ometry of the formally six-coordinate Hg atom and eight-coordinate potassium ions. This aspect of the crystal structure will not be elaborated upon here.

Supplementary Material Available: fractional atomic coordinates, thermal parameters, bond angles between carbon, nitrogen, and oxygen atoms, and tabulated observed and calculated structure factor amplitudes (53 pages). Ordering information is given on any current masthead page.

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Ultraviolet and γ-Ray-Induced Free-Radical Reactions of Nucleic Acid Constituents. Selectivity of Some Reactions for Purines. Suppression of the Reactivity of Pyrimidines

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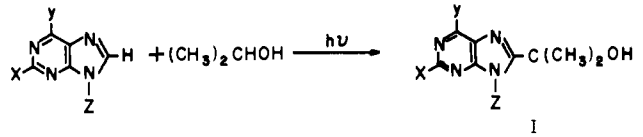
Received December 22, 1975

Abstract: The photochemical reactions of purine and pyrimidine bases, nucleosides, nucleotides, and polynucleotides with 2-propanol, employing di-*tert*-butyl peroxide as initiator, were found to be selective for purines. The selectivity was shown to result from the suppression of the reactivity of the pyrimidines due to the presence of the purines. This was evidenced by comparison of the quantum yields for product formation, when the bases were allowed to react separately, to those in mixtures. In equimolar mixtures of pyrimidines and purines, the pyrimidine reactivity was suppressed, whereas that of the purine remained unchanged. The concentration and temperature dependence and the effect of anions on the degree of suppression of pyrimidine's reactivity in the presence of purines, as well as the correlation with changes in the osmotic coefficients upon dilution of mixtures, suggest that heteroassociation in the form of base-stacking is responsible for the effect. The thermally initiated reactions exhibit the same effect, thus indicating the general scope of the phenomenon.

The multiplicity of products formed in irradiated DNA complicates the chemical identification of products and further interferes with the correlation between a given photoproduct and the accompanying biological effect. The induction of selective photochemical modifications of specific moieties in the nucleic acid can serve as a most powerful tool for this correlation. The pyrimidine bases have been regarded as the light-sensitive sites in nucleic acids; accordingly, until recently, the study of the photochemistry of nucleic acid constituents concentrated mainly on the reactions of pyrimidines.[2] The major

photoproducts identified were the cyclobutane-type dimers. Photosensitization, usually with ketonic photosensitizers, was utilized for the selective production of these dimers in nucleic acids.[3] It has recently been shown that purines also undergo photochemical reactions.[4,5] The reported reactions involve free-radical intermediates and, in the case of nucleic acid occurring purines, result in the substitution of the appropriate moiety for the hydrogen atom at the C-8 position of the purine system,[5] e.g., the reaction with 2-propanol (Scheme I).

Scheme I



I

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

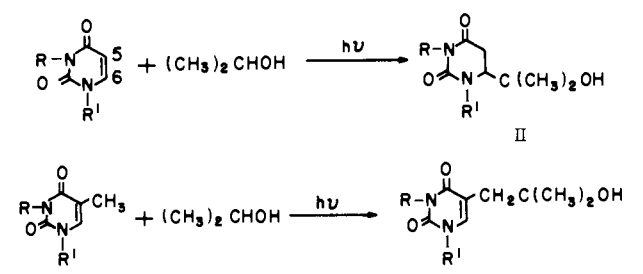
The fact that photosensitive sites other than pyrimidines are present in nucleic acids increases the number and types of expected photoproducts in irradiated nucleic acids. It is necessary, therefore, to sort out the various photochemical reactions for the appropriate bases in a given nucleic acid. In this research we examine the selectivity of photochemical reactions of nucleic acid constituents, with the aim of developing selective reactions for purines and purine moieties in nucleic acids.

The present publication describes the development of some selective photochemical reactions for purines, purine nucleosides, and nucleotides.[6] These include reactions of a variety of purine–pyrimidine systems with 2-propanol, initiated photochemically with di-*tert*-butyl peroxide [$(t\text{-BuO})_2$]. The reactions were found to be selective for purines, and it was shown that this selectivity results from the suppression of the reactivity of the pyrimidines due to the presence of the purine. We also present evidence that heteroassociation of the bases is responsible for this effect.

Results and Discussion

Initially we investigated the photochemical reactions of 2-propanol with uracil and thymine, as well as with their nucleosides and nucleotides.[6a] In these reactions uracil and its derivatives yielded the corresponding 5,6-dihydro-6-α-hydroxyalkyl derivatives II. On the other hand, thymine and its derivatives usually underwent substitution at the C-5 methyl group (III), though in some cases addition of the alcohol across the 5,6 double bond took place[7] (Scheme II).

Scheme II



II

III

R=R^1=H; R=R^1=Me; R=H, R^1=Ribose or deoxyribose

These reactions were carried out in the presence of a variety of photosensitizers or free-radical initiators. It was found that when $(t\text{-BuO})_2$ was used as a photoinitiator, the pyrimidine–alcohol adducts were formed exclusively; whereas photosensitization with acetone led to a mixture of photoproducts which included pyrimidine dimers and 5,6-dihydropyrimidines,

Table I. Photochemical and γ-Ray-Induced Reactions of Pyrimidines and Purines with 2-Propanol[a]

Pyrimidine + purine	Product, %[b] Pyrimidine	Purine
DMU + caffeine	4	60
DMT + caffeine		90
Uracil + adenine	37	35
Thymine + adenine	3	82
Thymine + adenine[c]		75
Thymine + purine	3	80
Thymidine + adenosine		98
Thymidine + adenosine[c]		50
Uridine + adenosine	5	60
Thymidine + guanosine	3	78
UMP[d] + AMP[e]	38	36
TMP[f] + AMP	7	46

[a] Concentrations of the heterocyclic bases ranged from 1 to 4.5 × 10^{-2} M. $(t\text{-BuO})_2$ was used as a photoinitiator. Hanovia 450W high-pressure mercury vapor lamps (Pyrex filters) were used as the radiation source. [b] Based on bases employed. [c] With γ-rays [no $(t\text{-BuO})_2$]. [d] Uridine 5′-monophosphate. [e] Adenosine 5′-monophosphate. [f] Thymidine 5′-monophosphate.

