Photochemical Formation and Degradation of Cephalosporins[1]

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Abstract: The mode of photodegradation of 3-cephem derivatives was clarified. UV irradiation of 3-cephem derivatives (**1**) in alcohols (methanol and ethanol) caused a novel photorearrangement leading to thiazole derivatives (**2** and **3**) which involves incorporation of alcohols into an intermediate photoproduct. This type of photoreaction is general for 7-acylamido-3-cephem derivatives. Dihydro-1,3-thiazinone (**6**) also underwent analogous photodegradation. Photolysis of 4-heterocyclic dithioazetidinones (**9**), which possess an *N*-(1-methoxycarbonyl-2-methylprop-2-enyl) side chain, resulted in the preferential formation of 3-methylenecepham derivatives (**10**) accompanied by 2-cephem derivatives (**11**). On the basis of a deuterium labeling experiment, the origin of the carbon at position 2 of **10** and **11** was confirmed to be the methylene carbon and not the methyl carbon in the isopropenyl moiety of **9**. Analogous irradiation of isomeric 4-dithioazetidinone (**13**), however, gave disulfide (**14**) rather than **10** and **11**. The present photoconversion provides a convenient preparative method of 3-methylenecephams from penams. Plausible mechanisms of the present photoreactions were presented and the results were discussed in connection with speculative biosynthetic routes to cephems.

In the chemical researches of β-lactam antibiotics, much of the current attention has been paid to the chemical conversion of the penam to the cepham skeleton.[3,4] The useful methods for the penam–cepham conversion are primarily based on the electrophilic addition of 4-sulfenic acid or its equivalent groups across the *N*-isopropenyl double bond in the azetidinone intermediate which can be produced via a thermal cleavage of the 1–2 bond of penicillin sulfoxides. Despite the abundance of these thermal conversions, photochemical transformation of penams to cephams has hitherto been unknown.

An extreme lability of cephalosporin C to UV light was observed in early investigations on this important class of antibiotics.[5] Because photolysis resulted in the destruction of cephalosporin antibiotic activity, the β-lactam dihydrothiazine nucleus (3-cephem nucleus) was suggested as the site of photoreactivity.

In view of these previous observations, we first attempted to clarify the mode of photodegradation of 3-cephem derivatives. It was found that UV irradiation through a Pyrex filter of 3-cephem derivatives in alcohols (methanol or ethanol) causes a novel photorearrangement, leading to thiazole derivatives, which involves incorporation of alcohols into an intermediate photoproduct.

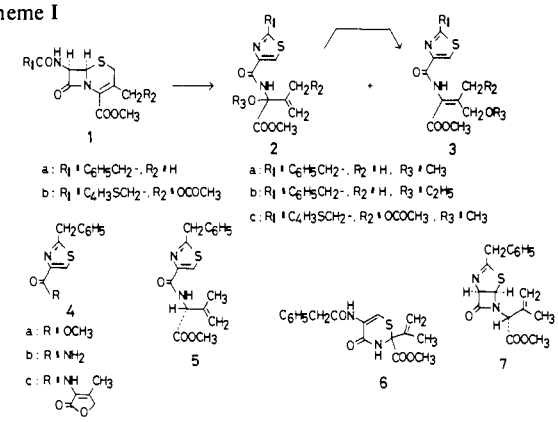
This type of photochemistry is general for 7-acylamido-3-cephem derivatives. In striking contrast to the photochemical behavior of 3-cephems, 3-methylenecephams and 2-cephems have proved to be essentially insensitive to Pyrex-filtered UV light (vide infra).

Accordingly, our efforts were focused on the photochemical preparation of 3-methylenecephams,[6] which can be thermally converted into 3-cephems and are versatile intermediates for the modification of cephalosporin antibiotics.[7] This purpose was achieved by irradiation of 4-heterocyclic dithioazetidinones,[4] which have been prepared by intermolecular trapping of the sulfenic acid intermediate formed thermally from penicillin sulfoxides with heterocyclic thiols.

