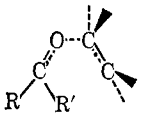
photosensitized reactions,[10] but our results define the scope and the limitation of such a mechanism in energy transfer to olefins; *i.e.*, this mechanism operates efficiently only with donors possessing a low-lying n,π^* triplet state.

Alternatively, the partially vacant n orbital of the n,π^* triplet state of the donor may polarize the π system of the olefin sufficiently during the energy transfer so that the olefin moiety in the transition state (III) is no longer planar. The dissociation follows to give back the carbonyl compound and the olefin triplet state in a nonplanar configuration. This point of view, which is favored by Hammond,[8] will also account for all of our current results. Our work suggests that such an energy transfer may occur efficiently only with a highly polarizing donor and a polarizable acceptor. Work is being continued with the hope of clarifying these points.



III

---

[10] G. O. Schenk and R. Steinmetz, *Bull. Soc. Chim. Belges*, **71**, 781 (1962).

[11] The authors wish to acknowledge the National Sciences Foundation for support of this work.

[12] National Institutes of Health Postdoctoral Fellow, 1967–1968.

N. C. Yang,[11] Jacob I. Cohen,[12] Arnon Shani
Department of Chemistry, University of Chicago
Chicago, Illinois 60637

Received March 9, 1968

Conformational Changes of the Ribose Group in Dinucleoside Mono- and Diphosphates. Temperature Dependence[1]

Sir:

A knowledge of the structure and interactions of simple, model oligo- and polyribonucleotides is essential if an understanding is to be achieved of the factors governing the conformational stability of nucleic acids in solution. Recent studies,[2–15] employing a variety of spectroscopic techniques, have shown that adjacent bases of purine oligo- and polyribonucleotides are capable of forming intramolecular stacks in aqueous solution under appropriate conditions of temperature and pH. Although it is reasonable to expect that the base-stacking interaction will be accompanied by a conformational change of the ribose phosphate backbone, evidence for such a change is lacking at present. In order to obtain information regarding this point we have studied the temperature dependence of the ribose proton spectra for a variety of mono- and dinucleotides. Since the magnitude of a vicinal proton–proton coupling constant is closely related to the dihedral angle between the protons,[16] a study of the temperature dependence of the ribose proton spectra should provide direct evidence for any conformational change of the ribose ring in the stacking process.

The ribose spectra[17] of the dimers[18] consist of two doublets in the range 365–410 cps (downfield from external TMS) and a complex group of signals located in the region of the residual HDO signal. The doublets can be assigned to the $H_{1'}$ protons of the nonequivalent ribose groups and are due to spin coupling with the respective $H_{2'}$ protons.

A summary of the $J_{H_{1'}H_{2'}}$ values for a number of mono- and dinucleotides, over a range of temperatures, is given in Table I. For mononucleotides the magnitudes of $J_{H_{1'}H_{2'}}$ lie between 5.0 and 5.5 cps and are in agreement with values reported in the literature.[19–21] No significant temperature dependence is noted for these couplings in the range studied. In contrast the $J_{H_{1'}H_{2'}}$ couplings for the dimers are all less than the values for corresponding 3′ and 5′ monomeric nucleotides. Furthermore, all dimer coupling constants, except that of the pA-c-p moiety of ApA-c-p, are markedly temperature dependent. In ApA, for example, there is approximately a twofold decrease in

Table I. $J_{H_{1'}H_{2'}}$,[a] of Several Mononucleotides and Dinucleoside Mono- and Diphosphates

Temp, °C	ApA[b]		ApA-c-p		GpA			
	Ap	pA	Ap	pA-c-p	Gp	pA	AMP-3′	AMP-5′
4	2.3	2.1			2.1	3.0	6.1	5.5
7			2.8	2.9				
15	2.5	2.2	3.7	3.0				
20								5.1
28	3.1	2.8	4.0	3.2			5.6	
35					4.0	4.0		5.2
55			4.6	3.2	4.5	4.2	5.2	5.1
62	4.5	4.1						
67				3.1			5.3	
71	4.7	4.5			5.4	4.3		5.3
80							5.1	

[a] Errors in the *J* values are ±0.10 cps. [b] AMP-3′ = adenosine 3′-monophosphate, ApA = adenylyl-(3′→5′)-adenosine; ApA-c-p = adenylyl-(3′→5′)-adenosine cyclic 2′,3′-monophosphate, etc.

---

[1] Work supported under the auspices of the U. S. Atomic Energy Commission.

[2] D. N. Holcomb and I. Tinoco, Jr., *Biopolymers*, **3**, 121 (1965).

[3] K. E. Van Holde, J. Brahms, and A. M. Michelson, *J. Mol. Biol.*, **12**, 726 (1965).

[4] D. Poland, J. N. Vournakis, and H. A. Scheraga, *Biopolymers*, **4**, 223 (1966).

