therein.
(6) 3-Methylenecepham derivatives have been synthesized by an electrochemical reduction and by chromium(II) salts, Raney nickel, or zinc–formic acid reduction of the corresponding cephalosporinic acids (M. Ochiai, O. Aki, A. Morimoto, T. Okada, K. Shinozaki, and Y. Asahi, *J. Chem. Soc., Perkin Trans. 1*, 258 (1974); M. Ochiai, O. Aki, A. Morimoto, T. Okada, and K. Morita, *Tetrahedron*, 115 (1975); R. R. Chauvette and P. A. Penington, *J. Org. Chem.*, **38**, 2994 (1973)). More recently two thermal conversions of penams into 3-methylenecephams have been reported (S. Kukolja, M. R. B. Gleissner, A. I. Ellis, D. E. Dorman, and A. W. Paschal, *J. Org. Chem.*, **41**, 2276 (1976); S. Kukolja, S. R. Lammert, M. R. B. Gleissner, and A. I. Ellis, *J. Am. Chem. Soc.*, **98**, 5040 (1976)).
(7) For example, 3-methoxy- (or chloro-) 3-cephems possessing potential antibiotic activity have been synthesized from 3-methylenecephams by a low-temperature ozonolysis of the 3-exomethylene function followed by methylation (or chlorination) (cf. R. R. Chauvette and P. A. Penington, *J. Med. Chem.*, **18**, 403 (1975); *J. Am. Chem. Soc.*, **96**, 4985 (1974)).
(8) For leading references, see (a) S. Nakatsuka, H. Tanino, and Y. Kishi, *J. Am. Chem. Soc.*, **97**, 5008, 5010 (1975); *Tetrahedron Lett.*, 581 (1976); (b) J. E. Baldwin, A. Au, M. Christie, S. B. Haber, and D. Hesson, *J. Am. Chem. Soc.*, **97**, 5975 (1975).
(9) Irradiation of **1a** in acetonitrile or ether followed by chromatographic separation did not give any crystalline products.
(10) R. D. G. Cooper and F. L. Jose, *J. Am. Chem. Soc.*, **92**, 2574 (1970).
(11) Analogous isomerization has been observed in 3-methylenecepham derivative (cf. M. Ochiai, O. Aki, A. Morimoto, T. Okada, and T. Kaneko, *Tetrahedron Lett.*, 2355 (1972)).
(12) Cf. A. Srivivasan, K. D. Richards, and R. K. Olsen, *Tetrahedron Lett.*, 891 (1976).
(13) R. B. Morin, M. E. Gordon, T. Mcgrath, and M. Shuman, *Tetrahedron Lett.*, 2159 (1973), and references cited therein.
(14) Examples of thermal thiazole cyclization by attack of a ring sulfur to an 6-acylamido group in penicillins are precedented (for a recent article, see R. Latree, *Justus Liebigs Ann. Chem.*, 1361 (1974); see also ref 10)).
(15) J. E. Baldwin, S. B. Haber, and J. Kitchin, *J. Chem. Soc., Chem. Commun.*, 790 (1973).
(16) Isomerization of the 3-cephem double bond to the 2-cephem position was well documented (cf. ref 3a, p 147). Isolation of the pure 2-cephem from an equilibrium mixture of 2-cephems and 3-cephems, however, is somewhat tedious. Irradiation of the mixture of 2-cephem (**11**) and 3-cephem (**1a**) obtained upon treatment of **1a** with triethylamine caused the complete destruction of **1a** during a short period. The unchanged **10** was easily isolable from the reaction mixture by silica gel chromatography (see Experimental Section).
(17) For reviews, see (a) T. Sheradsky in "The Chemistry of Thiol Group", Part II, S. Patai, Ed., Interscience, New York, N.Y., 1974, p 702; (b) D. Elad in "Organic Photochemistry", Vol. 2, O. L. Chapman, Ed., Marcel Dekker, New York, N.Y., 1969, p 181.
(18) J. K. Kochi, "Free Radicals", Vol. 2, Wiley, New York, N.Y., 1973, p 711.
(19) We did not attempt to optimize the reaction conditions.
(20) The NMR spectrum of the reaction mixture did not show the presence of detectable amount of other products.
(21) (a) R. D. G. Cooper, *J. Am. Chem. Soc.*, **92**, 5010 (1970); (b) D. H. R. Barton, F. Comer, D. T. C. Greig, G. Lucente, P. G. Sammes, and W. G. E. Underwood, *J. Chem. Soc., Chem. Commun.*, 1059 (1970).
(22) For our recent work on photolysis of aromatic disulfides, see Y. Maki and M. Sako, *Tetrahedron Lett.*, 851 (1976).
(23) Pyrolysis of dithioazetidinones **9** under certain conditions gave a resinous mixture of products. No formation of 3-methylenecepham **10** in the mixture was detected by TLC. Detailed results will be reported in the near future.