in addition to the pyrimidine–alcohol photoadduct.[6a] It is noteworthy that thymine, 1,3-dimethylthymine (DMT), and thymidine gave substitution products with 2-propanol, as represented by III, while TMP underwent addition across its 5,6 double bond.[7] In an analogous study of the photochemical reactions of purines and alcohols, which result in the alkylation of the purine at the C-8 position, we found that the use of $(t\text{-BuO})_2$ as initiator resulted in high yields of the purine–alcohol adducts.[5b] Since single products of either purine or pyrimidine with 2-propanol could be obtained in reaction with the peroxide photoinitiation, it was felt that these could serve as a proper system for the study of the selectivity of reactions for the various heterocyclic bases. Mixtures of equal amounts of purines and pyrimidines or their nucleosides and nucleotides were irradiated with light of $\lambda > 290$ nm in the presence of 2-propanol, employing $(t\text{-BuO})_2$ as a photoinitiator, or exposed to γ-radiation (in the absence of an initiator). The products were analyzed quantitatively, and it was found that in most cases the purine photoproducts predominated in the irradiated mixtures. These results are summarized in Table I. Progress of the reactions was followed by periodic analyses of samples by TLC, GLC, ultraviolet or NMR spectroscopy, or by the incorporation of ^{14}C-labeled 2-propanol into the reactants.

The NMR analyses of the uracil derivatives were based on the disappearance of the bands at τ 2 region (vinyl proton) and the appearance of bands at τ 7.2 ($-COCH_2-$) and 9 [$(CH_3)_2COH$]. In 1,3-dimethyluracil (DMU) the absorption of the NCH_3 protons at τ 6.7 and 6.8 served as the standard, while with the nucleosides and nucleotides the band of H-1′ at τ 4 served as standard. The analyses of TMP derivatives were based on the disappearance of the bands at τ 2.2 (vinyl proton), and the appearance of the absorption at τ 8.7 [$(CH_3)_2COH$]. With the purines, it was based on the disappearance of the H-8 proton absorption at τ 1–1.8 and the appearance of the band at τ 8.1 [$(CH_3)_2COH$]. The absorption of the H-2 protons served as standard. In adenine derivatives the analyses could be followed further by the appearance of the absorption of H-1′ in the modified purine at τ 3–3.2 and the disappearance of the absorption of H-1′ at τ 4 in the starting purine nucleoside. Photoproducts of the bases and their nucleosides were isolated either by column chromatography or by preparative paper chromatography. They were characterized by their physical properties and by comparison with authentic samples. Isolation of the nucleotidic photoproducts

Table II. Formation of Pyrimidine Photoproducts in the Absence and in the Presence of Purines[a]

Heterocyclic base		Solvent mixture, %				Pyrimidine photoproduct, %		
Pyrimidine	Purine	Acetone	Water	*tert*-Butyl alcohol	2-Propanol	Dimers	Pyrimidine–alcohol	Purine photoproduct, %
DMU		25	10	65		90		
DMU	Caffeine	25	10	65		7		
DMU		80		20		85		
DMU	Caffeine	80		20		24		
DMU		100				82		
DMU	Caffeine	100				55		
DMU	Caffeine	30	5		65	1	5	90
DMU	Caffeine	70	5		25	40	2	70
DMT		25	10	65		70		
DMT	Caffeine	25	10	65		10		
Thymidine		25	5		70	26	64	
Thymidine	Adenosine	25	5		70	4		42
DMU[b]						66		
DMU[b]	Caffeine					40		

[a] Hanovia 450W high-pressure mercury vapor lamp (Pyrex filter) was used as the radiation source. [b] In Me_2SO–acetone mixture (7:3).

was achieved either by paper chromatography or by electrophoresis. Spots were located by a mineralight lamp, while UMP and TMP photoproducts were detected according to the procedure described by Hanes and Isherwood.[8]

The presently described reactions have been shown previously[5a,c] to involve free-radical intermediates resulting from the photolysis of the peroxide, which absorbs most of the incident light. The oxy radicals, derived from the fragmentation of the peroxide, abstract a hydrogen atom from C-2 of 2-propanol to yield ketyl radicals $\dot{C}(CH_3)_2OH$; these are subsequently scavenged by the purine or the pyrimidine to yield the adduct. Quantum yield measurements (moles of adduct formed per quanta of light absorbed by the peroxide[6b]) showed that the quantum yields for the formation of pyrimidine–alcohol photoadducts were usually higher than those of the purine–alcohol photoproducts, when irradiated separately under the reaction conditions described above. For example, a quantum yield of 7.6×10^{-2} was observed for the formation of the DMU–2-propanol photoadduct, while that for the formation of the caffeine–2-propanol photoproduct was 3.6×10^{-2}. Similarly, the uridine–2-propanol photoadduct was formed in a quantum yield of 5×10^{-2}, while that of adenosine–2-propanol photoproduct was 1.5×10^{-2}. Upon mixing the pyrimidines with equivalent amount of purines, the formation of the pyrimidine photoproduct was inhibited, while the purine reacted with the same quantum yield as when separated. In the uracil–adenine and the UMP–AMP systems the pyrimidine–alcohol and the purine–alcohol photoproducts were formed in nearly equal amounts (see Table I). It should be noted, however, that uracil itself possessed an exceptionally high reactivity in its photoreaction with 2-propanol (quantum yield for the formation of the photoadduct was 4.7×10^{-1}). In the presence of adenine, the formation of the uracil photoadduct was suppressed 100-fold, as determined by quantum yield data (quantum yield 4×10^{-3}). Similarly, a 50–70-fold decrease in the formation of UMP–2-propanol adduct resulted in the presence of AMP. Here, too, the presence of the pyrimidine did not affect the reactivity of the purine. These results indicate, therefore, that the selectivity of the photochemical reactions of 2-propanol for the purines results primarily from the suppression of the reactivity of the pyrimidine due to the presence of the purine.

We further examined the effect of the presence of purines on the photosensitized dimerization of pyrimidines.[9] The acetone-photosensitized reaction, in the absence of a purine, leads usually to high yields of the dimers.[10] We irradiated with ultraviolet light of $\lambda > 290$ nm various pyrimidine–purine mixtures in the presence of acetone, which served as a triplet photosensitizer, and determined their conversion to products.

Our results are summarized in Table II, from which we can see that (i) in *tert*-butyl alcohol as solvent, the presence of a purine in the reaction mixture results in a pronounced inhibition of the dimerization of the pyrimidine; (ii) the suppression of dimerization in acetone or Me_2SO as solvents is less pronounced; and (iii) when 2-propanol is present in concentrations higher than 15%, purine photoproducts are formed in higher yields than the pyrimidine dimers and other pyrimidine photoproducts. These results indicate that the presence of a purine suppresses the reactivity of pyrimidines in the photodimerization reaction as well, and that in solvents like acetone and Me_2SO the effect of suppression of the reactivity of the pyrimidines is less dramatic. As discussed below, this effect results from heteroassociation of the bases; it should be noted that in these solvents base association is weak;[11] thus, our results are compatible with this observation.

