to dryness, dissolving the residue in *ca.* one-half its original volume of aqueous 50% methanol, and chromatographing the solution in system A. Spots were eluted into aqueous 50% methanol and assayed spectrophotometrically at 260 m$\mu$. Rate constants were calculated for hydrolysis of isopropylideneuridine and cyclopentylideneuridine, and both reactions were found to follow pseudo-first-order kinetics.

*Acknowledgments.* Dr. George Bosworth Brown is thanked for his encouragement and interest throughout this study, and Mr. D. Lamontonaro is thanked for capable assistance.

# Luminescence of Purines[1]

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*Contribution from Whitmore Chemical Laboratory, Pennsylvania State University, University Park, Pennsylvania. Received June 17, 1965*

*The phosphorescence and polarized phosphorescence excitation spectra have been studied for purine, the purine anion, adenine, and some alkyl-substituted adenines. The results indicate that, in all cases, the triplet → singlet transition moment is perpendicular to the molecular plane, and that the lowest singlet excited state in purine is (n, π\*), and in the purine anion and adenines, (π, π\*). The emission properties of 9-n-butyladenine in nonpolar media are reported and briefly discussed in terms of an energy-transfer process.*

There is considerable interest in the location of the n → π\* absorption bands in isolated nucleic acid bases, as such knowledge is important in understanding both energy transfer and hypochromism in polynucleotides. Clark and Tinoco[2] found a weak absorption between 290 and 320 m$\mu$ in purine in hydrocarbon solvent at 353°K., which they assume to be an n → π\* transition from its disappearance in polar solvent. A similar phenomenon is observed in 9-methylpurine.[3] Stewart and Davidson[4] have definitely identified an n → π\* band in 9-methyladenine, by studying the polarized absorption spectra in the crystalline adenine–thymine dimer. Borresen[5] concluded that the lowest singlet excited state in both adenine and purine should be (n, π\*), from their nonfluorescence in water at room temperature. His reasoning involved the well-known rule that (π, π\*) states fluoresce, whereas (n, π\*) states do not.[6] However, the number of established exceptions are sufficiently numerous to indicate that no general statement can be made regarding nonfluorescence of (n, π\*) states. N-Heterocyclics which have fluorescent (n, π\*) states are 3,4-benzocinnolin[7a] pyridazine and its derivatives,[7b] s-tetrazine,[8] and pyrimidine.[5] Thus the character of the lowest excited state cannot be conclusively identified by the presence or lack of fluorescence. In the case of adenine,

Callis, Rosa, and Simpson[9] have quite rigorously ascertained, by a polarized fluorescence excitation experiment, that the lowest singlet excited state in ethylene glycol–water solvent at 196°K. is (π, π\*). However, this experiment cannot distinguish between a higher energy n → π\* absorption and higher π → π\* absorptions.

As the first step in a program of the characterization of excited states in polynucleotides, it is necessary to have an unequivocal assignment of the lowest energy bands in the simple bases. The present work is an attempt to conclusively identify the n → π\* bands in the bases, purine, adenine, 9-n-butyladenine, and 9-methyladenine through a study of polarized phosphorescence excitation spectra.

## Experimental Section

*Materials.* Purine and adenine, A grade, were obtained from the California Corp. for Biochemical Research, and used without further purification. Adenine, obtained from the Nutritional Biochemicals Corp., was recrystallized twice from water. These two samples of adenine gave identical results. 9-Methyladenine, grade I, was purchased from the Cyclo Chemical Corp., and the sample of 9-n-butyladenine was generously donated to us by Dr. John A. Montgomery of the Southern Research Institute. These were used without further purification.

EPA (5:5:2 parts by volume of ether, isopentane, and ethanol), methylcyclohexane–isopentane (5:1), ethanol–NaOH (20:1),[10] butanol–isopentane (3:7), and mixtures of hydrocarbon and triethylamine were used as solvents for emission studies. All concentrations were between $10^{-3}$ and $10^{-5}$ M.