In this report, we describe a mode of photodegradation of 3-cephem derivatives involving the novel photorearrangement and the unique photochemical formation of 3-methylenecepham nucleus.

On the basis of a number of facts, some biosynthetic pathways to β-lactam antibiotics have been proposed[3] and tested in in vitro experiments.[8] The present results are also intriguing in connection with these speculative biosynthetic routes.

Scheme I



Results and Discussion

Photodegradation of 3-Cephems. Methyl 7-phenylacetamido-3-methyl-3-cephem-4-carboxylate (**1a**) in methanol[9] (0.01 M) was irradiated by a 400-W high-pressure mercury arc lamp surrounded by a water-cooled jacket through a Pyrex filter under nitrogen until the disappearance of **1a** (monitored by TLC) was complete (about 8 h). The solution was concentrated under reduced pressure to leave an oily residue which was submitted to chromatography on silica gel. Evaporation of the initial eluate and recrystallization of the residue gave 2-benzylthiazole-4-carboxamide derivative (**2a**) in 50% yield. Further elution afforded a small amount of an isomeric compound (**3a**) (vide infra). Attempts to isolate other minor products from further eluates failed.

No interconversion between the isolated products, **2a** and **3a**, was observed under analogous conditions. Microanalytical and mass spectral data of both the products established the molecular formula as $C_{18}H_{20}O_4N_2S$. These products were optically inactive.

Cooper et al.[10] have reported the transformation of penicillin V sulfoxide into the 2-phenoxymethylthiazole-4-carboxamide derivative. Analogously, penicillin G sulfoxide methyl ester was converted to optically active 2-benzylthiazole-4-carboxamide derivative (**5**) in 80% yield.

The NMR spectrum of **5** is similar to that of **2a**, except for the presence of a methine proton signal at δ 5.26 (1 H, d, J = 8 Hz, coalesced to a singlet by deuterium exchange) instead

of a methoxy signal in **2a**. The UV spectrum of **2a** (λ_{max} (MeOH) 228 nm (sh, ϵ 8000)) is superimposable on that of **5**.

Further structural proof was obtained upon treatment of **2a** with methanolic hydrochloric acid at room temperature. Silica gel chromatography of the reaction mixture led to the isolation of the isomeric product **3a** and oily methyl 2-benzylthiazole-4-carboxylate (**4a**) in 96 and 2% yields, respectively. The major product **3a** thus obtained was identical in every respect with the compound isolated as a by-product upon irradiation of **1a** in methanol. The NMR spectral change going from **2a** to **3a** is consistent with isopropenyl-isopropylidene isomerization.[11]

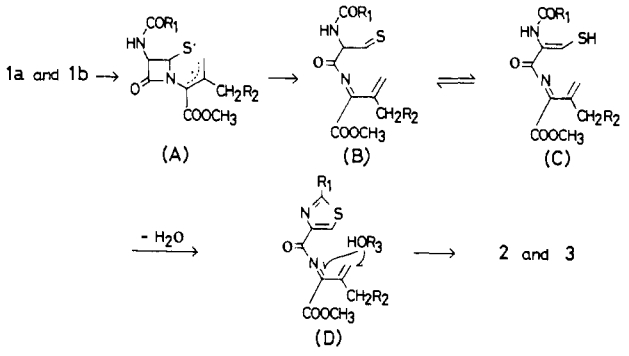
Upon treatment of **2a** with aqueous dioxane containing hydrochloric acid, α,β-unsaturated lactone **4c** (IR (film) 1760 cm^{-1}; NMR ($CDCl_3$) δ 4.83 (2 H, s, $-OCH_2O=$)) and 2-benzylthiazole-4-carboxamide (**4b**) were obtained in 21 and 72% yields, respectively. The formation of **4b** and **4c** can be rationalized by postulating the intermediacy of α,β-unsaturated acylimine (vide infra, D in Scheme II) which could be produced from **2a**.

