to the comparable positions in $6\cdot^-$ (*cf.* the α hfs), the effect could not even qualitatively be attributed to increased spin density at, *e.g.*, positions 1 and 6 in $1\cdot^-$, relative to those same positions in $6\cdot^-$. Noting that the odd electron in each of **1–3**$\cdot^-$ occupies a symmetrical orbital just as in the case of the indane radical the impressive enhancement of a_β in these three species must surely also be attributed to 1,3 p–σ interactions of very considerable potency.

Systems **1–3**$\cdot^-$ are exceptionally well constituted for a quantitative treatment of the relative magnitudes of 1,3 *vs.* 1,2 p–σ overlap. Equation 1 is an elaborated version

$$a_{CH_2} = B_2(c_i \cos\theta_i + \lambda c_j \cos\theta_j)^2 \quad (1)$$

of Whiffen's equation[3] giving the hfs of a proton engendered by its interactions with spin from two sites (i and j) in terms of the MO coefficients c_i and c_j of the singly occupied MO, the dihedral angles θ_i and θ_j, and the relative overlap efficiency λ of the two interactions. In the present case the overlap with site i is 1,2 and that with site j is 1,3. Further, in the present instances $c_i = \pm c_j$, $\theta_i = 26.5°$, and $\theta_j = 0°$, thus leading to eq 2.

$$a_{CH_2} = B_2c_i^2(0.89 \pm \lambda)^2 = B_2\rho_i\,(0.89 \pm \lambda)^2 \quad (2)$$

The values of a_{CH_2} are experimental; the values of $B_2\rho_i$ were determined as follows. First, an approximate $B_2\rho_i$ was obtained from the a_{CH_3} of the appropriate methyl analog **6–8**$\cdot^-$ and the simple relation $a_{CH_3} = {}^1/_2B_2\rho_i$. Then differences in spin distribution between **1–3**$\cdot^-$ and **6–8**$\cdot^-$ were corrected for by subtracting the sum of the α hfs of the olefinic or aromatic protons at unsubstituted positions of **1–3**$\cdot^-$ from the corresponding sum for **6–8**$\cdot^-$ and adding this difference to the approximate $B_2\rho_i$. Equation 2 can then be solved independently for λ for each of the three systems (**1–3**$\cdot^-$). For $1\cdot^-$, $a_{CH_2} = 5.30$ G, $B_2\rho_i$ (approx) = 4.00 G, $B_2\rho_i = 3.84$ G, and $\lambda = 0.31$. For $2\cdot^-$, $a_{CH_2} = 5.85$, $B_2\rho_i$ (approx) = 3.50, $B_2\rho_i = 4.04$, and $\lambda = 0.31$. For $3\cdot^-$ $a_{CH_2} = 7.65$, $B_2\rho_i$ (approx) = 4.00, $B_2\rho_i = 5.12$, and $\lambda = 0.33$. These three values agree reassuringly well; the value $\lambda = 0.32$ has been adopted for further use. In conclusion, *at the prevailing 1,3 C–C distances in cyclobutanes* **1–3**$\cdot^-$, *optimal* ($\theta_j = 0°$) *1,3 p–σ overlap is 0.32 as efficient as optimal* ($\theta_i = 0°$) *1,2 p–σ overlap.*

That 1,3 p–σ overlap is indeed the correct explanation for the observed enhancement in **1–3**$\cdot^-$ is also vividly attested by results from the anion radical of bicyclo[6.2.0]deca-1,3,5,7-tetraene ($4\cdot^-$). As with parent cyclooctanetetraene (COT), *s*-$4\cdot^-$ and *a*-$4\cdot^-$ are degenerate in the HMO approximation. From eq 2, their respective hfs should be 9.37 and 2.05 G. This greater than fourfold disparity is, incidentally, *solely* the result of 1,3 overlap. A rapidly equilibrating 50:50 mixture of *s*-$4\cdot^- \rightleftarrows$ *a*-$4\cdot^-$ should have $a_{CH_2} = 5.71$ G. The result is, interestingly, that the 1,3 effect is exactly expunged, giving an ideal model for cyclobutyl behavior in the absence of the 1,3 effect. The experimental value of $a_{CH_2} = 5.40$ G (electrochemical, acetonitrile, *n*-Bu_4NClO_4, ambient) is in relatively good agreement. The enhancement of 68% compared to the free rotation value is virtually exactly that predicted for a purely conformational effect. The olefinic protons in $4\cdot^-$ are characterized by $a_H = 3.25$ G (6 H). The similarity of this value to that for COT (3.21 G) assures the absence of significant ring spin density differences between the two systems.