*Procedure.* The emission and polarization spectra were obtained with a Baird-Atomic fluorescence spectrophotometer, Model SF1. Radiation from a 150-w. xenon source was dispersed by a double-grating monochromator and irradiated the sample which was at 77°K. The emission, observed perpendicularly to the excitation beam, was focused through a second double-grating monochromator and was detected with an RCA Type 1P28 phototube. The signal was recorded on an X–Y recorder. Polarization measure-

(1) Supported by a grant from the National Science Foundation.
(2) L. B. Clark and I. Tinoco, Jr., *J. Am. Chem. Soc.*, 87, 11 (1965).
(3) S. F. Mason, Special Publication No. 3, The Chemical Society, London, 1955, p. 139.
(4) R. F. Stewart and N. Davidson, *J. Chem. Phys.*, 39, 255 (1963).
(5) H. C. Borresen, *Acta Chem. Scand.*, 17, 921 (1963).
(6) M. Kasha, *Discussions Faraday Soc.*, 9, 14 (1950).
(7) (a) E. Lippert and W. Voss, *Z. Physik. Chem.* (Frankfurt), 31, 321 (1962); (b) B. J. Cohen, H. Baba, and L. Goodman, *J. Chem. Phys.*, 43, 2902 (1965).
(8) M. Chowdhury and L. Goodman, *ibid.*, 38, 2979 (1963).

(9) P. R. Callis, E. J. Rosa, and W. T. Simpson, *J. Am. Chem. Soc.*, 86, 2292 (1964).
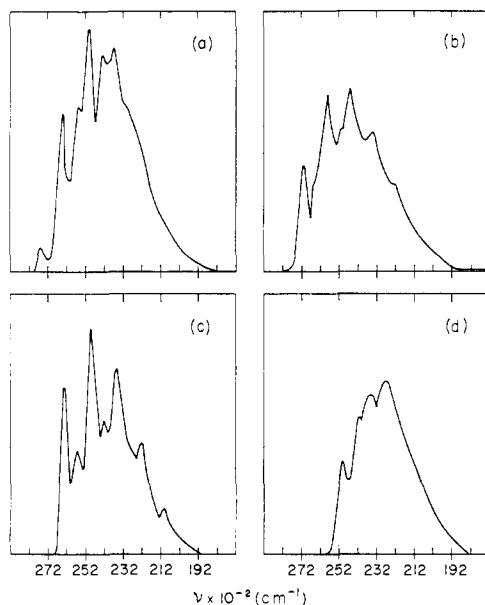(10) The NaOH solution is 0.5% by weight of NaOH.

Figure 1. Phosphorescence spectra of (a) purine, (b) adenine, (c) 9-$n$-butyladenine in EPA at 77°K., and (d) phosphorescence of purine anion in ethanol–NaOH (~20:1) at 77°K.

ments were obtained using a Glan-Thomson prism oriented to pass only exciting light (band width, 8 m$\mu$) polarized with the electric vector perpendicular to the plane formed by the excitation and observation beam. An HNPB UV polaroid was used to analyze the emission. In obtaining the polarized excitation spectra, because of intensity considerations, it was not possible to utilize the most highly polarized portion of the emission, (*vide infra*). In particular, for purine the emission monochromator was set on the second emission band, and for the adenines, the band maximum. The polarized excitation spectrum was recorded point by point in order to allow the phosphorescence to reach a steady intensity at each wave length. The polarization was corrected for scatter and instrumental effects by method c described by Azumi and McGlynn.[11] (The dewar holds liquid nitrogen for a sufficient time to record a series of four excitation spectra point by point.)

Correction factors varied smoothly from 0.98 at 405 m$\mu$ to 1.08 at 380 m$\mu$. The excitation spectrum falls rapidly in intensity below 270 m$\mu$, owing to the decrease in lamp intensity. However, reliable data could be obtained down to 240 m$\mu$. The polarization values in this paper are the data obtained from three separate measurements on either two or three different glasses.