Kinetics and Mechanism of Carbon-8 Methylation of Purine Bases and Nucleosides by Methyl Radical[1]

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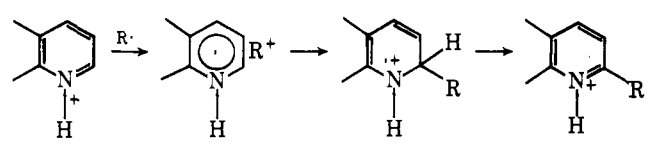
Abstract: The kinetics of homolytic methylation of the model purine compound caffeine at carbon-8 were determined as a function of several reaction variables. The methyl radical was generated from *tert*-butyl peracetate (BPA) either thermally (65–95 °C) or photochemically (>300 nm, 25 °C). The thermal reaction k (25 °C) was found to be 3.09×10^{-8} s^{-1} from the linear log k (pseudo-first-order) vs. $1/T$ plot. The light reactions using the 450- and 1200-W mercury lamps proceeded with k (25 °C) 450- and 2160-fold greater, respectively. The derived activation energies are consistent with an S_EAr reaction. Increasing the concentration of caffeine from 0.25 M to 1.67 M in the presence of 3 molar equiv of BPA did not cause any side reaction. The pH–rate profile as shown in Figure 1 can be predicted by rate equations (1a–c), which are derived on the basis of an electrophilic substitution occurring on the free base and conjugate acid of a heteroaromatic system. A competition study using tetrahydrofuran reveals the presence of a radical σ complex IIIa and a charge transfer complex IIIb as intermediates for methylation under neutral and acidic conditions, respectively. Their rate-determining nature was indicated by the small positive kinetic isotope effect and the inverse solvent isotope effects; $k_{H_3O^+}/k_{D_3O^+} = 0.87$ and $k_{H_2O}/k_{D_2O} = 0.32$. Thus, in acidic medium, a preequilibrium proton transfer to form the caffeine conjugate acid precedes the rate-controlling formation of IIIb. In neutral solution, the rate-determining step appears to be the protonation of the radical nitrogen in IIIa converting it to III. The acid-catalyzed caffeine–BPA reaction was shown to be general for other purines such as adenine, adenosine, guanine, guanosine, hypoxanthine, and inosine. Their reaction kinetics were found to be similar. Quantitative comparisons of these BPA methylation reactions with those using the *tert*-butyl hydroperoxide–iron(II) system for generation of the methyl radical reveal that these two series of homolytic methylation reactions proceeded in largely the same manner.

A working hypothesis in chemical carcinogenesis involves the metabolic conversion of certain carcinogens to free-radical species followed by covalent binding to nucleic acids.[2] This includes the carcinogenic actions of carbon tetrachloride and the like,[3] quinoline *N*-oxides,[4] and aromatic amines.[5] The latter amines are particularly well known for their unusual reactions with DNA and RNA. Thus, rats treated with the carcinogenic 2-acetylaminofluorene (AAF) were found to yield nucleic acids containing 8-(*N*-2-fluorenylacetamido)guanine.[5] The synthetic *N*-acetoxy-2-acetylaminofluorene also reacted with DNA in vitro at the same guanine C-8 position.[5] This unique C-alkylation has spurred interest in defining the nature and scope of possible radical reactions with nucleosides and bases. Thus, many 6-substituted purines and nucleosides were shown to react with alcohols and ethers under UV light, with

Scheme I



or without sensitizers, to yield the C-8 alkylated products.[6] Radical intermediates were postulated. Maeda et al.[7] reported the carbon methylation of purines and nucleosides by the methyl radical produced in the presence of iron(II).[7] The mechanism was postulated by analogy to radical alkylations at the α,γ positions of the conjugate acid of a pyridine or a quinoline compound as illustrated in Scheme I.[8] The methyl radical was considered to be nucleophilic, yielding an S_EAr

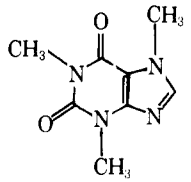
Table I. Rate Constants for the Thermal and Photoinduced C-8 Methylation of Caffeine with *tert*-Butyl Peracetate[a]

Reaction	Temp, °C/Hg lamp	$k \times 10^5$, s^{-1}
Thermal	58	*b*
Thermal	65	0.40 (±0.01)
Thermal	80	1.67 (±0.18)
Thermal	95	7.61 (±0.37)
Light[c]	450-W	1.39 (±0.02)
Light[c]	1200-W	6.67 (±0.36)

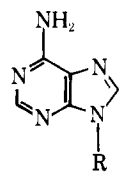
[a] 1 M of caffeine and 3 M of *tert*-butyl peracetate in D_2O-CF_3CO_2D (2:1). *k* was determined by following the appearance of 8-methylcaffeine by both ^1H NMR and HPLC. [b] No reaction after 21 h. [c] 25 °C, Pyrex filter.

type σ intermediate. However, for these alkylations of π-electron-deficient heterocycles (as opposed to the involvement of the π-electron-rich imidazole ring in purine C-8 alkylation), only relative rates of a series of alkyl radicals with substituted vs. unsubstituted heterocycles were reported.[8]

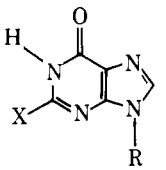
This paper presents for the first time the kinetics and mechanism of free-radical methylation of the purine nucleus. The purines studied include caffeine (**1**), adenine (**2**), guanine (**3**), hypoxanthine (**4**), and the corresponding nucleosides,