We assumed that the observed suppression of the chemical reactivity of the pyrimidine was due to their heteroassociation with the purines[12,13] and set out to test this hypothesis in several different ways. Our assumption was first tested in experiments with mixtures of equimolar amounts of purines and pyrimidines at different concentration, assuming that the degree of association would decrease upon dilution and would be reflected in an increased reactivity of the pyrimidine in dilute solution. We found (see Figure 1) that the reactivity of some pyrimidine bases, nucleosides, or nucleotides, which is suppressed considerably in concentrated solutions, is regained upon dilution of the reaction mixtures, as evidenced by the lower values of the ratios of purine to pyrimidine photoproducts in more dilute solutions. It is noteworthy, however, that the reactivity of DMT in the presence of caffeine and that of thymine in the presence of adenine is not regained even at a concentration of 3×10^{-3} M.[14]

Robinson and Grant[15] have shown that the presence of various anions affects the activity coefficients of purines and pyrimidines in aqueous solutions. Thus, the presence of sulfate, phosphate, and chloride ions was shown to increase the activity coefficients, i.e., decrease the association of the heterocyclic bases; whereas perchlorate ions decrease the activity coefficients. We have, therefore, examined the effect of the presence of these anions on the reactivity of pyrimidines in the photochemical reactions with 2-propanol in purine–pyrimidine mixtures. Our results, presented in Figure 2, indicate that the presence of sulfate, phosphate, or chloride ions in the DMU–

Table III. Photochemical Reactions of Mono- and Polynucleotides with 2-Propanol[a] [(t-BuO)$_2$ as Photoinitiator]

Irradiation time, h	Separated nucleotides, products, %				1:1 mixtures, products, %				R_M/R_B	
	UMP	AMP	Poly (U)	Poly (A)	UMP + poly(A)		AMP + poly(U)		UMP + poly(A)	AMP + poly(U)
1		14	35				14	20		1.75
2	67	19	60	30	36	17	18	21	1.05	2.7
6	72	25	95	34	48	22	21	21	0.97	3.8

[a] Nucleotide concentration 5×10^{-2} M.

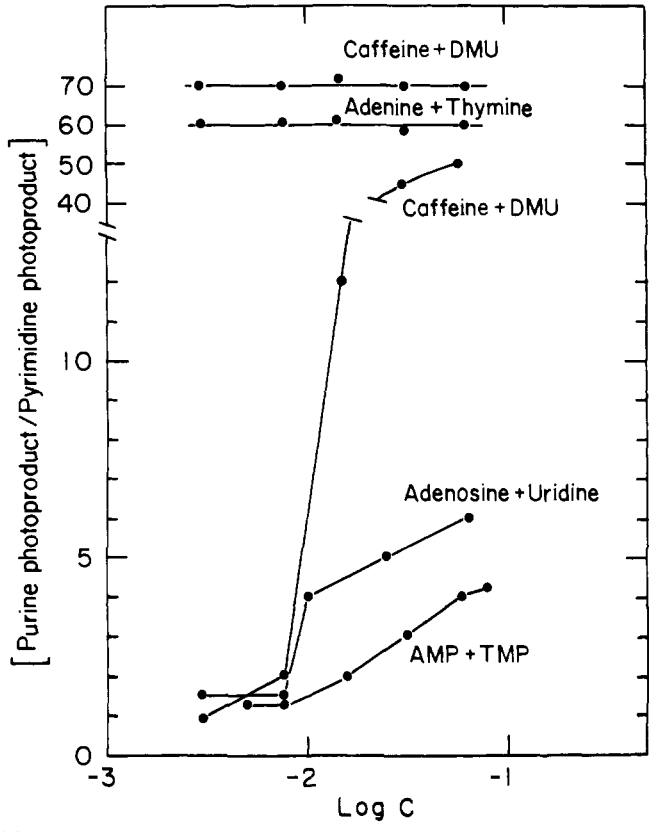


Figure 1. Concentration dependence of the ratio (purine photoproduct-pyrimidine photoproduct) in the photochemical reactions of mixtures of purines and pyrimidines with 2-propanol in the presence of (t-BuO)$_2$.

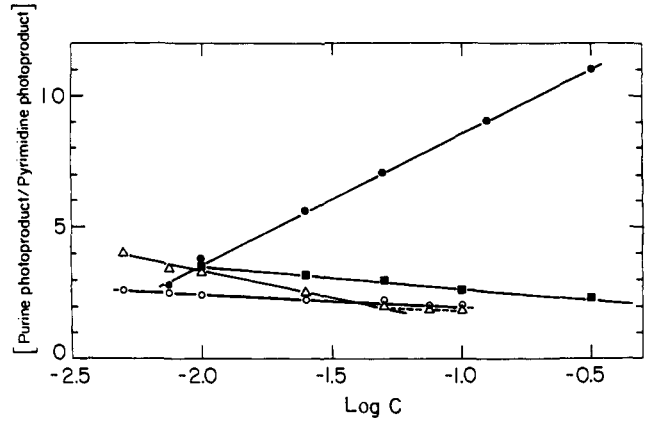


Figure 2. Salt effect on the ratio (purine photoproduct-pyrimidine photoproduct) in the photochemical reactions of mixtures of purines and pyrimidines with 2-propanol in the presence of (t-BuO)$_2$. Caffeine-DMU (1×10^{-2} M): Na_2HPO_4 (○); Na_2SO_4 (△); NaCl (■). Adenosine-uridine (1×10^{-2} M): $NaClO_4$ (●).

caffeine reaction mixture resulted in the enhanced formation of the DMU-2-propanol photoadduct. Indeed, the addition of even small amounts of these salts (7×10^{-3} M) reduces the purine-pyrimidine photoproduct ratio from 50 to 2.5. On the other hand, the presence of perchlorate ions in the uridine-adenosine mixture led to a decrease in the reactivity of uridine.

