progressively slower. As expected for such kinetics,[8] the reciprocal optical density is approximately linear with $(\text{time})^{1/2}$. Most indicative is that the lifetime does not depend on initial concentration. If the fraction of the initial optical density remaining at any time is plotted against time, then the resulting curves for runs differing 30-fold in dose can be superimposed over as much as 70% of the decay.[9]

The rate of neutralization, moreover, depends on temperature. For example, $(\tau_{1/2})_0$, the time for the initial optical density to decrease by 50%, changes from 4 msec to <2 μsec as the temperature is raised from -165 to $-70°$. A phenomenological activation energy of 2–4 kcal/mole is estimated from an observed linear dependence of $\log (\tau_{1/2})_0^{-1}$ on T^{-1}.

Comparison of the electron decay in squalane and in 3-methyloctane, where this species has also been observed, suggests that medium viscosity is an unimportant kinetic factor. At $-150°$ $(\tau_{1/2})_0$ is the same in both systems even though the viscosity of the latter is only $\sim 10^{-10}$ as high as that of the former.[10] Furthermore, the activation energy for decay in either case (~8 kcal/mole in 3-methyloctane) is much less than the respective Arrhenius term for viscosity change.

The observation of an electron absorption at these temperatures implies the persistence of suitable binding sites, even in the thermodynamically stable liquid phase. These sites presumably are always present and do not necessarily correspond to the ultimate stabilization depth achieved following electron trapping. A temperature-dependent, dynamic distribution of sites of different depths, which conceivably widens and shifts to lower energies with increasing temperature, should be considered.

The observation of a G for e_s^- that is smaller than G for total ion pairs[11] means that, unless most of the e_s^- escape detection, only some of the thermalized electrons become solvated. Possibly either the concentration of binding sites is too low or the stabilization process is too slow for solvation to compete effectively against recombination. As a consequence of inefficient trapping, the mean separation distance from the positive ions for the e_s^- is most likely longer than the corresponding distance for the original thermalized electrons.

The observation of an electron decay representing neutralization of correlated, inhomogeneously distributed ion pairs in the 10^{-6}–10^{-3}-sec time range under these conditions, and apparently with negligible viscosity dependence, tends to rule out structural diffusion as a means for migration of charge. It may be that there are in equilibrium bound and unbound electrons, e_s^- and e_m^-, the latter migrating and reacting, or that charge migration occurs by a tunneling mechanism.

The kinetic data obtained in this study, moreover, should provide a means for testing recent theoretical and empirical descriptions[8,12] of such neutralization reactions, since electron concentration–time profiles are directly indicated. Assessment of certain of these theories is in progress.

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Observation of Phosphorescence in Pyridines[1]

Sir:

Pyridine may be considered as the prototype nitrogen heteroaromatic, yet its electronic properties have remained for many years an enigma to the spectroscopist.[2] The apparent absence of phosphorescence has been one of the most puzzling items. The lowest triplet state was observed in absorption[3] under oxygen pressure and labeled $^3A_1(\pi\pi^*)$. The intersystem crossing yield has been measured to be 0.3 by photochemical studies.[4] Moreover, it might be expected that the lowest $\pi\pi^*$ triplet states of pyridine and benzene should have similar radiative capabilities. In spite of attempts by many researchers, using many different samples, methods of purification, and deoxygenating procedures, no authentic phosphorescence of pyridine has been reported.

Since pyridine has several low-lying $n\pi^*$ singlet states, it is possible that the lowest triplet state is in fact $n\pi^*$ and not $\pi\pi^*$. To investigate this possibility a careful study was made of the luminescence properties of a series of substituted pyridines, especially with cyano and methyl groups. We have observed either strong $\pi\pi^*$ (long lifetime) or strong $n\pi^*$ (short lifetime) phosphorescence with appropriate substitution. These results lead to the conclusion that the lowest triplet state in pyridine is the orbitally[5] forbidden $^3A_2(n\pi_5^*)$ state. The explanation of the absence of phosphorescence in pyridine itself, and its presence in substituted pyridines, is then given in terms of specific state ordering.

The low-lying triplet states of pyridine (Figure 1) in orbital classification are $^3A_1(\pi_2\pi_4^*-\pi_3\pi_5^*)$, $^3B_1(n\pi_4^*)$, and $^3A_2(n\pi_5^*)$. The n-orbital electron after excitation is localized partly on the N atom in the $^3B_1(n\pi_4^*)$ state, and on carbon atoms 2,6 in the $^3A_2(n\pi_5^*)$ state.