1



2, R = H
2a, R = β-D-ribofuranose



3, X = NH_2, R = H
3a, X = NH_2; R = β-D-ribofuranose
4, X = R = H
4a, X = H; R = β-D-ribofuranose

adenosine (**2a**), guanosine (**3a**), and inosine (**4a**). The methyl radical was generated by photochemical or thermal homolysis of *tert*-butyl peracetate (BPA).[9] Caffeine was chosen for the detailed mechanistic study because (1) there is only one ring carbon position which can be methylated, and (2) it has reasonable solubility in both organic and aqueous media, unlike the other purines studied herein, thereby permitting more variations of reaction parameters. Thus, by observing the rate constants of the caffeine–BPA reaction as a function of temperature, concentration, pH, tetrahydrofuran competition, and the kinetic as well as the solvent isotope effects, a detailed radical methylation mechanism is proposed. This mechanism is also applicable to similar methylation of other purines and nucleosides. Hence, this paper provides a quantitative model which will further our understanding of the molecular basis of chemical carcinogenesis via radical–nucleic acid reactions.

Results and Discussion

C-8 Methylation of Caffeine. Thermal and Photoinduced Reaction of Caffeine with *tert*-Butyl Peracetate. The methyl radical was generated from *tert*-butyl peracetate (BPA)[9] according to Scheme II. The rate constant for the rate-determining peroxy bond homolysis is 2.31×10^{-8} s^{-1} at 60 °C in

Table II. Concentration Effects on the Reaction of Caffeine with BPA[a]

	^1H NMR data		
Caffeine concn, M	% C-8 H reacted	% C-8 Me formed	$k \times 10^5$, s^{-1}
0.25	89.0	87.6	1.70
0.50	77.5	75.0	1.59
1.00	74.0	72.3	1.39
1.67	54.1	52.9	1.39

[a] In D_2O-CF_3CO_2D (2:1) medium with 3 equiv of BPA under 450-W mercury lamp (Pyrex filter, 25 °C) for 22 h.

chlorobenzene,[10] while subsequent β-scission of either the acetoxy radical[11] or the *tert*-butoxy radical[12] is much faster. The homolysis of BPA to yield CH_3· was achieved by thermal

Scheme II

$$CH_3CO_2\cdot \longrightarrow CH_3\cdot + CO_2$$

$$CH_3CO_3C(CH_3)_3 \longrightarrow +$$

$$(CH_3)_3CO\cdot \longrightarrow CH_3\cdot + CH_3COCH_3$$

or photochemical (λ >300 nm) means. It is unlikely that caffeine, λ_{max} 272 nm, is electronically excited at wavelengths >300 nm; hence photolysis and thermolysis reactions should be comparable. The first-order rates are shown in Table I for the thermal reactions at 58, 65, 80, and 95 °C, and the two light reactions conducted at 25 °C using 450- and 1200-W mercury lamps. From the linear log *k* vs. $1/T$ plot, the thermal reaction *k* (25 °C) was calculated to be 3.09×10^{-8} s^{-1}. Thus, the rate-enhancing effects of the two lamps are 450- and 2160-fold, respectively. Also, comparison of the 1200-W light reaction using di-*tert*-butyl peroxide with that using *tert*-butyl peracetate shows that methylation of caffeine is 28 times faster with the peracetate as the source of methyl radical.

The activation energies of the caffeine–BPA reaction, calculated from the rate data shown in Table I by applying the Arrhenius equation, are $\Delta H^{\ddagger}$ = 23.5 kcal mol^{-1} and $\Delta S^{\ddagger}$ = −11.7 eu at 25 °C. These values are consistent with an S_EAr reaction. The negative $\Delta S^{\ddagger}$ is especially suggestive of a σ-complex intermediate.[13] Although radical methylations of protonated quinolines and pyridines were postulated to proceed via an S_EAr type mechanism (cf. Scheme I), no activation energies or individual rates are available for comparison.[8] Therefore, caffeine was replaced with 2-methylquinoline in the 1200-W photoinduced methylation reaction. For the formation of 2,4-dimethylquinoline,[14] the rate constant found was 4.43×10^{-5} s^{-1}, similar to that of 6.67×10^{-5} s^{-1} for caffeine. It therefore seems likely that radical methylation of C-8 of purines or the α,γ positions of pyridines and quinolines may share a mechanism similar to that depicted in Scheme I.

Application of high-pressure cation exchange liquid chromatography allowed comprehensive analysis of these reactions. The disappearance of caffeine (t_R 2.65 min) and the concomitant increase of a peak (t_R 3.45 min) identified as 8-methylcaffeine (**5**)[15] were followed. No other caffeine products, i.e., no N- or O-methylation, were detected in any of these methylation reactions. These observations were corroborated by ^1H NMR spectroscopy which permits monitoring of the decreasing C-8 hydrogen (δ 8.57) and an emerging peak at δ 2.78 due to the C-8 methyl group. With reference to Table II, the percent reactions, as measured by the C-8 H decrease or C-8 methyl increase, are essentially the same for a wide range of reactant concentrations. Thus, the possibility of generating a caffeine C-8 radical which may undergo dimerization or combination with the oxy radicals is not indicated. The slightly

Table III. Product Distribution from the Reaction of Caffeine with BPA in the Presence of Tetrahydrofuran at 80 °C

Caffeine, mmol × 10	BPA, mmol × 10	THF, mmol × 10	Medium, mL	HPLC data,[a] % 1	5	6	Product ratio 5:6
3	9	1.5	D_2O, 0.3	86.7	11.5	1.8	6.39
3	9	1.5	D_2O–TFA 0.2:0.1	34.8	23.4	41.8	0.56
3	3	1.5	D_2O–TFA 0.2:0.1	67.7	13.6	18.7	0.73
3	3	3.0	D_2O–TFA 0.2:0.1	61.5	8.2	30.2	0.27

[a] On Bondapak μC_{18} column eluted with MeOH–0.1 N NH_4OAc (1:4), t_R of **1**, **5**, and **6** are 2.85, 4.35, and 10.40 min, respectively. Analyses of the reaction mixtures were also verified by using the ^1H NMR method.

