

rotary dispersion data and molecular models are consistent with a proposed conformation whereby the phosphate-metal moiety folds back onto the rest of the molecule so as to form a chelate-type structure by binding of the metal ion to the amino group and N-7 of the adenine ring.⁴ Since the nuclear magnetic resonance spectrum of ATP has been determined recently,⁵ it occurred to us that the structure of metal-ATP complexes could be investigated readily utilizing this experimental method. As will be shown, our results indicate that the proposed chelate conformation is extremely unlikely.

Experimental.—Solutions of 0.2 M ATP were prepared in D₂O using the disodium salt obtained from Nutritional Biochemicals Corp. The *pD* of the solution (as measured with a glass electrode) was adjusted with solid Na₂O₂ to about 9.6 so as to insure an uncharged amino group and completely ionized phosphate groups. Solid MgCl₂ or CaCl₂ then was added to a final concentration of 0.1 or 0.2 M MgCl₂ or 0.1 M CaCl₂. Nuclear magnetic resonance spectra were measured with a standard 60 megacycle high resolution spectrometer from Varian Associates (V 4300 B, equipped with a superstabilizer and a field homogeneity control unit) immediately after making up the solutions.

Results and Discussion.—The chemical shifts (in cycles per second) of the C₈, C₂ and C₁' protons with respect to the proton resonance of the residual water are presented in Table I. The standard

TABLE I
PROTON CHEMICAL SHIFTS IN ATP

	H ₈	H ₂	H ₁ '
ATP	-217	-193	-73.2
MgATP	-215	-192	-72.4
CaATP	-215	-191	-71.4

deviation of the many spectra analyzed was about ± 2 cps. Within experimental error, the chemical shifts are identical for all the samples. (The chemical shifts for ATP are also consistent with those obtained by Jardetzky and Jardetzky⁵ if the fact that their spectra were obtained at 40 megacycles rather than 60 megacycles is taken into account.) Since protonation of the pyrimidine ring in ATP results in a shift of the H₂ peak 26 cps. to lower field (at 60 Mc) and protonation of N-7 in guanosine triphosphate shifts the H₈ resonance 54 cps. to lower field (with a corresponding but smaller shift in the H₁' peak),⁵ it seems highly improbable that either Mg⁺⁺ or Ca⁺⁺ is significantly associated with the adenine portion of the ATP molecule. A word of caution should be inserted here: this interpretation assumes implicitly that adding positive charge with Mg⁺⁺ would produce the same general effect as adding a proton to one of the rings. Since the protonic charge is thought to distribute itself throughout the pyrimidine ring in ATP,⁶ Mg⁺⁺ might conceivably form a loose type of complex with the amino group where no charge transfer to the pyrimidine ring occurs;

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

(6) R. M. Bock in "The Enzymes," (P. D. Boyer, H. Lardy, K. Myrback, ed.), **2**, Academic Press, Inc., New York, N. Y., 1960, pp. 3-38.

thus the chemical shift of the C₂ proton would not be affected. Although such a situation cannot be ruled out, the possibility of forming a stable conformation without some appreciable charge transfer seems slight. A similar situation is also possible for the N-7 site, but it is even more difficult to think of a type of chemical bonding leaving the chemical shift of the C₈ proton unaltered.

These results indicate, therefore, that the structure of the metal-ATP complex in solution is likely to be somewhat extended. This offers the attractive possibility that the amino group and/or N-7 and the metal-phosphate moiety can provide separate binding sites for enzymes. Further experiments are being planned to investigate the effect of an ATP-requiring enzyme on the n.m.r. spectrum.⁷