Irradiation of **3a** in methanol caused photoisomerization to give a mixture of **3a** and its geometrical isomer. The newly formed isomer showed a vinylic methyl signal at δ 2.12 which is more deshielded than that of **3a** (δ 1.96). Thus, the cis orientation of an ester group to a methoxymethyl group in **3a** was proposed.[12]

When irradiation of **1a** was carried out in ethanol, ethoxy derivative **2b** and its isomer **3b** were obtained in 16 and 0.5% yields, respectively. The minor isomer **3b** was identical in every respect with the product obtained upon treatment of **2a** or **2b** with ethanolic hydrochloric acid. On employing isopropyl alcohol as a solvent, irradiation of **1a** did not give detectable amounts of pure products. In a similar manner, irradiation of methyl 7-(thiophene-2-acetamido)-3-acetoxymethyl-3-cephem-4-carboxylate (**1b**) resulted in the formation of thiazole derivative **2c**. It is notable that while dihydro-1,3-thiazinone (**6**)[13] also rearranged to give **2a** and **2b** in moderate yields by irradiation in alcohols, thiazoline-azetidinone derivative **7**[10] was stable under analogous conditions.

The present photorearrangement was not suppressed by addition of acetophenone in various concentrations and was almost completely quenched in the presence of piperylene. Accordingly, a triplet excited state of **1** may be involved in the initial stage of homolytic cleavage of a sulfur–allylic carbon (C_2) bond to give a biradical (A). Although some routes have been considered for the subsequent reactions leading to **2** and **3**,[14] we tentatively propose a reaction sequence as shown in Scheme II. Homolytic cleavage of β-lactam in the biradical

Scheme II



(A) could give thioaldehyde (B) which isomerizes to thioenol (C). An ultimate intermediate (D) could be easily formed by loss of water from C. The final step presumably proceeds via a thermal or light-induced 1,4-addition of the alcohols to an α,β-unsaturated acylimine moiety of the intermediate (D). The observed stability of thiazoline-azetidinone (**7**) to UV light eliminates an alternate route to D from A involving **7** as a possible intermediate. The formation of **2** and **3** from the dihydro-1,3-thiazinone (**6**) can also be accounted for by the intermediacy of D.

The acid treatment of **2a** (vide supra, HCl in aqueous dioxane) could give the intermediate D as a result of loss of methanol. Analogous 1,4-addition of water to D followed by the C–N bond cleavage or lactonization leads to the formation of **4b** or **4c**. Thus, D is again a reasonable intermediate for the conversion of **2a** to **4b** or **4c**.

A variety of oxidative transformations have to be envisaged for the biological conversion of a tripeptide, δ-(L-α-aminoadipoyl)-L-cystenyl-D-valine, into the penicillins and cephalosporins. Several speculative chemical schemes have been proposed[3] and tested in in vitro experiments;[8] e.g., Baldwin et al.[15] have suggested an electrocyclic addition of the thioaldehyde intermediate (B) for the biosynthesis of cephalosporins. The present photoreaction can be considered to be an illustration of intra- and intermolecular trapping of the speculative biosynthetic intermediate (B) by an amide side chain and alcohols.

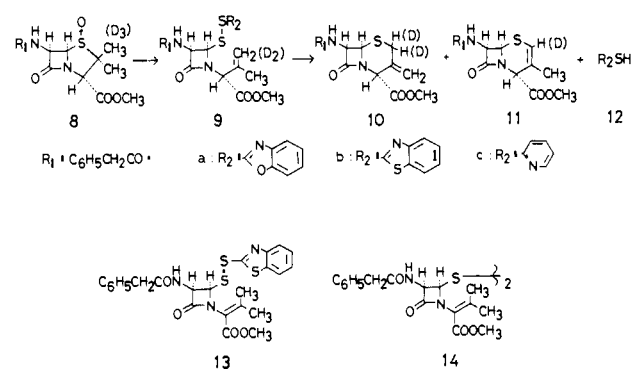
Photochemical Formation of 3-Methylenecephams. Contrary to 3-cephem (**1a**) (UV λ_{max} (MeOH) 260 nm (ϵ 8 × 10^3)), isomeric 3-methylenecepham (**10**)[6] (UV λ_{max} (MeOH) 251 nm (ϵ 1 × 10^3), 258 (sh, 8 × 10^2), 264 (sh, 6 × 10^2)) and 2-cephem (**11**)[3] (UV λ_{max} (MeOH) 246 nm (sh, ϵ 5 × 10^3)) were insensitive to analogous UV irradiation.[16] Accordingly, the photochemical procedure appears to be advantageous for the preparation of 3-methylenecepham **10** and 2-cephem **11** rather than the naturally occurring 3-cephem **1**.

