present in small amounts in the above dismutation reaction. Solutions of ADP or $AP_4$ in anhydrous pyridine also undergo rapid dismutation to products containing both longer and shorter polyphosphate chains. The reaction is not, however, unique to nucleoside 5′-polyphosphates since a similar dismutation readily occurs with *p*-nitrobenzyl triphosphate[4] in pyridine to give the corresponding mono- to pentaphosphates.

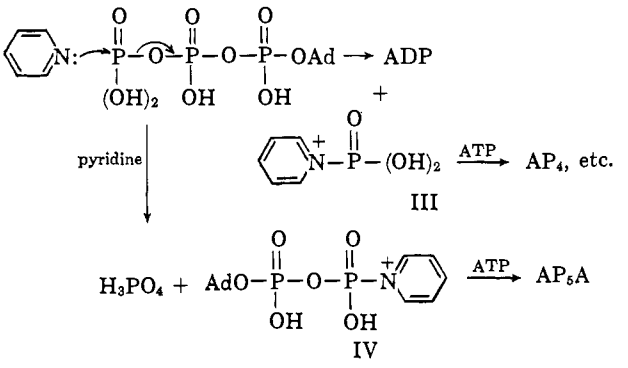
The addition of five molar equivalents of tributylammonium phosphate or pyrophosphate to the reaction mixture greatly changes the product pattern and the ATP then is converted quite rapidly into ADP and then AMP with very little formation of higher polyphosphates. This explains the degradation of initially formed product during attempted synthesis of ATP from adenosine 5′-phosphoromorpholidate and an excess of pyrophosphate in pyridine.[1,2]

The addition of small amounts of water (1–5%) to the pyridine also results in a marked decrease in the amounts of higher polyphosphates (ATP and higher) and an accumulation of ADP and AMP. A similar effect is observed on conducting the reaction in pyridine containing a large molar excess of an alcohol. In the presence of 100 molar equivalents of *p*-nitrobenzyl alcohol, for example, after 6 days, the reaction mixture contained 20% AMP, 61% ADP, 15% ATP, 3% $AP_4$, and one molar equivalent of *p*-nitrobenzyl phosphate. In the presence of methyl alcohol, the accumulation of methyl phosphate was observed.

Some insight into the over-all path of the reaction was achieved through studies of the dismutation of specifically $P^{32}$-labeled ATP. Phosphorylation of 2′,3′-O-isopropylidene adenosine with $P^{32}$-cyanoethyl phosphate[5,6] gave $P^{32}$-AMP which was converted into $\alpha$-$P^{32}$-ATP *via* reaction of the phosphoromorpholidate[1] with pyrophosphate in anhydrous dimethyl sulfoxide.[7] Dismutation of this material in pyridine gave the usual spectrum of products all having identical specific activities (c.p.m./adenosine) and giving $P^{32}$-AMP and no observable labeled inorganic phosphates on degradation with purified venom phosphodiesterase. Thus, the $\alpha$-phosphorus atom is not separated from the adenosine moiety during dismutation. On the other hand, exclusively $\gamma$-$P^{32}$-ATP[8] gives rise to a mixture of nucleoside 5′-polyphosphates which, after ion-exchange separation, contain nearly equal amounts of $P^{32}$ in the $\gamma$-, $\delta$-, and $\epsilon$-positions. Roughly 10% $P^{32}$ was found in the $\beta$-phosphorus of ADP, but no label was present in the $\alpha$-position. The distribution of label in the various phosphorus atoms was determined by controlled degradation of the nucleoside 5′-polyphosphate with *E. coli* alkaline phosphatase. Thus, for example, treatment of $AP_4$ (0.5 $\mu$mole) with 2.2 $\mu$g. of the purified enzyme at pH 8 for 50 min. at 35° gave roughly equal amounts of adenosine, AMP, ADP, ATP, and $AP_4$ which were cleanly separated on a micro ion-exchange column. The relative specific activities of the last three compounds were 0.16:1.00:2.04 showing equal labeling of the $\gamma$- and $\delta$-phosphorus atoms and relatively little in the $\beta$-position. Thus it is clear that the higher polyphosphates are built primarily by transfer of the terminal phosphate from ATP (or perhaps from other nucleoside polyphosphates).

In contrast to the ready reaction of ATP in pyridine, the $\gamma$-monomethyl ester of ATP, which was prepared in good yield by the reaction of triethylammonium ATP in methanol with dicyclohexylcarbodiimide, was largely unchanged, and independently synthesized[9] $AP_3A$ and $AP_4A$ were completely inert.