We further examined the reactivity of purine and pyrimidine polynucleotides in these reactions and found that when irradiated ($\lambda > 290$ nm) with 2-propanol in the presence of (t-BuO)$_2$, poly(A) or poly(U) was modified in high yields (>90% of the residues). Poly(A), however, was modified with a slower rate as compared with that of poly(U). Subsequently, we used mixtures of AMP and poly(U) or UMP and poly(A) for the study of the possible suppression of reactivity (chemical protection) of the pyrimidines in these systems. We define the amount of "chemical protection" as the ratio of the percent of modification of the purine to that of the pyrimidine in the mixture (R_M) divided (normalized) by the same ratio obtained from the modification of the components when irradiated separately (R_B), under identical reaction conditions. Our results, summarized in Table III, show that protection of poly(U) by AMP takes place, while no protection of UMP by poly(A) occurs. This indicates that a certain interaction occurs between AMP and poly(U) under our reaction conditions, while there is no indication for a similar interaction between UMP and poly(A).[12,16] These results could be interpreted assuming the interaction in the AMP-poly(U) system to be analogous to that previously reported for the monomeric adenosine and poly(U), which is of base-stacking nature at room temperature.[16c,d] Ts'O and Huang[16e] claim, however, that the formation of a soluble complex of poly(U), AMP, and Mg^{2+} at room temperature could not be demonstrated, although an insoluble complex was produced in the presence of moderate concentrations of Mg^{2+}. Therefore, it is presently difficult to conclusively correlate our observation with the previously reported data concerning the interactions in the AMP-poly(U) system. This point needs further clarification.

Some insight regarding the nature of the heteroassociates formed can be deduced from the observation that in both 1:1 and 2:1 mixtures of DMU-caffeine protection occurred. In 3:1 mixtures, however, this protection is reduced substantially. Hence, components of the associate need not be in a 1:1 ratio in order to observe protection. We have further examined whether the substitution at the C-8 position of the purine with the α-hydroxyisopropyl group affects the degree of protection of the pyrimidine in the above photochemical reactions. We, therefore, irradiated 1:1 mixtures of DMU-8-α-hydroxyisopropylcaffeine and uridine-8-α-hydroxyisopropyladenosine with 2-propanol and followed the formation of the pyrimidine-alcohol adduct. It was found, indeed, that no protection occurred. It seems, therefore, that the substitution at the C-8 position of the purine interferes, somehow, with the stacking of the bases, which, in turn, prevents protection.[17]

In order to examine whether the effect of the suppression of the reactivity of the pyrimidines in the presence of purines is of a more general scope, and is not limited solely to photo-

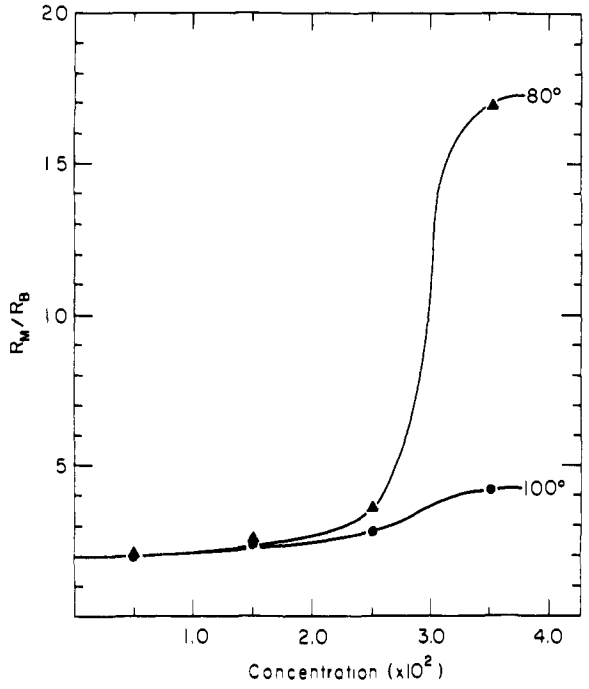


Figure 3. Concentration dependence of the amount of protection (R_M/R_B) in the thermal reactions of mixtures of caffeine and DMU with aqueous 2-propanol in the presence of $(t\text{-BuO})_2$.

chemical reactions, we have studied the thermally initiated free-radical reaction of the DMU-caffeine system with 2-propanol. We found that both DMU and caffeine reacted with 2-propanol when heated in the presence of $(t\text{-BuO})_2$,[5a] and that the products were the same as those obtained in the analogous photochemical reaction, i.e., II and of type I, respectively. For the determination of the effects concerned, equimolar amounts of DMU and caffeine in concentrations varying from 3.5×10^{-2} to 5×10^{-3} M were dissolved in solvent mixtures composed of 2-propanol, water, and $(t\text{-BuO})_2$. Blanks consisting of only DMU or only caffeine dissolved in these solvent mixtures were also prepared. For each concentration, the mixture and the two blanks were heated in sealed tubes at 80 and 100 °C until a small amount (~20%) of the caffeine had been converted to the corresponding 8-α-hydroxyisopropyl derivative. At the same time DMU was converted in varying amounts, depending on temperature, concentration, and absence or presence of caffeine, to its 2-propanol adduct. Typically, after heating 3.5×10^{-2} M solutions of DMU and caffeine for 57 h at 80 °C, 3% of the caffeine in the mixture and the blank was converted to adduct.[18] At the same time, 44% of DMU in the blank, but only 2% of DMU in the mixture, was converted to product.

In order to evaluate the amount of protection in the mixture, the same procedure as described above for the mononucleotide-polynucleotide system was applied; i.e., the ratio of the percent conversion of purine to product to the percent conversion of pyrimidine to product in the mixture (R_M) was divided by the same ratio obtained from the two blanks (R_B). The data presented in Figure 3 indicate that at higher concentrations (3×10^{-2} M) and lower temperatures (80 °C), the amount of protection, as measured by R_M/R_B, is substantial, but decreases sharply with dilution and increasing temperatures.[19] This is to be directly correlated with the melting of associates at elevated temperatures or upon dilution.[12] When the reaction is allowed to proceed to higher conversions of caffeine to product, the amount of protection decreases as shown in Table IV. Thus, it is the caffeine alone, and not its product with 2-propanol, which protects. As previously described, a similar effect was observed in the photoinduced reaction, where no protection of DMU was observed in the presence of 8-α-hydroxyisopropylcaffeine.