Methyl substitution at the 2,6 positions should blue shift the A_2 state more than the B_1 state. If the A_2

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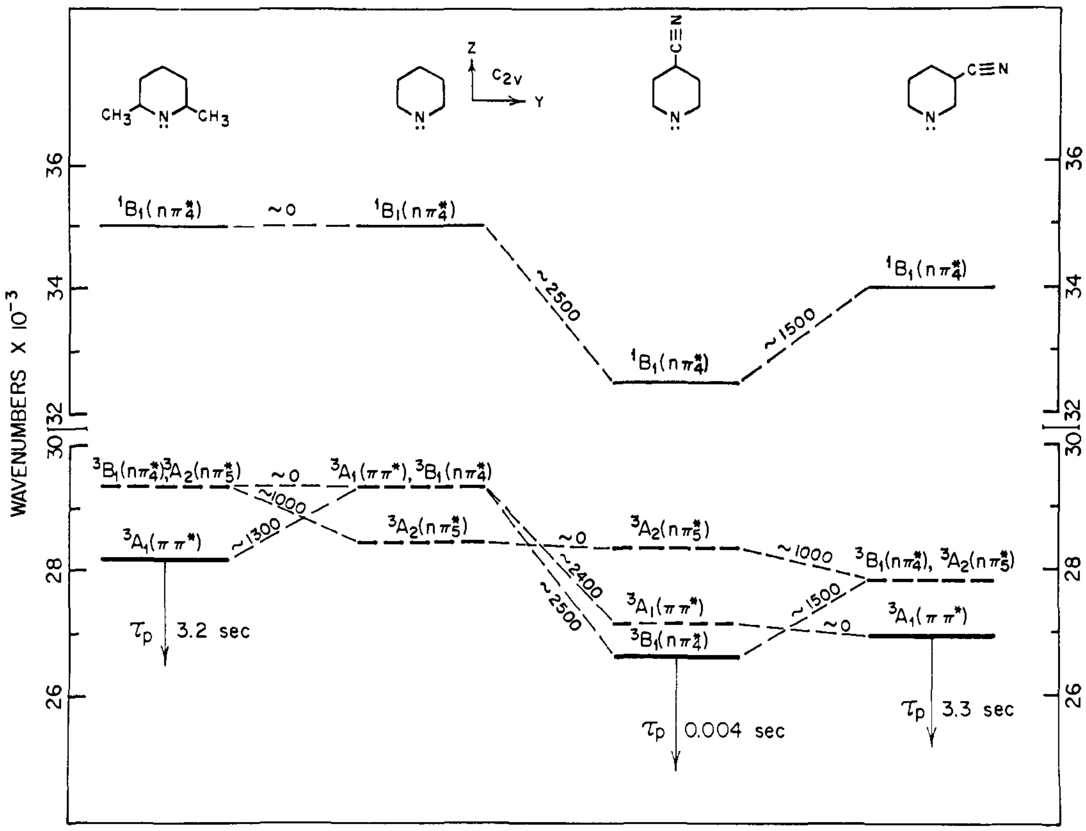

Figure 1. Lowest excited electronic states of pyridines: solid levels, observed triplet and singlet states; dashed levels, extrapolated estimated state positions.

state were 2500 cm^{-1} (or less) lower in energy than the A_1 state, then 2,6-dimethyl substitution could invert the state ordering (Figure 1) (assuming 500-cm^{-1} blue shift for the A_2 state and 650-cm^{-1} red shift for the A_1 state, per CH_3 group). Experimentally 2,6-lutidine yields strong phosphorescence (band onset 28,160 ± 200 cm^{-1}, ethanol glass, 77°K) with a lifetime of 3.2 sec. Fluorescence (probably $\pi\pi^*$) is observed in alcohol glass but not hydrocarbon glass.