An antisymmetric anion radicals, that of *cis*-1,2,3,4-tetraphenylcyclobutene ($5\cdot^-$), was also investigated. Here, interference between 1,3 and 1,2 overlap should and does engender an abnormally low hfs. The hfs of $5\cdot^-$ (K^+, THF, $-60°$) are 4.25 (4 H, $2p + 2$ saturated methines), 2.78 (2 H, ortho), 2.15 (2 H, ortho), 0.75 (2 H, meta), and 0.55 (2 H, meta). The dimethyl analog was not studied in this instance, but $B_2\rho_i$ could still be obtained, as follows. The sum of all observed phenyl ring hfs in $5\cdot^-$ (taking a_m as positive and $a_{o,p}$ as negative) was subtracted from an assumed total splitting of 27 G (tantamount to assuming $Q_\alpha = -27$). This difference was divided by two to obtain a_α resulting from spin density at each of the benzylic carbons in the stilbene-like π system. Assuming $Q_\beta = 27$, this is also the free rotation value of a_β in $5\cdot^-$, and is thus equal to ${}^1/_2B_2\rho_i$. Substituting the value of $B_2\rho_i$ thus derived (11.6) into eq 2 then generates the following interesting predictions. Hypothetical *s*-$5\cdot^-$ should have $a = 16.8$ G; $a = 9.3$ should obtain for a purely conformational effect (negligible 1,3 interaction); *a*-$5\cdot^-$, the experimental species, should have $a = 3.7$ G. The agreement (with 4.25 G) is only moderately good, but the qualitative effect of interference is clearly evident. It seems likely that the phenyl rings in $5\cdot^-$ are actually not instantaneously equivalent, thus causing $|c_i| \neq |c_j|$ and slightly diminishing the effect of the 1,3-interaction.

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Photochemical and γ-Ray-Induced Reactions of Nucleic Acid Constituents. Suppression of the Reactivity of Pyrimidines in the Presence of Purines

Sir:

We have shown recently that some photochemical reactions of nucleic acid bases in partially aqueous solutions are selective for purines.[1] The selectivity has been examined in reactions of mixtures of the pyrimidines and purines or their nucleosides with 2-propanol, employing light of $\lambda > 290$ nm and di-*tert*-butyl peroxide [$(t\text{-BuO})_2$] as a photoinitiator. In these reactions uracil and its derivatives yield 6-α-hydroxyalkyl-5,6-dihydrouracil (I), thymine undergoes substitution at the C-5 methyl group to yield II, while purines give the appropriate 8-α-hydroxyalkyl derivatives (III). Irradiation of mixtures of purines and pyrimidines led to the predominant formation of the purine photoproducts. We wish to report that this decrease in the reactivity of the pyrimidines is due to the presence of the purines and that such an effect is of a more general scope, as the presence of purines also affected the extent of the photodimerization of pyrimidine bases.

We found that the quantum yields for the formation of the pyrimidine–alcohol photoproducts were usually

(1) D. Leonov, J. Salomon, S. Sasson, and D. Elad, *Photochem. Photobiol.*, 17, 465 (1973).

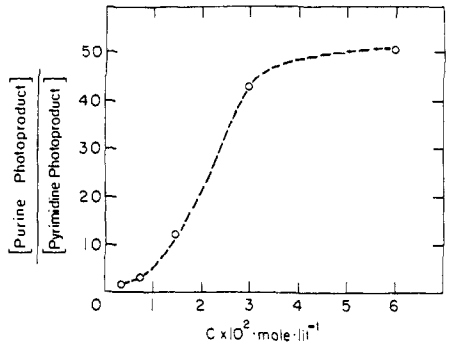
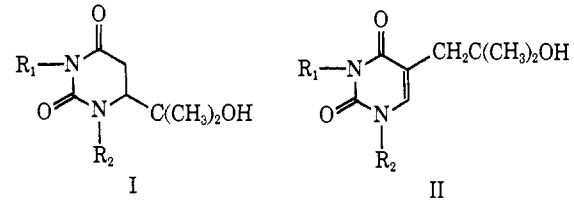


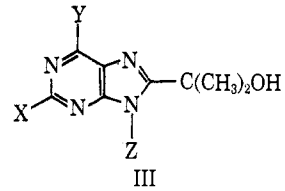
Figure 1. Concentration dependence of the ratio (purine photoproduct)/pyrimidine photoproduct) in the photochemical reactions of mixtures of caffeine and DMU with 2-propanol in the presence of $(t\text{BuO})_2$.