A definitive clue as to the mechanism of the reaction comes from the observation that whereas ATP shows very similar patterns of dismutation in pyridine and in $\beta$- and $\gamma$-picolines, it is completely stable in $\alpha$-picoline. A similar, but less dramatic, stability is found in quinoline as compared with isoquinoline. Since the various picolines are of very similar basicities, these observations are taken to indicate a steric hindrance of the dismutation reaction in $\alpha$-picoline. We propose a mechanism for the reaction involving nucleophilic attack of the pyridine nitrogen upon the terminal phosphorus atom of ATP (or of other polyphosphates) with formation of ADP and of the presumably very reactive phosphorylating species III which then reacts with unchanged ATP to form $AP_4$. In a similar way III can react with $AP_4$ to give $AP_5$, etc. The inhibition of the normal build up of higher polyphosphates upon the addition of excess inorganic orthophosphate or *p*-nitrobenzyl alcohol is explained by preferential attack on III by these species with the formation of the observed inorganic pyrophosphate and *p*-nitrobenzyl phosphate, respectively. The appearance of small amounts of $AP_2A$, $AP_3A$, $AP_4A$, etc., must indicate the occurrence of a less favored attack by pyridine upon the $\alpha$- or $\beta$-phosphorus of ATP with release of an inorganic phosphate and formation of an activated nucleotide, *e.g.*, IV. The latter can then form diesters by reaction with a nucleoside polyphosphate.




From a synthetic point of view, it is important that the presently described dismutation reactions can be avoided by use of solvents such as dimethyl sulfoxide in which ATP is stable,[7,8] rather than the commonly used pyridine. Under these conditions many complicating features of synthetic reactions involving nucleoside polyphosphates can be obviated.

(4) Prepared by reaction of *p*-nitrobenzyl phosphoromorpholidate with pyrophosphoric acid in anhydrous dimethyl sulfoxide.

(5) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

(6) An improved synthesis of this compound will be described shortly (K. E. Pfitzner and J. G. Moffatt, in preparation).

(7) J. G. Moffatt, *Can. J. Chem.*, in press.

(8) D. L. M. Verheyden, W. E. Wehrli, and J. G. Moffatt, *J. Am. Chem. Soc.*, **86**, 1253 (1964).

(9) J. R. Reiss, D. L. M. Verheyden, and J. G. Moffatt, unpublished results.

(10) Financial support for this work from the "Stiftung für Stipendien auf dem Gebiete der Chemie" (Switzerland) is gratefully acknowledged.

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## Hydrogen Bonding Effects on Triplet Energy Transfer in Solution

*Sir:*

We wish to report a pronounced hydrogen bonding effect on the triplet energy transfer in the benzophenone–biacetyl system. Previous authors working on

this[1] or similar[2] systems used nonpolar solvents such as hexane or benzene. Bäckström and Sandros observed that pure alcohols are poor solvents for energy transfer in benzophenone–biacetyl, probably due to the quenching effect[3] of alcohols on biacetyl.

In this work benzophenone and 4,4′-dimethylbenzophenone are the triplet donors and biacetyl is the acceptor. The solvents are hexane and mixtures of hexane and alcohols. The results are summarized in Fig. 1. It can be seen from the figure that a pronounced increase in biacetyl phosphorescence occurs with the addition of small amounts of alcohol to hexane. The maximum intensity is at about 0.5 vol. % of alcohol, and is more or less independent of the simple alcohols chosen. At high alcohol concentrations, the energy transfer becomes quite inefficient.

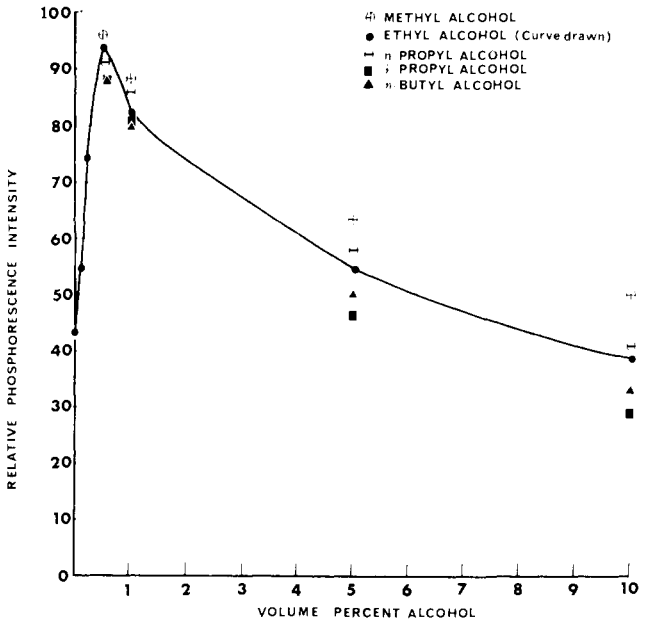


Fig. 1.—Biacetyl phosphorescence intensity (λ 524 mμ) at 25° *vs.* volume per cent alcohol in the hexane–alcohol solvent, $\lambda_{EX}$ 366 mμ. Concentration of 4,4′-dimethylbenzophenone = $1.70 \times 10^{-2}$ *M*; concentration of biactyl = $6.90 \times 10^{-3}$ *M*. Curve drawn through ethyl alcohol points. Similar results are obtained for the benzophenone–biacetyl system.