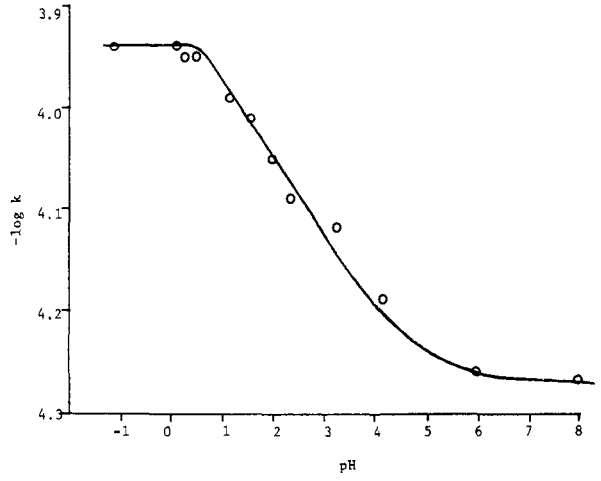


Figure 1. Experimental rate profile for C-8 methylation of caffeine with *tert*-butyl peracetate under irradiation by a 1200-W mercury lamp with Pyrex filter at 25 °C.

decreasing rate of the more concentrated reactions may be attributed to the increasing viscosity of the system as homolysis of BPA is viscosity dependent.[9a] The proton spectrum also showed the by-products of BPA decomposition. At 37% methylation of **1**, the presence of *tert*-butyl alcohol (δ 1.21), acetic acid (δ 2.06), and acetone (δ 2.20) was found in the ratio of 1:17.1:1.3. In the same aqueous acid, but in the absence of caffeine, BPA yielded these three products in the ratio of 1:9.6:2. The larger amount of acetic acid produced during methylation may be due to the involvement of $CH_3CO_2\cdot$ in abstracting the C-8 hydrogen from the σ intermediate. Also, gas chromatography of the gases evolved in a sealed tube caffeine–BPA reaction showed the presence of carbon dioxide (t_R 0.5 min) and methane (t_R 6.8 min). The latter probably also reflects the assisted removal of the C-8 hydrogen in the final step of the S_EAr reaction.

pH–Rate Profile. In radical alkylations of pyridines and quinolines, N-protonation was found to have an enhancing effect on reactivity and selectivity.[8] For pyridine, this rate increase ranges from 1.66 for phenylation with benzoyl peroxide to 13.2 for methylation with *tert*-butyl peroxide. The bipyridyls detected in nonacidic solutions were not formed in acidic media. In the present case, the dependence of the pseudo-first-order rate of the light-initiated methylation on pH was determined in unit ionic strength buffers and threefold excess of BPA at 25 °C as shown in Figure 1. The only product formed in the range of $H_0 - 1.1$ to pH 8.0 was 8-methylcaffeine. The caffeines decomposed beyond pH 8. Also, attempts were made to obtain a pH–rate profile for the thermolytic reaction. In the pH range of 0.1–7.8 at 80 °C, its shape is similar to that shown in Figure 1. However, no values could be obtained below pH 0.1 at 80 °C because of the decomposition of the caffeines. The pH–rate profile shown in Figure 1 is consistent with eq 1 derived for an electrophilic substitution occurring on the free base as well as the conjugate acid of a heteroaromatic system[16]

$$k_{obsd} = \{k_1K_a[E] + k_2[E][H^+]\}/\{K_a + [H^+]\} \quad (1)$$

where k_1 and k_2 are rate constants for methylation of the free base and conjugate acid, respectively; K_a is the acid dissociation constant of caffeine; and E is the electrophile. Imposing the limits of $[H^+] > K_a$, $[H^+] < K_a$, and $[H^+] \rightarrow 0$, and substituting $CH_3\cdot$ for E, eq 1 gives rise to eq 1a, 1b, and 1c, respectively:

$$k_{obsd} = \{k_1K_a/[H^+] + k_2\}[CH_3\cdot] \quad (1a)$$

$$k_{obsd} = \{k_1 + k_2[H^+]/K_a\}[CH_3\cdot] \quad (1b)$$

$$k_{obsd} = k_1[CH_3\cdot] \quad (1c)$$

The $[CH_3\cdot]$ term can be assumed to be constant under photoinduced homolysis of a threefold excess of BPA. Thus, these rate equations predict a level profile in the alkaline region (eq 1c) which increases linearly with medium acidity until pH = pK_a = 0.61 (eq 1b), then levels off or declines depending on the relative importance of k_1 and k_2 (eq 1a). Since the experimental profile shows a plateau at pH <0, it appears that k_2 is greater than k_1.