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(7) Since our original manuscript was submitted, a recent communication [K. Hotta, J. Brahm and M. Morales, *J. Am. Chem. Soc.*, **83**, 997 (1961)] has interpreted titration curves of ATP obtained spectrophotometrically in terms of a curled structure involving metal binding at the pyrimidine ring. The presence of Mg⁺⁺ caused a lowering of the apparent *pK* of the amino group (~ 4.5). The n.m.r. spectra at *pD*'s below about 5 are also changed by Mg⁺⁺—a striking feature being the marked broadening of the C₁ and C₃ proton resonances. The lowering of the apparent *pK* by Mg⁺⁺ is similar to that caused by a shortening of the phosphate chain: *cf.* ATP vs. ADP vs. AMP; in the latter two substances a folded structure is not possible. Therefore the observed ultraviolet and n.m.r. spectral changes are probably due to the disturbance of a water bridge structure between the amino and phosphate groups. Since at higher *pD*'s (>6) the n.m.r. (and apparently ultraviolet) spectra are unchanged by the presence of Mg⁺⁺, the assumption of a folded conformation on the basis of the above mentioned data is not warranted.

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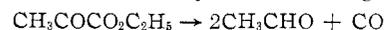
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DIRECT AND SENSITIZED PHOTOLYSIS OF ETHYL PYRUVATE

Sir:

We have observed that irradiation of benzene solutions of ethyl pyruvate leads to photolysis of the α -ketoester. The principal products are carbon monoxide and acetaldehyde, indicating the path



A small amount of carbon dioxide also is produced.

It was of interest to learn whether or not the fragmentation reaction involved a singlet excited state or a long-lived triplet. The latter possibility is suggested by the fact that irradiation of ethyl pyruvate in ether-pentane-alcohol glass at 77° K. gives rise to a weak, blue phosphorescence. In order to establish the decomposition mechanism we used benzophenone as a photosensitizer for the decomposition of the ester. Hammond, *et al.*,¹ and Bäckström and Sandros² have shown independently that excitation of benzophenone in solution is followed by quantitative intersystem crossing; therefore, the chemically significant excited state

(1) G. S. Hammond and W. M. Moore, *J. Am. Chem. Soc.*, **81**, 6334 (1959); G. S. Hammond, W. M. Moore and R. P. Foss, *ibid.*, in press.

(2) H. L. J. Bäckström and K. Sandros, *J. Chem. Phys.*, **23**, 2197 (1955).

of benzophenone is a triplet. It is reasonable to expect that transfer of energy from benzophenone triplets to other molecules will excite the latter to triplet states. In fact, the observation that just such a process occurred with biacetyl was a key part of the study by Bäckström and Sandros,² who observed induced phosphorescence of biacetyl. The use of benzophenone and other sensitizers to effect selective excitation of other molecules to triplet states offers both a new tool for the study of mechanism and the possibility of effecting new photochemical reactions. A nice example seems to be provided by the recent report by Schenck and Steinmetz³ that benzophenone serves as a photosensitizer for the addition of maleic anhydride to benzenoid nuclei.

Table I contains the results of a series of experiments in which solutions of ethyl pyruvate were irradiated (a) with no other solute present, (b) in the presence of excess benzophenone and (c) in the presence of both benzophenone and benzhydrol. Appropriate filters were used, and concentrations were adjusted so that 5% or less of the light was absorbed by ethyl pyruvate in the experiments with benzophenone. Sensitized decomposition of ethyl pyruvate not only occurs but has a higher quantum yield than the direct excitation process. Decomposition of the ester must involve triplet states in the sensitized experiment, and the evidence that direct photolysis follows the same path is presumptive. It is also certain that production of the triplet state of ethyl pyruvate by energy transfer from benzophenone triplets is a more efficient process than the intersystem crossing from the excited singlet produced by direct excitation of the ketoester. Additional evidence for the efficiency of intermolecular energy transfer is quenching of photoreduction of benzophenone by benzhydrol in the presence of ethyl pyruvate. The quantum yield for disappearance of benzophenone becomes immeasurably small in the presence of 0.050 *M* ethyl pyruvate.