Since the donor benzophenones do not luminesce in fluid solutions at room temperature, phosphorescence spectra were obtained in rigid methylcyclohexane–isopentane and methylcyclohexane–isopentane–alcohol mixtures at 77°K. The results are shown in Fig. 2. Although the phosphorescence intensity continues to increase past the addition of about 1% alcohol, by far the most pronounced change in intensity occurs when less than 1% alcohol is added. Some emission shifts to shorter wave lengths are also observed upon the addition of alcohol to these systems. No such pronounced effect is observed with biacetyl at low temperatures, and at room temperatures we have verified that alcohols quench[3] the biacetyl phosphorescence. Energy transfer from benzophenone to biacetyl is also markedly enhanced when small amounts of alcohol are added to methylcyclohexane–isopentane at 77°K., but will be reported at a later time.

(1) H. Bäckström and K. Sandros, *Acta Chem. Scand.*, **14**, 48 (1960).

(2) (a) J. Dubois and B. Stevens, "Luminescence of Organic and Inorganic Materials," John Wiley and Sons, Inc., New York, N. Y., 1962, p. 115; (b) J. Dubois and M. Cox, *J. Chem. Phys.*, **38**, 2536 (1963); (c) J. Dubois and F. Wilkinson, *ibid.*, **38**, 2541 (1963).

(3) H. Bäckström and K. Sandros, *Acta Chem. Scand.*, **12**, 823 (1958).

Preliminary studies of biacetyl phosphorescence intensity *vs.* donor concentration show that the slopes of the curves are also increased when small amounts of alcohol are added to hexane. A study that remains to be performed is direct lifetime measurements of benzophenones in the presence of small amounts of alcohol.

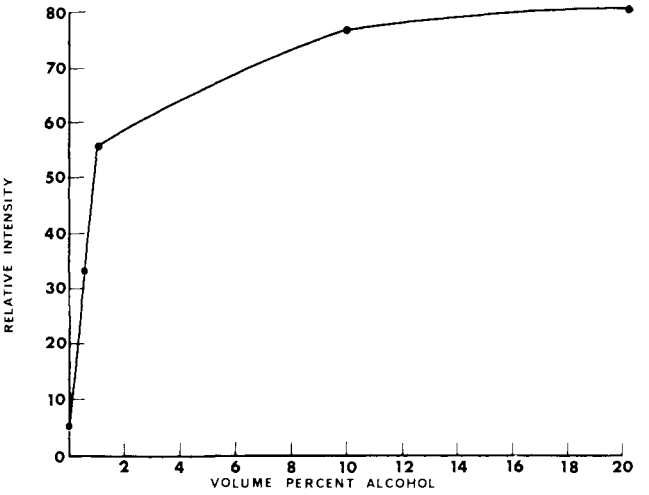


Fig. 2.—Relative phosphorescence intensity of 4,4′-dimethylbenzophenone at 77°K. *vs.* volume per cent alcohol in the methylcyclohexane–isopentane–alcohol solvent, λ (peak) 446–452 mμ, $\lambda_{EX}$ 366 mμ. Concentration of 4,4′-dimethylbenzophenone = $1.70 \times 10^{-3}$ *M*.. Similar results are obtained for the benzophenone system.

An increase in the benzophenone lifetime associated with intermolecular hydrogen bonding would partially account for these results. Since the viscosity of the solvent increases upon addition of alcohol to hexane as does the amount of biacetyl quenching, the energy transfer efficiency must eventually decrease when the viscosity and quenching effects overcome the hydrogen bonding effect, since the process is at least partly diffusion controlled[4,2] in a fluid medium. Intermolecular hydrogen bonding may also impart some additional solution structure to the system, enabling energy transfer to occur over greater distances than the $R_0$ predicted by the Förster theory.[5] In those systems studied it appears that the major hydrogen bonding contribution is to the donor and not to the acceptor. Similar results are obtained with an isooctane–alcohol solvent system.

Further work is presently in progress on the above points.

(4) W. Melhuish, *J. Phys. Chem.*, **67**, 1681 (1963).

(5) T. Förster, *Discussions Faraday Soc.*, **27**, 7 (1959).

(6) Esso Educational Foundation Fellow, 1962–1963.

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## Classification of Alcohols by Nuclear Magnetic Resonance Spectroscopy

*Sir:*

In the common n.m.r. solvents, deuteriochloroform and carbon tetrachloride, alcohols are often sparingly soluble, and their hydroxyl resonances are obscured by methylene and methyl resonances. The traces of acid always present in these solvents catalyze