I

II

$R_1 = R_2 = H$ or CH_3
$R_1 = H$; R_2 = ribose



III

X = H; Y = NH_2; Z = H, ribose, or deoxyribose
X = NH_2; Y = OH; Z = H, ribose, or deoxyribose

higher than those of the purine–alcohol photoproducts when each base was irradiated separately; however, upon mixing the pyrimidines with equivalent amounts of purines, the formation of the pyrimidine photoproduct was inhibited completely for DMU (1,3-dimethyluracil), DMT (1,3-dimethylthymine), and uridine. With uracil, which alone showed an exceptionally high reactivity, the formation of the photoproduct was also suppressed to a considerable extent. Our results, as summarized in Table I, indicate that the selectivity of the photochemical reactions of 2-propanol for the purines results primarily from the suppression of the pyrimidine reactivity by the presence of the purines. In typical experiments, 3.5×10^{-2} and 3.5×10^{-1} M aqueous 2-propanol solutions of the heterocyclic bases and $(t\text{-BuO})_2$, respectively, were employed. The formation of photoproducts was followed by nmr as well as by vpc, and the light flux was measured by the ferrioxalate method.

Table I. Quantum Yields of Product Formation of Pyrimidines and Purines with 2-Propanol (*Di-tert-butyl* Peroxide as Photoinitiator)

Separated bases	Φ	Mixtures	Φ	
		Purine + pyrimidine	Purine	Pyrimidine
DMU	7.6×10^{-2}	Caffeine + DMU	3.5×10^{-2}	0
DMT	2.2×10^{-2}	Caffeine + DMT	4.1×10^{-2}	0
Uracil	4.7×10^{-1}	Adenine + uracil	4×10^{-3}	4×10^{-3}
Caffeine	3.6×10^{-2}	Adenosine + uridine	1.5×10^{-2}	0
Adenine	4×10^{-3}			
Uridine	5×10^{-2}			
Adenosine	1.5×10^{-2}			

These results prompted us to examine the effect of the presence of purines on the reactivity of pyrimidines in other photoreactions, *e.g.*, the acetone-photosensitized dimerization of pyrimidine bases. We found that in *tert*-butyl alcohol as solvent,[2] the addition of equivalent amounts of caffeine or adenosine to the reaction mixture led to a pronounced inhibition of the dimerization of DMU and DMT (from 90 to *ca.* 10%). With 2-propanol as solvent, adenosine–2-propanol adducts were the predominant photoproducts formed (42%), while the amount of thymidine photoproducts was reduced from 90% (26% dimer and 64% of thymidine–2-propanol adduct) in the absence to 4% in the presence of the purine. Increasing the amount of acetone (from 25 to 100%) resulted in an increase in the formation of pyrimidine photodimers. Similarly, in DMSO considerable amounts of DMU dimers were formed even in the presence of caffeine (65% in the absence and 40% in the presence of caffeine). In typical experiments, solutions of the pyrimidine (5×10^{-2} M) or mixtures of the pyrimidine (5×10^{-2} M) and the purine (5×10^{-2} M) with acetone were irradiated in Pyrex tubes with Hanovia 450-W lamp for *ca.* 5 hr through solution of the purine (5×10^{-2} M), in order to filter out the light absorbed by the purine.

We assume that the observed suppression of the chemical reactivity of the pyrimidines is due to their association with the purines. Heterocyclic bases, as well as some amino acids, are known to form associates in protic solvents.[3,4] These associates have been studied by the use of physical methods;[5] however, the effect of base association on the chemical or photochemical reactivity of the partners in the associate has not been described. Our assumption has been tested in some experiments with mixtures of equal amounts of purines and pyrimidines at different concentrations, assuming that the degree of association will decrease upon dilution. Results with DMU and caffeine, for example (Figure 1), indicated that the reactivity of DMU, which was totally suppressed in concentrated solutions, was regained upon dilution.[6]

Additional evidence to our assumption can be derived from the relatively small decrease in the degree of the dimerization of the pyrimidines in the presence of

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(3) D. L. Dimicoli and C. Helene, *J. Amer. Chem. Soc.*, **95**, 1036 (1973), and references cited therein.

(4) N. I. Nakano and S. J. Igarashi, *Biochemistry*, **9**, 577 (1970), and references cited therein.

(5) P. O. P. Ts'O in "Fine Structure of Proteins and Nucleic Acids," G. D. Fasman and S. N. Timasheff, Ed., Marcel Dekker, New York, N. Y., 1970, p 49, and references cited therein.

(6) In DMT–caffeine mixtures, no reaction of the pyrimidine could be observed even in very dilute solutions. The increased association with DMT results from the presence of an additional alkyl group in the pyrimidine. *Cf.* G. K. Helmkamp and N. Kondo, *Biochim. Biophys. Acta*, **157**, 242 (1968).

the purines in acetone and DMSO, in which, as aprotic solvents, base association is less significant.[7]

We conclude that the selectivity observed in the photochemical reactions of the heterocyclic bases for the purines is a result of the suppression of the reactivity of the pyrimidines due to the presence of the purines, and we assume that base association (stacking) is responsible for this effect. These findings are now being studied further and extended to associates of heterocyclic bases with amino acids. The possible "protection" of sensitive sites in biopolymers through the appropriate association agent is also under investigation.