Table IV. Dependence of the Amount of Protection (R_M/R_B) on the Extent of Caffeine-2-Propanol Product Formation at 100 °C

Concn, M	% caffeine product	R_M/R_B
3.5×10^{-2}	18	4.2
	26	3.3
	58	1.5
2.5×10^{-2}	11	2.8
	43	2.0
	80	1.1
1.5×10^{-2}	8	2.6
	65	1.5
	94	1.1

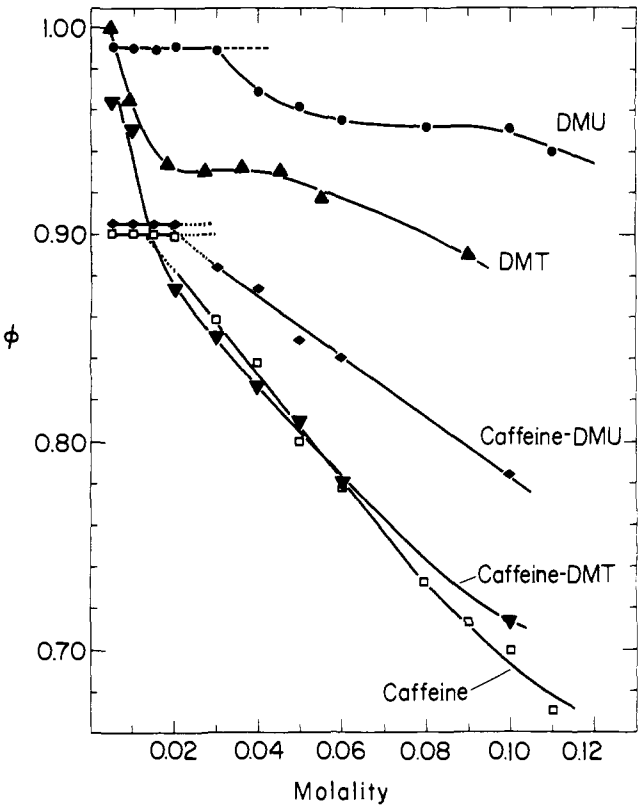


Figure 4. Concentration dependence of ϕ, the molal osmotic coefficient (i.e., the observed molality divided by the theoretical molality), for aqueous solutions of DMU (●), DMT (▲), caffeine (□), and 1:1 mixtures of caffeine-DMU (♦) and caffeine-DMT (▼).

In order to correlate these effects with the state of association at the various concentrations, we determined the molal osmotic coefficients ϕ[12,13] of aqueous solutions of DMU, DMT, caffeine, and 1:1 mixtures of DMU-caffeine and DMT-caffeine by the vapor pressure osmometry method at 50 °C (Figure 4). We have found that the associates in some cases do not simply melt gradually with increasing dilution, but a sudden leveling off often occurs for very dilute solutions. In particular, we have found that for DMU, caffeine, and 1:1 caffeine-DMU mixtures, the value of ϕ remains constant at concentrations up to approximately 2×10^{-2} M and then drops sharply continuing downward with increasing concentration, indicating a rapid increase in the degree of association. The cause of this leveling off phenomena is not clear; it may result from the melt of oligoheteroassociates at this point. It is noteworthy that the rapid decrease in reactivity of DMU in the presence of caffeine, which also occurs around 2×10^{-2} M,

may be correlated and, perhaps, be explicable by this sharp increase in heteroassociation. Of importance as well is the observation that in DMT-caffeine mixtures, where no photochemical reaction of the pyrimidine could be observed even at very dilute solutions, no such leveling off in the value of ϕ was observed even at concentrations of 5×10^{-3} M.

Conclusion

In contrast to the general notion that pyrimidines are the more susceptible sites to light-induced transformations than purines,[2d] our results indicate that in many cases purine susceptibility predominates, since, when reacted separately, the pyrimidines exhibit higher reactivity than the purines, while the reactivity of the purines in the mixtures does not differ substantially from that of the separated purines. The observed "enhanced" reactivity of the purines in the mixture is thus due to the suppressed reactivity of the pyrimidine. It was further demonstrated that this phenomenon is of a general scope, since the same effect was observed in the corresponding thermally initiated reactions. We propose that this phenomenon results from the heteroassociation of the bases[12,13,16,20-24] and stress the point that chemical reactivity can serve as a most efficient tool for the study of the state of heteroassociation of nucleic acid constituents. The reactions described in this publication can serve as a handle by which both purine and pyrimidine ends in the heteroassociate can be observed simultaneously,[24] and it has been shown that a great deal of information regarding heteroassociates of polynucleotides can be derived from the chemical reactivity of the monomer units in the appropriate associates. Furthermore, the material described here presents a step toward the development of selective photochemical reactions for modifying the purine moieties in nucleic acids and the understanding of the role that purines play in a variety of photochemical transformations of nucleic acids, including crosslinks with proteins.[25]

Experimental Section

Caffeine (Schuchardt, Munich) was recrystallized from water before use. Pyrimidines and pyrimidine nucleosides (Schuchardt, Munich), as well as purines and purine nucleosides (Fluka, CHR grade), were used without purification. Polyuridylic acid Type II (mol wt >100 000), polyadenylic acid Type I (mol wt >100 000), adenosine-5′-monophosphoric acid sodium salt, thymidine-5′-monophosphoric acid disodium salt, and guanosine-5′-monophosphoric acid disodium salt were purchased from Sigma. Uridine-5′-monophosphoric acid disodium salt was purchased from Fluka & Buchs. 2-Propanol, acetone, Me_2SO, and *tert*-butyl alcohol (Frutarom, Haifa) were freshly distilled before use, while di-*tert*-butyl peroxide (Merck-Schuchardt, Munich) was used without purification. [1,3-^{14}C]-2-propanol (9.1 mCi/mmol) was purchased from The Radiochemical Centre, Amersham. DMU and DMT were synthesized from uracil and thymine, respectively, according to the procedure described by Davidson and Baudisch.[26]