### Results and Discussion

The phosphorescence spectra of purine, adenine, and 9-$n$-butyladenine in EPA solvent at 77°K. are presented in Figure 1. The spectrum of 9-methyladenine is almost identical with that of 9-$n$-butyladenine, and, therefore, is omitted. The lifetimes of the phosphorescence were estimated visually, and in all cases were ~1 sec. The singlet–triplet invervals are given in Table I. As the 0–0 band of the first $\pi \rightarrow \pi^*$ absorption cannot be located precisely, the singlet–triplet interval was calculated from the difference between the maxima of the absorption and phosphorescence.

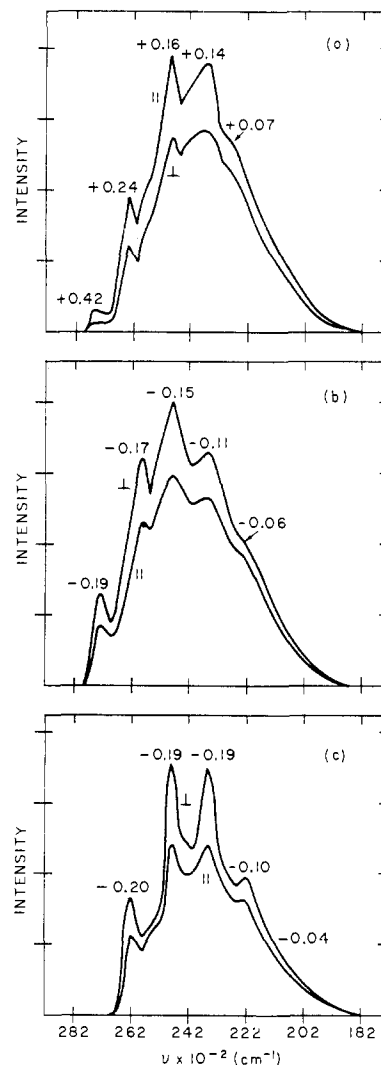(11) T. Azumi and S. P. McGlynn, *J. Chem. Phys.*, 37, 2413 (1962).



Figure 2. Polarized phosphorescence emission spectra in EPA at 77°K. of (a) purine with respect to excitation at 300 m$\mu$, (b) adenine with respect to excitation at 280 m$\mu$, (c) 9-$n$-butyladenine with respect to excitation at 280 m$\mu$, corrected for instrumental and scatter effects. The degree of polarization is shown for the principal peaks.

It is evident from the long lifetimes and the large singlet–triplet intervals (greater than 10,000 cm.$^{-1}$) that in all cases phosphorescence is from a ($\pi$, $\pi^*$) triplet state, an assignment similar to that made by Rahn, *et al.*,[12] from lifetime and e.s.r. studies for the triplet state of adenosine.

Table I. Purine Singlet–Triplet Intervals

| | Absorption max., cm.$^{-1}$ | Phosphorescence max., cm.$^{-1}$ | $^1E - {}^3E$, cm.$^{-1}$ |
|---|---|---|---|
| Purine | 38,200 ± 150 | 25,000 ± 130 | 13,200 |
| Adenine | 38,500 ± 150 | 24,600 ± 120 | 13,900 |
| 9-$n$-Butyladenine | 38,500 ± 150 | 24,900 ± 120 | 13,600 |
| 9-Methyladenine | 38,500 ± 150 | 24,800 ± 120 | 13,700 |
| Purine anion | 36,900 ± 140 | 22,730 ± 100 | 14,200 |

Examination of the polarized emission spectra in Figure 2 shows that the polarization retains the same sign throughout the entire emission band. However,