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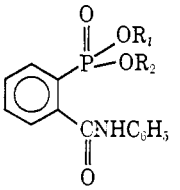
Interaction of Amides and Phosphates. Intramolecular Catalysis of Amide Hydrolysis by a Phosphonic Acid

Sir:

The association of nucleic acids and proteins has been recognized to be of biochemical significance.[1] We have sought evidence for chemical interactions between the functional groups serving as linkages in these molecules and have recently demonstrated the reactivity of an amide functionality toward a phosphate center in acid solution.[2] We have now observed a reaction due to a complementary interaction, catalysis of the hydrolysis of an amide brought about by the presence of a neighboring phosphate derivative.

Compound **1**, 2′-phosphonobenzanilide, was prepared by hydrolysis of the diethyl ester, **2**, in acetone–



1, $R_1 = R_2 = H$
2, $R_1 = R_2 = C_2H_5$

water (1:1) containing 6 *M* hydrogen chloride.[2] Crystals of **1** (colorless plates, mp 213–214°) precipitate from the solution in over 99% yield. Spectral characteristics are consistent with the proposed structure (nmr m, δ 7.0–8.9; ir (KBr) 1600 (C=O), 1160 cm^{-1} (P=O)) as is the elemental analysis. *Anal.* Calcd for $C_{13}H_{12}NO_4P$: C, 56.32; H, 4.36; N, 5.05; P, 11.17. Found: C, 55.99; H, 4.48; N, 5.07; P, 11.19. The hydrolysis of the amide to 2-carboxyphenylphosphonic acid[4] and aniline hydrochloride (products were determined in a large scale preparative reaction) was followed by monitoring the decrease in absorbance due to the starting material at 270 nm. All kinetics were performed using stoppered quartz cells to hold solutions in the constant-temperature cell housing of a Unicam SP1800A spectrophotometer at 50 ± 0.10°. All acid concentrations were confirmed by titration; NBS buffers were used.[5]

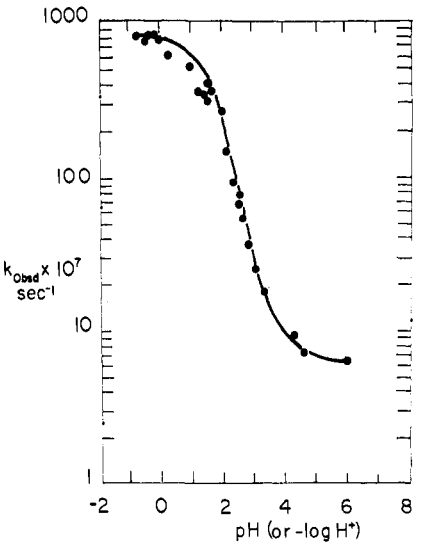


Figure 1. Observed rate constants for hydrolysis of **1** at 50.0°. The curve is a plot of eq 1. Experimentally determined points are indicated. Substrate concentration was 10^{-6} *M*. From pH 1 to pH 6 ionic strength was maintained at 0.5 *M*.

Figure 1 is a compilation of the observed first-order (buffer independent) rate constants for hydrolysis of **1** as a function of the acidity of the hydrolysis medium. The empirical kinetic equations for the reaction involving participation of the phosphonic acid do not involve an explicit term in solvent lyonium ion concentration at acidities up to 5 *M*. The plotted curve is of the equation

$$k_{obsd} = k(H_2A)/(H_2A + HA^-) + k'(HA^-)/(HA^- + H_2A) \quad (1)$$

where H_2A and HA^- are **1** and its conjugate base, respectively; $k = 8.6 \times 10^{-5}$ sec^{-1} and $k' = 6.9 \times 10^{-7}$ sec^{-1}. The pK' for **1** that gives a best fit for the curve is 1.6 and this agrees with values obtained by extrapolation from related literature values.[6] The corresponding para-substituted compound monoethyl ester[2] (prepared by hydrolysis of the para isomer of **2** in lithium hydroxide) hydrolyzes (without involvement of a plateau region) at a much slower rate, according to the equation

$$k_{obsd} = k''(H+) \quad (2)$$

at 50° where $k'' = 1.5 \times 10^{-6}$ sec^{-1} M^{-1}. Similar behavior is observed for benzanilide ($k'' = 1.1 \times 10^{-6}$ sec^{-1}) so that the phosphonate group has only a small inductive effect. For comparison, the diacid form of **1** hydrolyzes with $k = 8.5 \times 10^{-5}$ sec^{-1} M^{-1}. In 1.0 *M* acid at 50° the hydrolysis of **1** proceeds with a value for k_{obsd} greater than that for benzanilide by a factor of 74. However, at pH 6, where reaction of **1** occurs through the monoanion in the plateau asso-

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