It is well known that addition of a thiyl radical to an olefinic double bond occurs preferentially in the anti-Markownikoff manner.[17] The photocleavage of a disulfide linkage provides one of the efficient methods for the generation of thiyl radicals.[18] Thus, 4-heterocyclic dithioazetidinones (**9a–c**) which set up appropriately an isopropenyl double bond and a disulfide linkage in the molecule for a intramolecular photocyclization are attractive candidates.

Kamiya et al.[4] have shown that 4-heterocyclic dithioazetidinones can be prepared by intermolecular trapping of the sulfenic acid intermediate produced thermally from penicillin sulfoxides with heterocyclic thiols, e.g., 2-mercaptobenzothiazole (**12b**), and utilized as a versatile intermediate for the preparation of 3-cephem derivatives.

According to Kamiya's procedure,[4] 4-(2′-benzoxazolyl and benzothiazolyl)dithioazetidinones (**9a, b**) were obtained quantitatively upon heating of penicillin G sulfoxide methyl ester (**8**) with 2-mercaptobenzoxazole (**12a**) or 2-mercapto-

Scheme III



benzothiazole (**12b**) in toluene. Synthesis of 4-(2′-pyridyl)-dithioazetidinone (**9c**) was achieved by employing 2,2′-pyridyl

disulfide instead of 2-mercaptopyridine (**12c**) as a trapping agent of the intermediary sulfenic acid.

Dithioazetidinone (**9a**) (2×10^{-3} M) in acetonitrile was irradiated by a 400-W high-pressure mercury arc lamp through a Pyrex filter under nitrogen for about 1 h. Chromatographic separation allowed the isolation of 3-methylenecepham methyl ester (**10**), 3-methyl-2-cephem methyl ester (**11**) and 2-mercaptobenzoxazole (**12a**) in 60, 15, and 58% yields, respectively.[19,20]

On the basis of the spectral data, microanalytical results, and base-catalyzed conversion into 3-methyl-3-cephem derivative,[7] the structure of **10** was established. The other products **11** and **12a** were identical in every respect with authentic samples. Analogous irradiation of **9b** and **9c** in acetonitrile gave **10** in 45 and 18% yields, respectively.[19]

No occurrence of photointerconversion between **10** and **11** under the same conditions indicates the competing formation of both the products.

It should be noted that 3-methyl-3-cephem derivative was not detected in the present photoreaction. As mentioned previously, 3-cephem derivatives are labile to UV light which allows the exhaustive degradation of the β-lactam dihydrothiazine nucleus. However, since the complete destruction requires a considerable long-period irradiation under the employed conditions, the possibility of further photodegradation of the initially formed 3-cephem derivative during irradiation is negligible.

Irradiation of isomeric 4-(2′-benzothiazolyl)dithioazetidinone (**13**) resulted in the formation of a disulfide (**14**) in 37% yield and did not give the cephem and penam derivatives. Microanalytical and spectral data fully supported the structure of **14**.