Charge Transfer Complex and Radical σ Complex as Intermediates. A competition study of the caffeine–BPA reaction using tetrahydrofuran (THF) lends some insight about the nature of the intermediates. The 2-tetrahydrofuranyl radical (THF·) was formed from THF in the presence of BPA as it underwent homolysis. As an α-alkoxy radical, THF· is more stable and more nucleophilic than $CH_3\cdot$. By adding small amounts of THF ($\leq$1 equiv relative to BPA) to the methylation reaction, varying mixtures of 8-methylcaffeine (**5**) and 8-(2-tetrahydrofuranyl)caffeine (**6**)[17] were obtained as shown in Table III. The new alkylated product was formed in minute amount in neutral media but in large amounts in acidic media. In neutral aqueous solution, the ratio of the products **5**:**6** is 6.39, similar to the starting BPA:THF ratio of 6. On the other hand, the trend is reversed for the same BPA:THF ratio in the aqueous trifluoroacetic acid reaction, yielding a product ratio of 0.56 in favor of THF alkylation. When the molar ratios of caffeine:BPA:THF 1:1:0.5 were employed in the acidic medium, the ratio of **5**:**6** was 0.73. Doubling the amount of THF to give a 1:1:1 starting mixture decreased the ratio of **5**:**6** by a factor of 2.7. These competitive effects of THF are discussed in terms of Scheme III. For the neutral reaction depicted in Scheme IIIa, the unprotonated purine is attacked by the more abundant $CH_3\cdot$ to form the σ intermediate IIIa. Reversibility of the addition is probably unimportant so that the product distribution of **5** > **6** is similar to the ratio of the starting radicals. In the acidic medium, a charge transfer complex IIIb

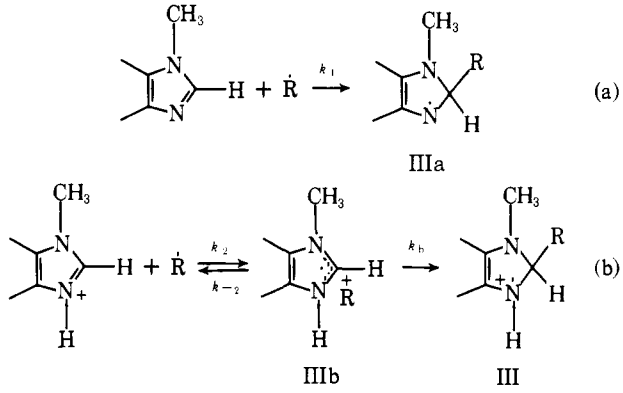
Table IV. Deuterium Effects in Caffeine-BPA Reaction

Caffeine	Medium	$k \times 10^5$, s^{-1}	k_H/k_D
	A. Kinetic Isotope Effect[a]		
8-H[b]	D_2O-CF_3CO_2D (2:1)	2.70 (± 0.28)	1.04
8-D	D_2O-CF_3CO_2D (2:1)	2.59 (± 0.18)	
	B. Solvent Isotope Effects[c]		
8-H	D_2O-CF_3CO_2D (2:1)	1.97 (± 0.26)	0.87
8-H	H_2O-CF_3CO_2H (2:1)	1.71 (± 0.28)	
8-H, 8-D	D_2O	0.34 (± 0.08)	0.32
8-H	H_2O	0.11 (± 0.03)	

[a] 0.13 M of caffeine and 3 equiv of BPA in the specified medium heated at 85 °C. [b] No deuteration of 8-H was observed. [c] 0.13 M of caffeine and 3 equiv of BPA in the specified medium heated at 80 °C.

involving a carbonium ion electrophile is postulated to be the first intermediate. A similar charge-transfer intermediate as shown in Scheme I was proposed to explain the relative reactivities of protonated pyridines and quinolines with a variety of radical species in the order of $\cdot CH_3$ < primary < secondary < tertiary.[8] Assuming that there is a prior equilibration between the caffeine conjugate acid and the charge transfer

Scheme III



complex IIIb, i.e., k_b and k_{-2} are comparable, the lone pair stabilized carbonium ion derived from THF is favored over CH_3^+. This will lead to the σ complex III which discriminates against the methyl adduct, thereby yielding more **6** than **5**. Alternatively, with $k_2 > k_1$ for eq 1a as indicated by the level profile at pH <0 (cf. Figure 1), THF· can be more competitive than the less nucleophilic CH_3· at the k_2 stage; hence more **6** than **5** could have formed without necessitating a reversible addition.

Kinetic and Solvent Isotope Effects. The rate-determining nature of the intermediates depicted in Scheme III is probed by observing the secondary deuterium isotope effects and the solvent isotope effects of deuterated water as shown in Table IV. The relative rate of methyl replacement of caffeine 8-H and caffeine 8-D in the acidic medium is 1.04 at 85 °C (cf. Table IVA). The small magnitude of this secondary α-deuterium effect is reminiscent of those determined for aromatic electrophilic substitution (1 ± 0.1) where the electrophile attacking step is rate determining.[18] Hence, for the acid-catalyzed reaction shown in Scheme IIIb, formation of either the charge transfer complex IIIb or the more developed σ complex appears to be rate determining. Likewise, the isotope effect for the neutral reaction should be small, but it was not directly determined owing to the deuterium exchange of caffeine 8-H in neutral D_2O. Also, the possibility of H-8 abstraction followed by combination with a CH_3· is ruled out since this would exhibit a primary isotope effect of >2.[19] Further delineation of the rate-determining aspect of the methylation mechanism