TABLE I
DIRECT AND SENSITIZED PHOTOLYSIS OF ETHYL PYRUVATE
IN BENZENE SOLUTION

Ethyl pyruvate mole l. ⁻¹	Benzophenone mole l. ⁻¹	Benzhydrol mole l. ⁻¹	ϕ_{ketone}	ϕ_{ester}
0.050	none	none	...	0.17
.050	none	none14
.050	0.100	none32
.010	.050	0.50	0.11	.27
.050	.100	.50	.00	.31
none	.100	.50	.80	...

A corollary of the theory presented is the prediction that triplet states which have excitation energies much lower than ethyl pyruvate triplets should be incapable of acting as sensitizers.⁴ The phosphorescence of β -acetonaphthone is green (maximum at 5100 Å.) so the triplets must

(3) G. O. Schenck and R. Steinmetz, *Tetrahedron Letters*, No. 21, 1 (1960).

(4) It is entirely possible that even though energy transfer is slightly uphill, the process may still be fairly efficient. We know only that benzophenone and ethyl pyruvate triplets have similar energies because of the qualitative similarity in the color (but not intensity) of their phosphorescence.

lie lower than ethyl pyruvate triplets. Irradiation of a benzene solution containing ethyl pyruvate and excess β -acetonaphthone results in no decomposition of the ketoester. The ketone acts only as an internal filter, thereby preventing direct photolysis of the ester.

Experimental: Determination of Quantum Yields.—The apparatus has been described previously.¹ The filter system consisted of a Corning 7-54 glass filter in series with a solution containing nickel sulfate and cobaltous sulfate. The transmission maximum is close to 3100 Å.; 2% of incident 3660 Å. light is transmitted. All reaction mixtures were degassed. Residual ethyl pyruvate was determined by vapor chromatography and benzophenone was determined spectrophotometrically.

Reaction Products.—One hundred milliliters of a solution containing 0.2 *M* benzophenone and 0.1 *M* ethyl pyruvate in toluene was irradiated in a Hanovia Type S 200-watt quartz immersion apparatus for 4 hr. using a filter with a cutoff at 2800 Å. A total of 175 ml. of gas, non-condensable at -78° , was collected. Infrared analysis of the gas showed it to be 90% carbon monoxide and 10% carbon dioxide. The liquid residue then was warmed to 40° , causing distillation of a volatile liquid which was shown by infrared analysis to be acetaldehyde. In other experiments the liquid residue was sampled directly and analyzed by vapor chromatography. Only one new constituent, acetaldehyde, was detected.

CONTRIBUTION No. 2686

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PHOTOSENSITIZED *CIS-TRANS*
ISOMERIZATION OF THE PIPERYLENES

Sir:

We have observed that a number of carbonyl compounds may be used as efficient photosensitizers for the *cis-trans* isomerization of piperylene (1,3-pentadiene). Thus, irradiation of a benzene solution originally containing 0.12 *M* benzophenone and 0.20 *M cis*-piperylene in a Hanovia quartz immersion reactor, using a filter with a cutoff at 2800 Å., converts the diene to a photostationary mixture within three hours. In this mixture the *trans/cis* ratio is about 1.3. A solution containing 0.05 *M* benzophenone and 0.10 *M cis*-diene was irradiated in the apparatus used in quantitative studies¹ for a time sufficient to convert 21% of the *cis*-diene to its *trans* isomer. The quantum yield calculated from the data was 0.29. The actual quantum yield must be slightly higher since the reverse reaction reduces the amount of net conversion. A similar mixture, except for the inclusion of 0.5 *M* benzhydrol, was irradiated and the rate of disappearance of benzophenone measured. The quantum yield for ketone destruction was 0.09

(1) G. S. Hammond and W. M. Moore, *J. Am. Chem. Soc.*, **81**, 6334 (1959); G. S. Hammond, W. M. Moore and R. P. Foss, *ibid.*, in press.