Column chromatography was performed on Kieselgel (Merck) using a modified "dry column" technique.[27] Mixtures of acetone-petroleum ether (bp 60-80 °C) were used as eluents for DMU, DMT, and caffeine, while methanol-chloroform mixtures were used for elution of the bases and the nucleosides. Aluminum plates of Kieselgel SIF or cellulose CEF (Riedel-de-Haen) were used for ascending TLC. Acetone-petroleum ether mixtures were used as eluents for DMU, DMT, caffeine, and their derivatives, while 1-butanol-NH_4OH-H_2O (7:1:2 v/v, solvent system A) and isobutyric acid-NH_4OH-H_2O (66:1:33 v/v, solvent system B) mixtures served as eluents for the other pyrimidines, purines, or their nucleotides. Whatman No. 1, No. 3 MM, and No. 17 papers were used for paper chromatography in 1-butanol-NH_4OH-H_2O (14:3:3 v/v, solvent system C) or 1-propanol-NH_4OH-H_2O (11:2:7 v/v, solvent system D). High-voltage paper electrophoresis was performed on a Shandon apparatus at pH 1.9 and 3.5. Spots were revealed by mineralight lamp or by the phosphomolybdate reagent.[8] GLC was performed with a Varian Aerograph 1200-2 instrument using a 10% SE-30 column (7 ft × ⅛ in.). NMR spectra were recorded with a Varian A-60 instrument in $CDCl_3$ solutions (for DMU, DMT, and caffeine) with Me_4Si as internal standard or in Me_2SO-d_6 and D_2O (for the bases and the nucleosides) with an external standard. Absorptions are reported in τ values. Ultraviolet measurements were performed with a Zeiss Spectrophotometer PMQ II. Radioactivity measurements were performed on a Packard Model 3890 liquid scintillation spectrometer. Aqueous solutions were counted in Bray's solution.[28] Electrophoretic papers were thoroughly dried, cut into stripes (1.5 cm wide), and counted in toluene scintillation liquid [4 g of 2,5-diphenyloxazole (PPO) and 0.05 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP) per liter of freshly distilled toluene]. Vapor pressure osmometry measurements were carried out with Hitachi Perkin-Elmer Model 115 molecular weight apparatus.

Hanovia 450W high-pressure mercury vapor lamps with an internal water cooled jacket served as the light source. Experiments were carried out in immersion apparatus, Pyrex test tubes, or capillaries. Reactions mixtures were flushed with nitrogen for 5 min prior to irradiation. Quantum yields were measured by ferrioxalate actinometry.[29] γ-Ray irradiation was conducted in a Gammacell 220 apparatus (Atomic Energy of Canada Ltd, Ottawa) with an internal air cooling device at a dose rate of 12 000 rad/min. Nitrogen was bubbled through the mixture prior to irradiation. Typical experiments are described.

Reaction of Caffeine and DMT with 2-Propanol. A mixture of caffeine (500 mg, 2.6 mmol), DMT (400 mg, 2.6 mmol), $(t\text{-BuO})_2$ (5 ml), water (20 ml), and 2-propanol (150 ml) was irradiated (Pyrex filter) at room temperature under nitrogen for 6 h. By that time most of the caffeine had reacted, as demonstrated by TLC. The solvent was removed under reduced pressure and the residue was chromatographed on Kieselgel (200 g). Acetone-petroleum ether (1:4) eluted 8-α-hydroxyisopropylcaffeine (585 mg, 90%), followed by unreacted DMT (395 mg, 99%), and unreacted caffeine (40 mg, 8%).

Products were identified by comparison with authentic samples. The purity of the recovered starting pyrimidine and purine as well as that of the photoproducts was further checked by TLC and their NMR spectra.

Irradiation of Adenine and Thymine with γ-Rays. A mixture of adenine (350 mg, 2.6 mmol), thymine (330 mg, 2.6 mmol), water (20 ml), and 2-propanol (110 ml) was exposed to γ-rays for 12 h. By that time most of the adenine had reacted. The solvent was removed under reduced pressure and the residue chromatographed on Kieselgel (200 g). Methanol-chloroform (1:19) eluted thymine (322 mg, 98%). The same solvent mixture (1:9) further eluted 8-α-hydroxyisopropyladenine (385 mg, 77%), followed by unreacted adenine (68 mg, 19%). Products were identified by comparison with authentic samples.

Irradiation of DMU and Caffeine with 2-Propanol in the Presence of Inorganic Salts. Mixtures of DMU (36 mg, 0.26 mmol), caffeine (50 mg, 0.26 mmol), water (3 ml), $(t\text{-BuO})_2$ (1 ml), 2-propanol (9 ml), and various amounts of the appropriate inorganic salt were irradiated for 3 h. Progress of the reaction was followed by TLC, GLC, and NMR spectroscopy. Results are described in Figure 2.

Photosensitized Dimerization of DMU in the Presence of Caffeine. A mixture of DMU (36 mg, 0.26 mmol), caffeine (50 mg, 0.26 mmol), acetone (2.8 ml), water (1.4 ml), and *tert*-butyl alcohol (9.8 ml) was irradiated in a Pyrex tube for 6 h. The formation of dimers was followed by GLC at temperature programming from 140 to 250 °C. The reaction mixture was shown to consist of dimers (7%), unreacted DMU (90%), and starting caffeine (quantitative). Under similar reaction conditions, but in the absence of caffeine, DMU dimers were formed in >90% yield.

Acetone-Photosensitized Dimerization of DMU in Me_2SO in the Presence of Caffeine. A mixture of DMU (36 mg, 0.26 mmol), caffeine (50 mg, 0.26 mmol), Me_2SO (9.1 ml), and acetone (3.9 ml) was irradiated for 115 h. The solvent was removed under reduced pressure and part of the residue was injected into a GLC instrument and was found to consist of 40% dimers and 58% unreacted DMU; caffeine was not changed during irradiation.

Photodimerization of DMU in Acetone in the Presence of Caffeine. A mixture of DMU (36 mg, 0.26 mmol) and caffeine (50 mg, 0.26 mmol) in acetone (13 ml) was irradiated for 3.5 h. Progress of the reaction was followed by GLC. The solvent was removed under reduced pressure and the residue was found to consist of unreacted DMU (45%) and DMU dimers (55%).

Reaction of DMU and Caffeine in the Presence of Acetone and 2-Propanol. A mixture of DMU (36 mg, 0.26 mmol), caffeine (50 mg, 0.26 mmol), acetone (45 ml), water (7.5 ml), and 2-propanol (95 ml)

was irradiated for 18 h. Progress of the reaction was followed by GLC (temperature programming 140–220 °C). The reaction was stopped when 90% of the caffeine had reacted. The solvent was removed under reduced pressure and the residue was analyzed by GLC and found to consist of DMU dimers (1%), DMU–alcohol photoadduct (5%), and 8-α-hydroxyisopropylcaffeine (90%).