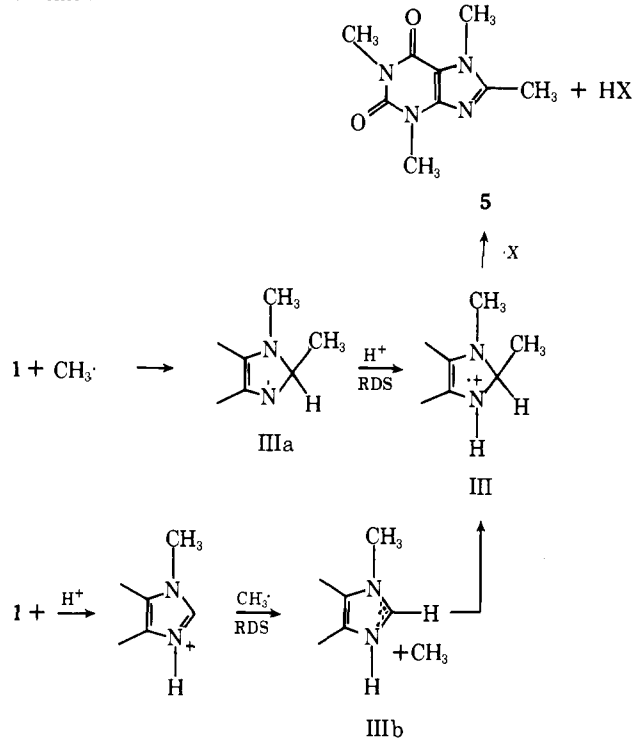
Table V. Rate Constants for C-8 Methylation of Purines and Nucleosides with BPA[a]

Purines, nucleosides	$k \times 10^5$, s^{-1}
Adenine (**2**)	1.18 (± 0.14)
Adenosine (**2a**)	1.87 (± 0.06)
Guanine (**3**)	3.85 (± 0.06)
Guanosine (**3a**)	4.30 (± 0.26)
Hypoxanthine (**4**)	4.02 (± 0.20)
Inosine (**4a**)	3.92 (± 0.20)

[a] 0.4 M of purine compound and 3 equiv of BPA in 2:1 D_2O-CF_3CO_2D mixture irradiated by a 1200-W mercury lamp with Pyrex filter at 20 °C. The reaction was followed by HPLC and k calculated via computer assisted least-squares curve fit.

is made possible by the inverse solvent isotope effects observed (cf. Table IVB). On the basis that D_3O^+ is a stronger acid than H_3O^+ by a factor of 3,[20] the magnitude of these solvent isotope effects permits an estimate of the timing of proton transfer to caffeine N-9 with respect to the rate-determining step. Thus, in aqueous trifluoroacetic acid, a $k_{H_3O^+}/k_{D_3O^+}$ of 0.87 observed at 80 °C indicates a preequilibrium proton transfer to give the caffeine conjugate acid preceding the rate-controlling formation of IIIb. In neutral aqueous solution, a k_{H_2O}/k_{D_2O} of 0.32 was observed at 80 °C whether caffeine 8-H or 8-D was used in D_2O.[21] This substantial inverse solvent isotope effect suggests the rate-determining nature of the protonation step for the radical nitrogen in IIIa converting it to III. This dual-pathway mechanism is summarized in Scheme IV.

Scheme IV



Carbon Methylation of Adenine, Guanine, Hypoxanthine, and Their Nucleosides. The mechanistic study of the caffeine-BPA reaction suggests that radical carbon methylation of purines should be a general phenomenon. Application of the photoinduced homolysis of BPA in aqueous trifluoroacetic acid to the purines adenine (**2**), adenosine (**2a**), guanine (**3**), guanosine (**3a**), hypoxanthine (**4**), and inosine (**4a**) yielded in all cases the C-8 methyl derivative as the major product. The rates of methylation of these purine compounds as shown in Table V are quite similar. The presence of the bulky 9-β-D-ribofu-

Table VI. Product Analyses of Purine-BPA Reactions and Purine-$HOOC(CH_3)_3$-Iron(II) Reactions

Purines, nucleosides	Reaction conditions (Maeda et al.)[d]	% distributions of purines			
		Starting purine	8-Me	2-Me	2,8-DiMe
Adenine (**2**)	A,[e] 24 h	25.7	52.4		21.8
Adenine (**2**)	B[e]	78.6	17.7		3.7
Adenine (**2**)	(Maeda et al.)	38.1	13.8	5.1	9.9
Adenosine (**2a**)[a]	A, 12 h	52.7	15.1	5.3	7.8
Adenosine (**2a**)[a]	B	84.5	4.8	3.3	4.9
Adenosine (**2a**)[a]	(Maeda et al.)	49.4	7.2	5.4	8.3
Guanine (**3**)	A, 8 h	28.5	71.5		
Guanine (**3**)	(Maeda et al.)	40.0	57.0		
Guanisine (**3a**)[b]	A, 8 h	12.1	81.1		
Guanosine (**3a**)[b]	B	18.6	81.4		
Guanosine (**3a**)[b]	(Maeda et al.)	7.0	68.0		
Hypoxanthine (**4**)	A, 9 h	46.4	48.2		4.8
Hypoxanthine (**4**)	B	47.4	38.3	4.5	8.9
Hypoxanthine (**4**)	(Maeda et al.)	19.5	19.0	2.1	3.6
Inosine (**4a**)[c]	A, 6 h	41.0	36.4		3.3
Inosine (**4a**)[c]	(Maeda et al.)	40.0	57.0		