(12) R. O. Rahn, J. W. Longworth, J. Eisinger, and R. G. Shulman, *Proc. Natl. Acad. Sci. U. S.*, 51, 1299 (1964).
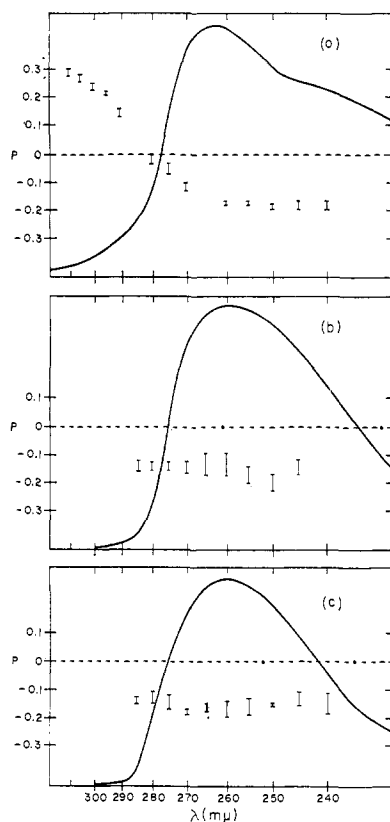
Figure 3. Absorption spectra in EPA at room temperature and polarized phosphorescence excitation spectra in EPA at 77°K. corrected for instrumental and scatter effects of (a) purine, (b) adenine, (c) 9-$n$-butyladenine. Degree of polarization ($P$) is defined as $P = (I_{||} - I_\perp)/(I_{||} + I_\perp)$ where $I_\perp$ and $I_{||}$ refer to the emission intensity with perpendicular and parallel electric vectors relative to the exciting light electric vectors. The values represent the scatter of three measurements.
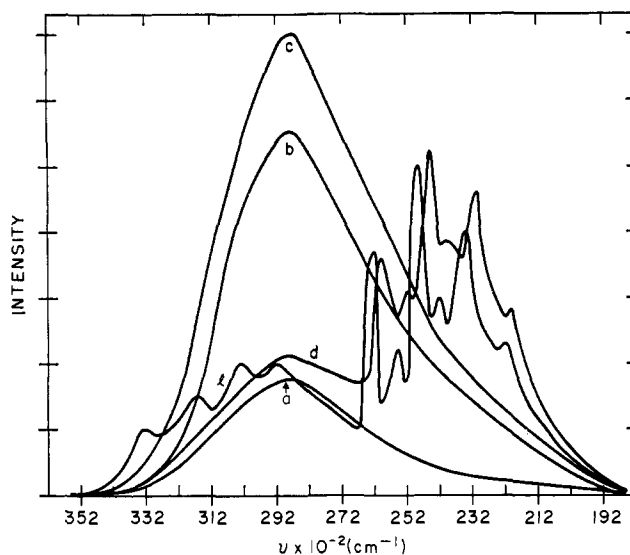


Figure 4. Total emission of 9-$n$-butyladenine at 77°K. in (a) iso-pentane–methylcyclohexane (5:1), (b) hydrocarbon–10% diethyl ether, (c) hydrocarbon–1% triethylamine, (d) hydrocarbon–10% triethylamine, (e) isopentane–butanol (7:3).

the magnitude distinctly decreases with increasing wave length. Notice also (Figure 1) that the phosphorescence spectra of purine and adenine are very similar, except that there is no counterpart in adenine to the highest energy vibrational band of purine. Such a band was searched for carefully in adenine in the region of 28,500 cm.$^{-1}$,[13] but none could be found. These observations, (suppression of the adenine "0–0" band and decrease in polarization magnitude) suggest a vibronic path for the intensification of the triplet → singlet transition.

It has already been shown that the transition moment of the $\pi^* \to \pi$ phosphorescence of a number of N-heterocyclic molecules is perpendicular to the molecular plane.[14–16] Thus, although the low symmetry of the purines ($C_s$) permits direct spin–orbit coupling of the $^3(\pi, \pi^*)$ state with all other states, the major contribution is expected to come from the atomic (one-center) spin–orbit coupling matrix elements with the out-of-plane polarized (n, $\pi^*$) and/or ($\sigma$, $\pi^*$) states.

