the complex along the evidence here outlined and plan eventual X-ray determination of the structure to unequivocally establish the mode of Ca^{2+} binding.

Acknowledgment. A research grant from the Rutgers University Research Council supporting this work is gratefully acknowledged.

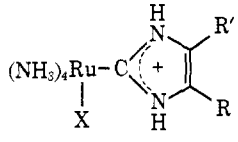
(7) J. F. Whitfield and J. P. MacManus, *Proc. Soc. Exp. Biol. Med.*, 139, 818 (1972).
(8) H. S. Loring and R. S. Waritz, *Science*, 125, 644 (1957).

Frank Jordan,* B. Y. McFarquhar
Department of Chemistry, Rutgers University
Newark, New Jersey 07102
Received July 10, 1972

Carbon-Bound Imidazolium Ylides as Ligands in Ruthenium(II) and Ruthenium(III) Complexes

Sir:

Investigation of the acid-catalyzed aquation of the ion $[(NH_3)_5Ru(II)Im]^{2+}$ [1,2] has shown that the rate is pH and ionic-strength dependent qualitatively like the hydrolysis of $[(NH_3)_5Ru(II)py]^{2+}$,[3] although the rate is some 500 times greater for the imidazole complex. The major product of acidic cleavage is $[(NH_3)_5Ru(II)H_2O]^{2+}$ isolated as $[(NH_3)_5Ru(III)Cl]Cl_2$ after oxidation. There is also formed an imidazole-containing species isolated (<10%) after air oxidation as a solid having the composition $(NH_3)_4Ru(III)ImCl_3$. We consider the imidazole in both the Ru(II) and Ru(III) states of this ion to be bound *via* C-2 of the imidazole ring as shown for **1a**. The proton nmr spectrum of the Ru(II) species in D_2O or H_2O displays a single line for carbon-bound protons at 7.2 ppm.[4] The ammine protons appear as a broad signal at 1.9 ppm.



1a, R = R′ = H
b, R = R′ = CH_3
c, R, R′ = benzo
d, R = H; R′ = CH_3

(1) Abbreviations: Im = imidazole; MIm = 4-methylimidazole, DMI = 4,5-dimethylimidazole; BZI = benzimidazole.
(2) This ion, characterized as the BF_4^- salt, was prepared by the procedure of R. G. Gaunder and H. Taube, *Inorg. Chem.*, 9, 2627 (1970). Proton nmr data show that it is a typical imidazole complex bound through the "pyridine nitrogen." Three C–H signals are observed at δ 7.04, 7.29, and 7.75 in D_2O. The uv spectrum consists of a band at 255 nm (ε 2800) with a prominent shoulder at 280 nm (ε 2700). Air oxidation gives the Ru(III) species isolated as the trichloride: λmax 300 nm (ε 1880), 430 (250).
(3) P. C. Ford, J. R. Kuempel, and H. Taube, *ibid.*, 7, 1976 (1968); R. E. Shepherd, Ph.D. Thesis, Stanford University, 1971.
(4) Chemical shifts are relative to sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$.