[a] Reaction mixture also contained adenine (1.8%) and 8-methyladenine (0.7%). [b] Reaction mixture also contained 6.9% of guanine. [c] Reaction mixture also contained hypoxanthine (19.2%) and 8-methylhypoxanthine (5.6%). [d] Reference 7. [e] A, 0.4 M of purine compound and 3 equiv of BPA in D_2O-CF_3CO_2D (2:1) irradiated by 1200-W mercury light with Pyrex filter at 20 °C; B, repeated after procedures reported by Maeda et al.[7a] Purine compound, 4 equiv of $FeSO_4 \cdot 7H_2O$, and 3 equiv of $HOOC(CH_3)_3$ in 1 N H_2SO_4 at 25 °C except for adenosine at 0 °C.

Table VII. HPLC Conditions for Analyses of the Methylation Reaction Mixtures

Reaction sample	Solvent system			Flow rate, mL/min
	% MeOH	% H_2O	% 0.1 N $NH_4H_2PO_4$	
Caffeine (**1**)	10		90	3.0
Adenine (**2**)	5		95	3.0
Adenosine (**2a**)			100	2.5
Guanine (**3**)			100	3.0
Guanosine (**3a**)		100		3.0
Hypoxanthine (**4**)			100	2.5
Inosine (**4a**)		100		1.5

ranosyl group apparently has no inhibitory effect on methylation at the adjacent C-8 site. The slower rate of adenine methylation may be due to the fact that it is principally protonated at N-1[22] rather than at N-7 as is the case with the other purines in this study. Since the only general method for purine C-methylation is the Fe^{II}-$HOOC(CH_3)_3$-aqueous H_2SO_4 system reported by Maeda et al.,[7a] we have made quantitative comparison of our BPA reactions with theirs. Several of the latter were repeated and analyzed. The percent distributions of the 8-methyl, 2-methyl, and 2,8-dimethyl derivatives as well as the unreacted starting purine in the reaction mixtures are shown in Table VI. Our HPLC analyses of the reaction mixtures did not reveal any N- or O-methylated products. Minor amounts of purine bases formed from hydrolysis of the nucleosides under these acidic reaction conditions were also determined. In this regard, the BPA homolysis was best induced at 20 °C by the 1200-W mercury lamp. Attempts to conduct the nucleoside-BPA reactions at 95 °C in D_2O-CF_3CO_2D (2:1) mixture or D_2O alone resulted in extensive hydrolysis. It can be seen from these comparisons that the methyl radicals generated either by BPA homolysis or by iron(II) oxidative decomposition of *tert*-butyl hydroperoxide reacted with the purine compounds in largely the same manner. In a preparative sense, the BPA reaction is more convenient since it does not involve a rather tedious isolation procedure to remove the ferrous and ferric ions inherent with the iron-hydroperoxide reaction.

Experimental Section

Instrumentation. ^1H NMR spectra were run on a Perkin-Elmer R12A spectrometer in D_2O or D_2O-CF_3CO_2D with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. The high-pressure liquid chromatograph (HPLC) system used was obtained from Waters Associates, and it includes a M-6000 pump, a U6K injector, and a Model 440 differential UV detector (254 nm). The column used for analysis of the reaction mixtures of caffeine, purines, and nucleosides was a Reeves Angel Partisil 10SCX column (4.6 mm i.d. × 25 cm). The solvent systems and flow rates used for the isocratic elution of the reaction mixtures are listed in Table VII. Analyses of the tetrahydrofuran-BPA-caffeine reaction mixtures were performed on a prepacked Bondapak μC_{18} column (4 mm i.d. × 30 cm) using isocratic elution with methanol-0.1 N NH_4OAc (20:80) at 5 mL/min. The analysis of the gases produced during the caffeine-BPA reaction was accomplished using a Hewlett-Packard Model 700 gas chromatograph. The column was 20 ft × 0.25 in. packed with 5A molecular sieves. The column temperature was 120 °C with an argon flow of 30 mL/min.

Materials. Caffeine was supplied by Eastman Organic Chemicals, guanine from Sigma Chemical Co., and the remaining purines and 2-methylquinoline were obtained from Aldrich Chemical Co. The *tert*-butyl peracetate (75% in benzene) was obtained from K&K division of ICN and di-*tert*-butyl peroxide was supplied by MCB. Caffeine-*8-d* was prepared from caffeine as follows. Caffeine (1.9 g, 1 mmol) was dissolved in 15 mL of D_2O and heated at reflux for 15 h. Upon cooling, caffeine-*8-d* (90% by integration of δ 7.77 in $CDCl_3$) was crystallized from solution.