[5] M. Leng and G. Felsenfeld, *J. Mol. Biol.*, **15**, 455 (1966).

[6] J. Brahms, A. M. Michelson, and K. E. Van Holde, *ibid.*, **15**, 467 (1966).

[7] C. R. Cantor and I. Tinoco, Jr., *Biopolymers*, **5**, 821 (1967).

[8] J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 481 (1967).

[9] C. C. McDonald and W. D. Philips, *Science*, **144**, 1234 (1964).

[10] J. P. McTague, V. Ross, and J. H. Gibbs, *Biopolymers*, **2**, 163 (1964).

[11] M. P. Schweizer, D. P. Hollis, and P.O.P. Ts'o, Abstracts, 9th Meeting of the Biophysical Society, San Francisco, Calif., Feb 1965.

[12] K. H. Scheit, F. Cramer, and A. Franke, *Biochim. Biophys. Acta*, **145**, 21 (1967).

[13] Y. Inoue and S. Aoyagi, *Biochem. Biophys. Res. Commun.*, **28**, 973 (1967).

[14] C. C. McDonald, W. D. Phillips, and J. Lazar, *J. Am. Chem. Soc.*, **89**, 4166 (1967).

[15] F. E. Hruska and S. S. Danyluk, *Biochim. Biophys. Acta*, in press.

[16] M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

[17] All of the spectra were measured in buffered D_2O solutions at pD 7.0; the solute concentrations were 0.02 *M* (in base). Spectra were recorded with a Varian DA 60I spectrometer locked on a capillary of TMS immersed in the solution. A C1024 CAT was used to enhance the signal-to-noise ratio.

[18] The spectra for the mononucleotides were similar to those reported by other workers.[19–21]

[19] C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).

[20] C. D. Jardetzky, *ibid.*, **82**, 229 (1960).

[21] C. D. Jardetzky, *ibid.*, **83**, 2919 (1961).

going from 71 to 4°. The most reasonable explanation of the results is a change in conformation of both ribose rings in the dimer.[22,23] Moreover, the lack of any significant $J_{H_{1'}H_{2'}}$ changes in the monomers also suggests that the conformational changes in the dimers are related to the base-stacking interactions. This is strongly supported by the linear correlation[25] between the $H_{1'}$ chemical shifts and the corresponding $J_{H_{1'}H_{2'}}$ coupling constants (see Figure 1). Earlier work[13-15] has shown that the downfield shift of the base ring and $H_{1'}$ proton signals with increasing temperature can be associated with a progressive intramolecular destacking of the base rings in the dimer.

A qualitative picture of the conformational changes can be deduced from a consideration of known ribose structures and the dependence of vicinal couplings upon dihedral angle, ϕ. X-Ray diffraction studies of the crystal structures of purine nucleosides and nucleotides,[26-28] the dinucleoside monophosphate ApU (2′→5′),[29] and a variety of polyribonucleotides[30] have shown that the ribose ring is in a puckered conformation with either the $C_{2'}$ or the $C_{3'}$ atom displaced out of the plane defined by the $C_{1'}$, $C_{4'}$, $O_{1'}$, and $C_{3'}$ (or $C_{2'}$) atoms. In general the out-of-plane atom is in an *endo* position, *i.e.*, located on the same side of the plane as the $C_{5'}$ atom. In only one known case, deoxyadenosine, does the out-of-plane atom ($C_{3'}$) lie in an *exo* position. A rough estimate of the $J_{H_{1'}H_{2'}}$ coupling constants expected for the 2′-*endo* (out-of plane) and 3′-*endo* (out-of-plane) conformations can be made from Karplus' equation for vicinal couplings.[31] Thus, for $C_{2'}$ *endo*, ϕ is approximately 150°[33] and $J_{H_{1'}H_{2'}}$ is estimated to be 6.9 cps, while, for $C_{3'}$ *endo*, $\phi \approx 115°$ and $J_{H_{1'}H_{2'}}$ is estimated to be 1.7 cps. Accordingly at low temperature where the bases are predominantly stacked (<25–30°), the ribose groups of the dimers favor a conformation in which the $C_{3'}$ is in an *endo* orientation, while at elevated temperatures, where the bases are unstacked, the preferred conformation is one in which $C_{2'}$ is *endo*.[34]

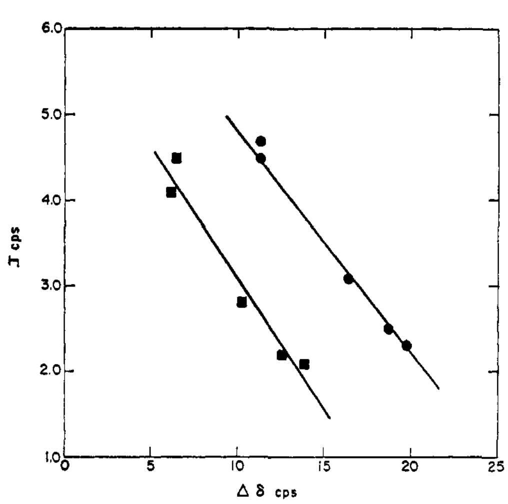


Figure 1. Plot of $J_{H_{1'}H_{2'}}$ *vs.* the respective $H_{1'}$, chemical shifts of the Ap (■) and pA (●) moieties of ApA (the shifts are relative to the values for the corresponding mononucleotides).