Cyano substitution red shifts benzene phosphorescence 2400 cm^{-1}. The pyridine $^1B_1(n\pi_4^*)$ state is red shifted 2500 cm^{-1} by 4-cyano substitution and *ca.* 1000 cm^{-1} for 2- and 3-cyano. We shall assume that the corresponding triplet state $^3B_1(n\pi_4^*)$ is shifted equivalently. The $^3A_2(n\pi_5^*)$ state should not be shifted significantly by 4-cyano substitution because of the π_5^* node, while a 3-cyano group should red shift this state by about 1000 cm^{-1}. Thus, if we assume that the $^3B_1(n\pi_4^*)$ and $^3A_1(\pi_2\pi_4^*-\pi_3\pi_5^*)$ states are nearly degenerate (as reasonable values of S–T splits predict) and the $^3A_2(n\pi_5^*)$ state is some 2000 cm^{-1} lower in energy, in 4-cyanopyridine $^3B_1(n\pi_4^*)$ should lie lowest, and in 3-cyanopyridine $^3A_1(\pi\pi^*)$ should be the lowest triplet (Figure 1). Experimentally, strong phosphorescence (diffuse) is observed in 4-cyanopyridine with a lifetime of 0.004 sec (band onset 26,650 ± 200 cm^{-1}, ethanol glass, 77°K); for 3-cyanopyridine strong phosphorescence (structured) is observed with a lifetime of 3.3 sec (0, 0 band at 26,946 ± 200 cm^{-1}, ethanol glass, 77°K). We assign the short lifetime phosphorescence of 4-cyanopyridine to the $^3B_1(n\pi_4^*)$ state, and the long-lifetime phosphorescence of 3-cyanopyridine to the $^3A_1(\pi_2\pi_4^*-\pi_3\pi_5^*)$ state.

The lowest triplet state of pyridine therefore must be the forbidden $^3A_2(n\pi_5^*)$ state, and must be about 28,000 cm^{-1} above the ground state. The absence of phosphorescence from the $^3A_2$ state is attributable to an exceptionally low *radiative* transition probability, and a high rate of *radiationless* transition to the ground state. It is easily shown that only ^1L_b and ^1B_b $\pi\pi^*$ states mix with the $^3A_2(n\pi_5^*)$ state *via* spin-orbital coupling. The out-of-phase coupling of $^3A_2$ with this pair of singlet states results in a nearly vanishing borrowed transition moment for radiation. This is in marked contrast to spin-orbital coupling for the $^3B_1(n\pi_4^*)$ state. Radiationless transition rates for the $^3A_2(n\pi_5^*)$ state to the ground state are contrariwise expected to be large owing to large expected molecular distortions, with expected large magnitudes for Franck–Condon factors.[6,7] The small spacing between triplet states would also be expected to lead to very large vibronic coupling between the states.[8]

Owing to the node through the nitrogen atom in the π_5^* orbital, a small singlet–triplet splitting in the $A_2(n\pi_5^*)$ state is expected. This suggests that the lowest singlet state of pyridine might be $^1A_2(n\pi^*)$. We have looked for the possible corresponding low-energy absorption but have not found a new band. The absorption is predicted to be exceedingly weak, and this may be why none was observed in pyridine (at 1.0 *M* in 10 cm of 3-methylpentane). However, the extensive

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charge rearrangement in this state may give a much larger singlet–triplet splitting than expected (*i.e.*, large electron correlation effects).

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Selective Phosphorylation of the 5'-Hydroxy Groups of Thymidine and Uridine

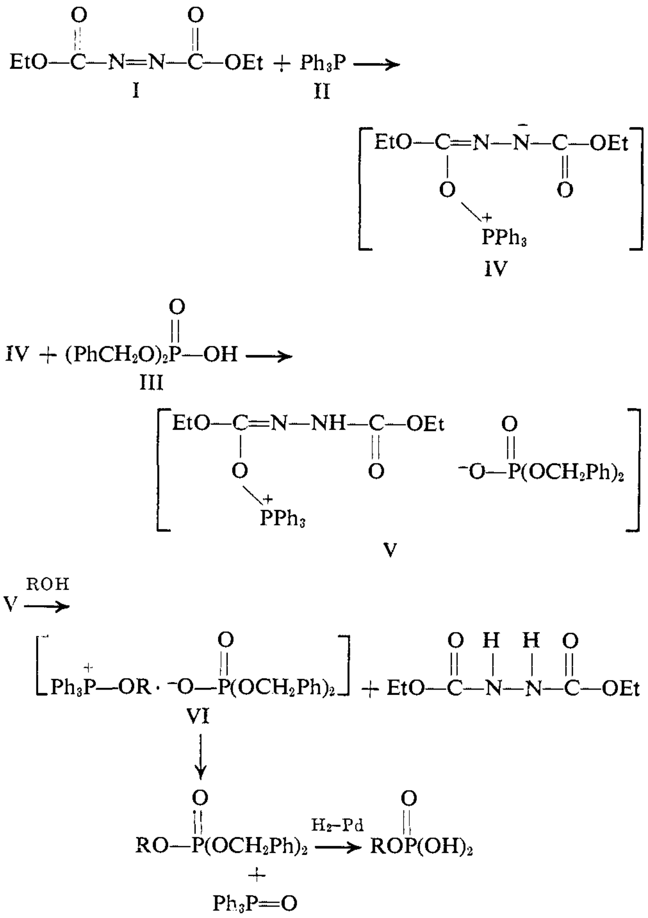
Sir:

An obvious disadvantage in the use of protected nucleosides for the syntheses of nucleotides and oligonucleotides is that undesirable side reactions take place in some instances during the conditions necessary for phosphorylation or for the removal of the protecting groups.[1-4] It would therefore be desirable to develop selective phosphorylation of unprotected nucleosides in connection with the specific synthesis of oligonucleotides. We wish to report the selective phosphorylation of the 5'-hydroxy groups of thymidine and uridine.

A number of other laboratories have reported the direct phosphorylation of unprotected nucleosides and nucleotides.[5-9] Thus, Tener[5] has reported that the phosphorylation of unprotected deoxyribonucleosides by the use of dicyclohexylcarbodiimide and 2-cyanoethyl dihydrogen phosphate resulted in the formation of the corresponding 5'-phosphates as major products. Similar results have been obtained in the ribonucleotide series.[6,7] Weimann and Khorana[10] have demonstrated that the rate of phosphorylation of the secondary hydroxy group of 5'-O-tritylthymidine is somewhat slower than that of the primary hydroxy group of 3'-O-acetylthymidine. Recently, the selective phosphorylations of thymidine and ribonucleosides by means of dibenzyl phosphorochloridate[8] and pyrophosphoryl chloride,[9] respectively, have been reported. Further, the 5'-hydroxy group of nucleoside is sulfonylated more readily than the 3'-hydroxy group.[8,11]

We have studied the phosphorylation of alcohols with diethyl azodicarboxylate (I), triphenylphosphine (II), and dibenzyl hydrogen phosphate (III), giving corresponding alkyl dibenzyl phosphates. A mechanistic pathway for this reaction involving quaternary phosphonium salts (IV–VI) is presumed to be as in Scheme I.[12]

Scheme I



It is well established that trityl chloride mainly attacks the 5'-hydroxy group of nucleosides to give corresponding 5'-O-trityl nucleosides. The selectivity of the tritylation of the 5'-hydroxy group of nucleosides is explained by the steric hindrance of the triphenylmethyl group. Since the phosphorus cation in the intermediate V similarly has three bulky phenyl groups, nucleophilic attack at the 3'-hydroxy group of the nucleoside would be expected to be much more hindered than that at the 5'-hydroxy group, the corresponding nucleoside 5'-phosphate being predominantly formed.[13]

A mixture of thymidine (242 mg, 1×10^{-3} mol), triphenylphosphine (393 mg, 1.5×10^{-3} mol), and dibenzyl hydrogen phosphate (417 mg, 1.5×10^{-3} mol) in dry tetrahydrofuran (THF, 1 ml) was stirred, and diethyl azodicarboxylate (261 mg, 1.5×10^{-3} mol) in dry THF (1 ml) was added at room temperature. Stirring was continued for 3 hr, and the solution was then kept standing overnight at room temperature. After the solution was concentrated, the residue was dissolved in 75% ethanol and hydrogenated on palladium. After absorption of hydrogen had ceased, the catalyst was removed by filtration. Paper chromatography (1-propanol–2 *N* HCl, 3:1) of the filtrate revealed two uv-absorbing components; one was the unreacted thymidine (R_f 0.86) and the other was thymidine 5'-phosphate (R_f 0.81). The filtrate was concentrated to a small bulk and adjusted to pH 7.5 by adding 1 *N* barium hydroxide solution. After removal of a precipitate by centrifugation,

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