Reaction of Thymidine and Adenosine in the Presence of Acetone and 2-Propanol. A mixture of adenosine (500 mg, 1.87 mmol), thymidine (455 mg, 1.87 mmol), water (7.5 ml), acetone (37.5 ml), and 2-propanol (105 ml) was irradiated for 8 h. Progress of the reaction was followed by TLC using solvent system A. The solvent was removed under reduced pressure, and the residue was chromatographed on Whatman No. 17 paper using solvent system B as eluent. Stripes containing nucleosidic material were thoroughly extracted with 0.1 N NH_4OH. The solvent was removed under reduced pressure and the NMR spectrum of the residue was determined. The mixture consisted of 8-α-hydroxyisopropyladenosine (252 mg, 42%), unreacted adenosine (250 mg, 50%), unreacted thymidine (390 mg, 86%), and thymidine dimers (20 mg, 4%).

Reaction of TMP with 2-Propanol. A mixture of TMP (163 mg, 0.4 mmol), water (8 ml), 2-propanol (7.5 ml), *tert*-butyl alcohol (1.5 ml), and $(t\text{-BuO})_2$ (three portions of 0.5 ml) was irradiated in a Pyrex tube for 22 h. Portions of $(t\text{-BuO})_2$ were added to the irradiated mixture after 6 and 12 h. Progress of the reaction was followed qualitatively by TLC on cellulose plates using solvent system B as eluent and quantitatively by measurements of the absorption at 270 nm and the NMR spectrum. The solvent was removed under reduced pressure and the residue was chromatographed on Whatman No. 3 1 MM paper in solvent system D. The product (130 mg, R_f 0.54) was located with the phosphomolybdate reagent[8] and extracted with 0.25 M NH_4HCO_3 solution: NMR (D_2O) τ 3.75 (t, $J_{1',2'}$ = 7.5 Hz, 1 H, C-1′-H), 5.55 (broad band, 1 H, C-3′-H), signal at 5.75–6.25 (broad band, 3 H, C-4′-H, C-5′-2H), 6.4 (broad band, 2 H, C-6-2H), 7.75 (broad band, $J_{2',1',3'}$ = 7 Hz, 2 H, C-2′-2H), and 8.75 [apparent singlet, 6 H, $C(CH_3)_2OH$]. Anal. Calcd for $C_{13}H_{21}N_2O_9PNa_2{\cdot}4H_2O$: C, 31.32; H, 5.82; N, 5.62; P, 6.22. Found: C, 31.40; H, 4.95; N, 5.71; P, 5.47.

Reaction of UMP with 2-Propanol. UMP (161 mg, 0.4 mmol) was irradiated under similar conditions to TMP. Progress of the reaction was followed by measurement of absorption at 260 nm (which disappeared after 2 h) and the NMR spectrum. The mixture was worked up as described above leading to 6-(α-hydroxyisopropyl)-5,6-dihydro-UMP (141 mg; R_f 0.46): NMR (D_2O) τ 4.1 (d, $J_{1',2'}$ = 6 Hz, 1 H, C-1′-H), signals at 5.4–6.4 (broad band, 5 H, C-2′-H, C-3′-H, C-4′-H, C-5′-2H), 6.4 (t, $J_{6,5}$ = 7 Hz, C-6-H), 7.2 (d, $J_{5,6}$ = 7 Hz, C-5-2H), and 8.8 [s, 6 H, $C(CH_3)_2OH$]. Anal. Calcd for $C_{12}H_{19}N_2O_{10}PNa_2{\cdot}2H_2O$: C, 31.03; H, 4.95; N, 6.07; P, 6.68. Found: C, 31.04; H, 4.80; N, 6.73; P, 6.97.

Reaction of AMP with 2-Propanol. AMP (623 mg, 1.5 mmol) was irradiated with 2-propanol for 15 h under standard conditions. By that time over 95% of the starting nucleotide reacted. Purification of 8-α-hydroxyisopropyl-AMP (486 mg, R_f 0.51) was achieved by the procedure described above: NMR (D_2O) τ 1.9 (s, 1 H, C-2-H), 3.25 (m, 1 H, C-1′-H), signal at 5.3–6.2 (broad band, 5 H, C-2′-H, C-3′-H, C-4′-H, C-5′-2H), and 8.3 [s, 6 H, $C(CH_3)_2OH$]. Anal. Calcd for $C_{13}H_{19}N_5O_8PNa{\cdot}2H_2O$: C, 33.83; H, 4.96; N, 15.18; P, 6.72. Found: C, 33.78; H, 4.65; N, 15.6; P, 6.92.

Irradiation of TMP and AMP with 2-Propanol. A mixture of TMP (72 mg, 0.18 mmol), AMP (75 mg, 0.18 mmol), water (1.7 ml), 2-propanol (1.6 ml), *tert*-butyl alcohol (0.6 ml), and $(t\text{-BuO})_2$ (0.15 ml) was irradiated in a Pyrex tube for 13 h. Additional $(t\text{-BuO})_2$ (0.15 ml) was added to the irradiated mixture after 4 and 8 h. Progress of the reaction was followed by TLC on cellulose plates using solvent system C as eluent and quantitatively determined by NMR measurements. The solvent was removed under reduced pressure and the residue was dried over KOH in vacuo. The mixture contained 8% of TMP photoadduct and 46% of AMP photoproduct. Results of other experiments are described in Table I.

Irradiation of Poly(U) and AMP with 2-Propanol. A mixture of poly(U) (22.2 A_{260} units), AMP (49 A_{260} units), sodium phosphate buffer (11 μl of 0.1 M, pH 6.8), water (66 μl), 2-propanol (66 μl), *tert*-butyl alcohol (24 μl), and $(t\text{-BuO})_2$ (24 μl) was divided into five Pyrex capillary tubes and irradiated. A capillary tube was removed after 1, 2, and 6 h. The solvent was removed under reduced pressure; the residue was dried over KOH, dissolved in water (60 μl), and chromatographed on Whatman No. 1 paper in solvent system D. Spots were located by mineralight lamp, the material was extracted with 0.25 M aqueous NH_4HCO_3, and the absorption at 260 nm measured. Blanks consisting of poly(U) and AMP were irradiated separately under similar conditions. Results are summarized in Table III.