The temperature dependence of $J_{H_{1'}H_{2'}}$ for the dimers therefore indicates a conformational change of the ribose group from the $C_{3'}$-*endo* form at low temperatures to a $C_{2'}$-*endo* form at high temperatures. The similarity in magnitude of $J_{H_{1'}H_{2'}}$ in the monomers and dimers at higher temperatures also suggests that the ribose conformations in the unstacked dimers are not significantly different from the monomers.

---

[22] It is highly improbable that the J changes are due to any perturbation of electron density at $C_{1'}$ and $C_{2'}$ either intramolecularly (*i.e.*, by inductive effects) or by solvent molecules.

[23] Temperature-dependent conformational changes are known to produce significant changes in coupling constants in many saturated ring systems.[24] The absence of coupling constant changes for pA-c-p suggests that conformational changes have been restricted by the 2′,3′-cyclic phosphate linkage.

[24] J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, Inc., New York, N. Y., 1965, p 575.

[25] A linear correlation is also noted between the coupling constants and the chemical shifts of the H_2 (and H_8) protons of the purine bases.

[26] M. Sundaralingam, *J. Am. Chem. Soc.*, 87, 599 (1965).

[27] M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 930 (1965).

[28] J. Kraut and L. H. Jensen, *Acta Cryst.*, **16**, 930 (1965).

[29] E. Shefter, M. Barlow, R. Sparks, and K. Trueblood, *J. Am. Chem. Soc.*, 86, 1862 (1964).

[30] D. M. Davies, *Ann. Rev. Biochem.*, **36**, 339 (1967).

[31] The equation is of the form[16] $J_{\text{vicinal}} = A \cos^2 \phi - 0.028$, where $A = 8.5$ for $0° \leq \phi \leq 90°$ and $A = 9.5$ for $90° \leq \phi \leq 180°$. Although the magnitudes of the coupling constants estimated by this equation may be subject to considerable error, the essential form and predicted trends have been confirmed by a number of experimental studies.[20,21,24,32]

[32] R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

[33] The dihedral angles were estimated from Koltun molecular models.

[34] A $C_{3'}$ *endo* conformation is supported in the crystalline state by X-ray diffraction studies on AMP-5′[28] and poly A[35] while the $C_{2'}$ *endo* conformation is indicated from nmr studies on a number of purine mononucleosides and nucleotides.[20,21] One can consider the stacked form of the dimer as approximating the structure of the polynucleotide in the solid state.

[35] A. R. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, 3, 71 (1961).

F. E. Hruska, S. S. Danyluk
Division of Biological and Medical Research
Argonne National Laboratory, Argonne, Illinois
Received February 15, 1968

Electron Spin Resonance of Nitrogen Dioxide (NO_2) Adsorbed on Zinc Oxide

Sir:

In the course of an investigation of the interaction of oxides of nitrogen with nonstoichiometric surfaces of metallic oxides, the electron spin resonance spectra of NO_2 adsorbed on zinc oxide has been investigated. High-purity ZnO samples supplied by the New Jersey Zinc Co. with surface areas of ~3 m^2/g were employed. Prior to treatment with NO_2, samples were outgassed for 2 hr at 500° at 10^{-6} torr. The NO_2 employed was of ultrahigh purity and was supplied by Cryogenic Rare Gas Laboratories Inc., Newark, N. J. Experiments were done with an X-band Varian spectrometer (Model V-4502) provided with a TE_{104} mode dual cavity. A wave meter was used to measure the correct frequency and 1,1-diphenylpicrylhydrazyl (DPPH) was used in the reference cavity as a standard for g measurements.

The esr spectrum of vacuum-outgassed ZnO at −195°, shown in Figure 1A, primarily consists of a signal, at $g \simeq 1.96$ ($\Delta H = 7.6$ G) which is variously assigned to Zn^+ ions,[1,2] oxide ion vacancies,[3] or to conduction electrons.[4] Following treatment with excess

---

[1] K. A. Muller and J. Schneider, *Phys. Letters*, 4, 2288 (1963).

[2] Y. Fujita and J. Turkevich, *Discussions Faraday Soc.*, **41**, 407 (1966).

[3] P. H. Kasai, *Phys. Rev.*, **130**, 989 (1963).

[4] R. J. Kokes, *J. Phys. Chem.*, **66**, 99 (1962).