Several authors have concluded that the broad 260-m$\mu$ band of the purines consists of two $\pi \to \pi^*$ transitions (analogs of $^1L_b$ and $^1L_a$), and that their transition moments are mutually perpendicular.[2–4] The weaker

$^1L_a$ band appears as a shoulder at 240 m$\mu$ in purine and is completely merged with the stronger $^1L_b$ band in the adenines.[2]

The polarized phosphorescence excitation spectra in EPA at 77°K. are presented in Figure 3. The polarization of the triplet–singlet emission is uniformly negative with respect to excitation into the region between 280 and 240 m$\mu$ in all instances. In the case of purine, excitation into the long wave length tail of the absorption gives positive polarization, indicating the existence of a separate electronic transition in the vicinity of 310 m$\mu$.

Since the phosphorescence is polarized negatively to the same extent with respect to excitation into both $\pi \to \pi^*$ bands, it may be concluded that the singlet–triplet transition moment is predominantly perpendicular to the molecular plane in accord with atomic spin–orbit coupling expectations. As the sign of the polarization is unchanged throughout the emission bands, the possible vibronic effects mentioned previously do not alter this conclusion. The transition moment of the 310 m$\mu$ of purine is hence directed out of plane, consistent with an $n \to \pi^*$ promotion. This result strongly confirms the assignment of Clark and Tinoco[2] to this band. Quite likely the n → $\pi^*$ absorptions in the adenine compounds lie under the strong $\pi \to \pi^*$ bands, and are of too low intensity to locate by this method.

Purine forms a negatively charged species by losing its proton from the N-9 position[17] (pH >9). In order to determine the effect of this dissociation on the low-lying states, the emission of the anion in ethanol–NaOH glass at 77°K. was studied. The room-temperature absorption spectrum in this solvent is red shifted by 1268 cm.$^{-1}$ from that of the neutral species in EPA. The phosphorescence spectrum is given in Figure 1. The phosphorescence lifetime of the anion is, to the accuracy of our observations, unchanged from purine itself ($\sim$1 sec.), and the singlet–triplet interval does not change significantly (Table I). These properties are

(13) There is no resonance absorption in this region.
(14) F. Dörr, H. Gropper, and N. Mika, Ber. Bunsenges. physik. Chem., 67, 202 (1963).
(15) F. Dörr and H. Gropper, ibid., 67, 193 (1963).
(16) M. A. El-Sayed and R. G. Brewer, J. Chem. Phys., 39, 1623 (1963).
(17) A. Albert and D. J. Brown, J. Chem. Soc., 2060 (1954).

characteristic of a $(\pi, \pi^*)$ triplet. Owing to the high degree of strain in the ethanol–NaOH glass, high polarizations could not be obtained. However, the polarization is always negative with respect to excitation throughout the region from 310 to 265 m$\mu$, consistent with a $\pi \rightarrow \pi^*$ assignment for the longest wave length absorption band.

Unlike purine itself, the anion fluoresces at $\sim$298°K. in ethanol–NaOH solution ($\nu_{max}$ 27,400 cm.$^{-1}$), in agreement with Borresen's observation.[5] At 77°K., this fluorescence shifts 2000 cm.$^{-1}$ to the blue. The excitation spectrum, however, is identical at both temperatures. Such blue shifts of fluorescence in rigid media are attributed to emission from molecules in a strained environment.[18]

*Emission of 9-n-Butyladenine in Hydrocarbon Media.* The emission spectra of 9-n-butyladenine ($\sim$10$^{-4}$ M) in hydrocarbon alone, and in solutions containing 1% triethylamine (N(Et)$_3$), 10% N(Et)$_3$, and 30% butanol (all at 77°K.) are given in Figure 4. In isopentane–methylcyclohexane (5:1), 9-n-butyladenine exhibits only a weak structureless fluorescence, $\nu_{max}$ = 28,600 cm.$^{-1}$. This emission is intensified by approximately a factor of 4 through the addition of either 1% N(Et)$_3$ or 10% diethyl ether to the solution. In the hydrocarbon–10% N(Et)$_3$ solution, a strong phos-

(18) D. M. Hercules and L. B. Rogers, *J. Phys. Chem.*, **64**, 397 (1960).

phorescence appears (Figure 4d) which is similar to that occurring in EPA, but red shifted by 400 cm.$^{-1}$. In butanol–isopentane (3:7), the fluorescence shifts to the blue by $\sim$1000 cm.$^{-1}$, and again phosphorescence appears strongly (Figure 4e).