Irradiation of Poly(A) and UMP with 2-Propanol. A mixture of poly(A) (170 A_{260} units), UMP (150 A_{260} units), water (80 μl), phosphate buffer (20 μl of 0.1 M, pH 6.8), [1,3-^{14}C]-2-propanol (100 μl, 7.4×10^6 cpm), and $(t\text{-BuO})_2$ (20 μl) was irradiated for 15 h. $(t\text{-BuO})_2$ (13 μl) was added to the irradiated mixture after 6 h. Samples (65 μl) were withdrawn after 2 and 6 h. The solvent was removed under reduced pressure; the residue was further dried over KOH in vacuo and dissolved in water (30 μl); 15 μl of each sample was applied to Whatman No. 3 MM paper and subjected to electrophoresis at pH 3.5 at 2 kV for 30 min. Spots were located by mineralight lamp, and the paper was cut into strips 1.5 cm wide and counted in toluene scintillation liquid. The UMP spots were also extracted with 0.25 M NH_4HCO_3 solution and their absorption at 260 nm was measured. Both methods indicated ca. 75% modification of UMP, while 35% modification of poly(A) could be observed from radioactivity counting. Blanks consisting of solution of poly(A) and UMP were irradiated separately under similar conditions and worked up similarly. The results are summarized in Table III.

Vapor Pressure Osmometry. Molal solutions of DMU, DMT, caffeine, and 1:1 mixtures of DMU–caffeine and DMT–caffeine were made up by weight. Calibration was carried out at 50 °C with aqueous solutions of sucrose (Schwartz/Mann, ultrapure). The technique of measurement was as follows: 1.5–2 min was assumed to be sufficient for equilibration after placing a fresh drop of solution upon the therminator probe. A reading was taken, the procedure repeated twice more, and an average taken. In general, there was no drift with time and the resistance readings were consistent to within ±0.5 units.

Quantum Yield Determination. A mixture of DMU (4×10^{-2} M), $(t\text{-BuO})_2$ (0.33 M), and 2-propanol was irradiated in Pyrex tubes. Light intensity and absorption were measured by ferrioxalate actinometry.[29] The formation of photoproduct was followed by the disappearance of the absorption band at 260 nm. After 2 h the solvent was removed under reduced pressure, the residue was dissolved in $CDCl_3$, and its NMR spectrum was recorded to show that 34% of the starting material was converted into DMU–2-propanol photoproduct. The calculated quantum yield was 0.076%. With other bases the progress of the reactions was followed qualitatively by TLC, using either solvent system A or B, and quantitatively by NMR.

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(1) (a) Weizmann Postdoctoral Fellow, 1974–1975; (b) in partial fulfilment for the requirements of a Ph.D. degree, to be submitted to Feinberg Graduate School; (c) in partial fulfilment for the requirements for a Ph.D. degree submitted to the Feinberg Graduate School, 1975.

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(18) In all cases, the percent conversion of caffeine to its 2-propanol photoproduct (I) was approximately the same in the presence or absence of DMU. The low conversion of caffeine to product at 80 °C results from the slow decomposition of $(t\text{-BuO})_2$ at this temperature.
(19) Though stable under VPC conditions (injector at 280 °C), under the reaction condition, II slowly decomposes back to starting material at the same rate at all concentrations, more slowly in the presence of caffeine and more rapidly as the temperature is raised. Hence, the values of R_M/R_B plotted in Figure 3 are lower limits.
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Fragmentation Reaction of Ylide. 5.[1] A New Metabolic Reaction of Aziridine Derivatives

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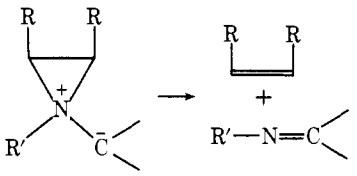
Abstract: Reactions of a number of substituted aziridines with rat liver microsomes under in vitro condition were carried out. Most of the aziridines employed gave olefin and nitrosoalkane as the fragmentation reaction products of the aziridine ring. This is a new metabolic reaction of aziridine, which has been discussed as an alkylating reagent or a precursor of amino alcohol formation in vivo. The fragmentation reaction of aziridine with enzyme(s) should be important in discussions on the biological character of aziridines hereafter.

In studies of the chemical properties of aziridine derivatives, known as strong carcinogenic substances, we found that aziridinium ylide, prepared by addition of carbene onto aziridine[2] or by abstraction of a proton from the N-substituent group of aziridine,[3] decomposed immediately after formation into fragments of olefin and heterounsaturated product.



The olefin formation reaction of the aziridinium ylide usually proceeded with stereospecific retention of the configuration and no other typical ylide reaction, for example, Stevens rearrangement, was observed.

Under the assumption that the metabolic course of aziridines might proceed through the formation of ylide by hepatic enzyme, we examined the in vitro biotransformation of aziridines by hepatic microsomes obtained from normal rats. Here we wish to report a new fragmentation reaction of aziridines by microsomal enzymes and the possibility of this hitherto unknown process of decomposing aziridines in vivo as a characteristic biological property.

The metabolic reactions of aziridines by liver microsomes were carried out in 50 mM Tris–HCl buffer (pH 7.4) containing 150 mM KCl, 10 mM $MgCl_2$, 1 mM nicotinamide, 1 mM pyrophosphate, 30 mg of protein of microsomes, 0.01 mM of aziridines, and NADPH-generating system (0.9 mM NADP, 10 mM glucose 6-phosphate, and 2.5 units of glucose-6-phosphate dehydrogenase) in a final volume of 5 ml. Unless otherwise stated, incubations were carried out aerobically at 37 °C for 60 min with moderate shaking.

After the reaction time was over, 2 N NaOH solution was added and the mixture was extracted with an appropriate amount of CH_2Cl_2 for VPC analysis. For the substrate, aziridine derivatives were converted into their tartarate immediately before use. In the case of compound **10**, we used the HCl salt of 2-chloro-*N*-isopropyl-2-(β-naphthyl)ethylamine instead of aziridine. This chloroamine was a precursor of aziridine **10** and was converted immediately into aziridine in the reaction solution.

In a preliminary experiment without introduction of the enzymatic system, aziridines were stable under the reaction conditions. First, tartarate of *cis*-2,3-diphenylaziridine (**1**) was used as a substrate. Fortunately, in the reaction mixture we found the formation of *cis*-stilbene by VPC analysis, although the yield was poor.

In our experiments, immediately after mixing the aziridines into the microsome solution, extraction with CH_2Cl_2 gave only 80–90% recovery of the aziridines. Thus, we calculated the reaction rate constant on the basis of the amount of aziridine recovered as the initial concentration and obtained 2×10^{-4} s^{-1} as the pseudo-first-order rate for the disappearance of *cis*-2,3-diphenylaziridine. The yield of *cis*-stilbene from compound **1** was approximately 4% at the half-life of the reaction.[4] We had expected a better yield, however, under the reaction conditions; olefin also smoothly disappeared from the mixture by another metabolic reaction.