Caffeine-*tert*-Butyl Peracetate Reaction Rate Studies. Thermal Reactions. Caffeine solution at 1 M was prepared in D_2O-CF_3CO_2D (2:1) mixture containing 1% DSS as an internal standard, and aliquots (0.3 mL) were placed in ^1H NMR tubes. *tert*-Butyl peracetate (3 molar equiv based on caffeine) was added to each sample. The tubes were heated in constant temperature baths maintained at 58, 65, 80, and 95 °C. The tubes were removed periodically for determination of the extent of reaction via ^1H NMR analysis of the residual C-8 of caffeine. Concurrent with the ^1H NMR analysis, samples were also removed for HPLC analysis. The rate constants were derived from the pseudo-first-order plot of log [residual caffeine] vs. time, and the data were submitted to a computer assisted least-squares curve fit routine. These rate constants are listed in Table I.

Photoreactions. One molar caffeine solutions containing 3 molar equiv of BPA were placed in clear ^1H NMR tubes. These were irradiated at 25 °C with either a 450-W or a 1200-W UV lamp. In the case of the 450-W irradiation, a Hanovia water-cooled immersion well (Pyrex filter) was set up at a distance of 1.2 cm from a tube holder in

Table VIII. The Effect of pH on the Pseudo-First-Order Rate Constant for the Reaction of Caffeine with BPA

pH	k, s^{-1}	$-\text{Log } k$
	A. Photoinduced Reaction at 25 °C	
−1.1	1.15×10^{-4}	3.94
0.1	1.15×10^{-4}	3.94
0.3	1.12×10^{-4}	3.95
0.5	1.13×10^{-4}	3.95
1.2	1.01×10^{-4}	3.99
1.6	9.72×10^{-5}	4.01
2.0	8.89×10^{-5}	4.05
2.4	8.06×10^{-5}	4.09
3.3	7.62×10^{-5}	4.12
4.2	6.50×10^{-5}	4.19
6.0	5.55×10^{-5}	4.26
8.0	5.33×10^{-5}	4.27
	B. Thermal Reaction at 80 °C	
0.1	1.71×10^{-5}	4.77
2.8	1.00×10^{-5}	5.00
4.0	0.66×10^{-5}	5.18
6.7	0.14×10^{-5}	5.85
6.8	0.11×10^{-5}	5.96
7.0	0.11×10^{-5}	5.96
7.8	0.10×10^{-5}	6.00

which the samples were placed. For the 1200-W irradiation, a Gates Instrument lamp was used and the samples were placed in a Pyrex water-cooled bath (25 °C) at a distance of 2.5 cm from the source. In both cases, the reaction rates were followed as was described for the thermal reaction. The results of the photoreaction are also listed in Table I.

The same procedure was used for reactions at other initial caffeine concentrations (Table II), and for reactions in solvents other than D_2O–CF_3CO_2D.

pH–Rate Profile. In the pH region, 0.13 M of caffeine solutions were prepared in buffered solutions at pH 0.1–8.0, and at unit ionic strength adjusted either by initial buffer concentration or by addition of KCl. In the $-H_0$ region, 0.13 M of caffeine solution was dissolved in 20 wt % of H_2SO_4 ($H_0 = -1.1$). A 3 M excess of BPA was added to each sample. The samples in clear ^1H NMR tubes were either irradiated with a 1200-W UV lamp (Pyrex filter) at 25 °C or were heated at 80 °C. Samples were removed periodically for HPLC analysis. The results of the study are shown in Figure 1 and in Table VIII.

Kinetic Isotope Effect. The determination of the secondary kinetic isotope effects at 85 °C was accomplished by comparison of the rate of reaction of caffeine and caffeine-*8-d* (0.13 M) in D_2O–CF_3CO_2D (2:1) mixture using a 3 M excess of BPA. The extent of reaction was followed by HPLC techniques. Table IVA lists the pseudo-first-order rate constants and the k_H/k_D calculated from them.

Solvent Isotope Effects. Solutions (0.13 M) of caffeine were prepared in D_2O, H_2O, D_2O–CF_3CO_2D (2:1), and H_2O–CF_3CO_2H (2:1), and they were transferred to ^1H NMR tubes. The D_2O and H_2O solutions were buffered to the equivalent of pH 6.8 with 1 M buffer solution. The pH meter reading for the D_2O solution was adjusted by the addition of 0.4.[23] A 3 M excess of BPA was added to each sample and the tubes were placed in a constant temperature bath of 80 °C. Periodically, samples were removed for HPLC analysis. The resulting rate constants as well as the k_H/k_D values derived from them are tabulated in Table IVB.

Methylation in the Presence of Tetrahydrofuran. Varying amounts of caffeine, BPA, and tetrahydrofuran were introduced into ^1H NMR tubes containing either D_2O–CF_3CO_2D (2:1) or D_2O as shown in Table III. The tubes were heated in a constant temperature bath at 80 °C. The reactions were followed by both ^1H NMR and HPLC techniques. The product distributions found are listed in Table III.

Reactions of Purines and Their Nucleosides with BPA. Solutions (0.4 M) of adenine, adenosine, guanine, guanosine, hypoxanthine, and inosine were prepared in D_2O–CF_3CO_2D (2:1), and were placed in ^1H NMR tubes. A 3 M excess of BPA was added to each tube and they were irradiated with a 1200-W UV lamp in a constant temperature bath of 20 °C. The reactions were followed by HPLC. The rate constants obtained are tabulated in Table V. The products of the reactions were identified by ^1H NMR analysis and by HPLC cochromatography with authentic samples prepared by the procedure given by Maeda and co-workers.[7] The product distributions are listed in Table VI.

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