The salient feature of these emission spectra is the absence of phosphorescence in hydrocarbon solvent. One possible interpretation is that the hydrocarbon solvent and the low temperature favor the formation of self-associated, hydrogen-bonded complexes. Exciton interaction in the complex leads to electronic energy transfer with a consequent quenching of emission. This phenomenon has, in fact, been observed in the acridine carbazole complex.[19] In the case of 9-n-butyladenine, phosphorescence is much more severely quenched than fluorescence. In strong hydrogen-bonding solvents, association of the solute with the solvent is favored, and phosphorescence from the non-associated species appears. To test this hypothesis, polarization measurements and low-temperature absorption studies are now being carried out on this and related molecules.

*Acknowledgment.* The authors acknowledge helpful conversations and the criticism of Professor Hiroaki Baba.

(19) M. A. El-Bayoumi and M. Kasha, *J. Chem. Phys.*, **34**, 2181 (1961).

# Communications to the Editor

## Carbethoxynitrene. Control of the Stereospecificity of an Addition to Olefins
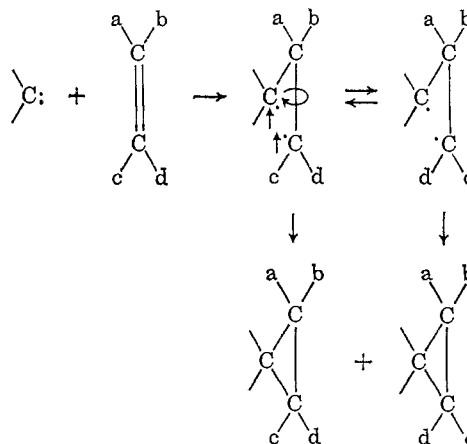
*Sir:*

Investigation of the stereospecificity of carbene addition to olefins has been used frequently to gain information on the mechanism of the reaction, especially to determine whether the addition takes place in one or two steps. Since its introduction by Skell,[1] the reliability and the theoretical basis of the method have been widely discussed.[2] Stereospecific addition is generally interpreted as indicating the reaction of a singlet carbene, while nonstereospecific addition indicates the intervention of a triplet carbene, at least in condensed phase. In the latter case, a triplet diradical is supposed to be the intermediate, and rotation about the former double bond, preceding ring closure, causes nonstereospecificity.

We have investigated the addition of carbethoxynitrene (produced both by photolysis of ethyl azidoformate[3] and by $\alpha$ elimination from N-p-nitrobenzene-



sulfonoxyurethan[4]) to *cis*- and *trans*-4-methylpentene-2.[5] While our work was in progress, Hafner[6] reported the addition of carbomethoxynitrene to *cis*- and *trans*-

(1) P. S. Skell and R. C. Woodworth, *J. Am. Chem. Soc.*, **78**, 4496 (1956).

(2) For a review see: P. P. Gaspar and G. S. Hammond in W. Kirmse, "Carbene Chemistry," Academic Press Inc., New York, N. Y., 1964, p. 258 ff.

(3) W. Lwowski and T. W. Mattingly, Jr., *Tetrahedron Letters*, 277 (1962); *J. Am. Chem. Soc.*, **87**, 1947 (1965).

(4) W. Lwowski and T. J. Maricich, *ibid.*, **86**, 3164 (1964); **87**, 3630 (1965).

(5) The aziridines produced were identified by their infrared and n.m.r. spectra, their elemental analyses, and by comparison with authentic samples prepared by a modification of the method of Hassner [A. Hassner and C. Heathcock, *J. Org. Chem.*, **29**, 3640 (1964); **30**, 1748 (1965)].

(6) K. Hafner, W. Kaiser, and R. Puttner, *Tetrahedron Letters*, 3953 (1964).