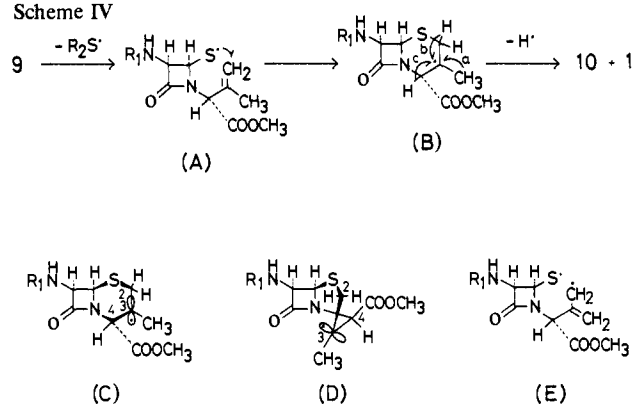
4-(2′-Benzothiazolyl)dithioazetidinone deuterated at the olefinic carbon (**9b-d**) (deuterium content ca. 30% by NMR) was prepared by reaction of 2-β-methyl-d_3-penam sulfoxide (**8-d**)[21] (deuterium content ca. 40%) with **12b**. The NMR spectrum of the photoproducts (**10-d** and **11-d**) derived from **9b-d** showed that deuterium was retained at the 2 position of **10-d** and **11-d** in ca. 30% deuterium content, respectively. Thus, the origin of the carbon at position 2 of the photoproducts **10** and **11** was confirmed to be the methylene carbon and not the methyl carbon in the isopropenyl moiety of **9**. On the basis of above facts, a reaction sequence is outlined as shown in Scheme IV.

Homolytic cleavage of the S–S bond of **9** in an excited state[18,22] could give a thiyl radical (A) and a heterocyclic thiyl radical ($R_2S\cdot$). Attack of the thiyl radical (A) on the olefinic carbon occurs intramolecularly in the anti-Markownikoff manner to give an intermediate radical (B). Although three possible ways (a, b, and c) for loss of a hydrogen in B can be considered, the most favorable one appears to be the hydrogen abstraction from a methyl grouping (a) mainly due to the steric reason; i.e., a Dreiding model experiment of possible conformations of B (see C and D in Scheme IV) showed that the C–H bond orbitals at position 2 or 4 and the spin orbital of the radical carbon (C_3) are not coplanar.

There have been many examples of five-, six-, and seven-membered ring formations by intramolecular addition of thiols to appropriately situated double bonds via the radical or ionic process,[17] which are not accompanied by the subsequent hydrogen abstraction.

The heterocyclic thiyl radical ($R_2S\cdot$) would play a role for the hydrogen abstraction from a methyl group and could capture the eliminated hydrogen atom to convert into heterocyclic thiol **12**. An alternate mechanism, involving the radical coupling of the biradical species (E) formed as a result of hydrogen abstraction from the allylic methyl group of A by the heterocyclic thiyl radical ($R_2S\cdot$), is unequivocally excluded based on the above-mentioned deuterium labeling experiment.

Scheme IV



The present result provides a convenient preparative method of 3-methylenecepham from penam in fairly high yields.[23]

This type of photoreaction is also anticipated in azetidinone derivatives which are equivalent to the disulfide **9** in respect to the photochemical generation of thiyl radicals. From this viewpoint, further investigations are currently in progress and advanced details will be reported in the near future. Additionally, we note that the present photoreaction suggests a chemically reasonable biosynthetic route to cepham from a dithioazetidinone intermediate, which might attach to a protein surface by the S–S linkage.

Experimental Section

All melting points were determined on a Yanagimoto micro hot stage apparatus and are uncorrected. Microanalyses were obtained from the microanalytical laboratory of this college. Infrared spectra were recorded on a Hitachi 215 spectrometer from samples as neat film or KBr pellets. NMR spectra were obtained on a Hitachi R20-B (60 MHz) spectrometer using $CDCl_3$ as solvent unless specified otherwise. Chemical shift values are presented in parts per million (δ) downfield from tetramethylsilane (Me_4Si) as an internal standard. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Ultraviolet spectra were obtained from samples in dilute solution (in methanol) on a Hitachi 323 spectrophotometer. Mass spectra were run on a Hitachi RMU-6L mass spectrometer. All irradiations were conducted in an immersion reaction equipped with a water-cooled jacket (Rikosha Model UVL 400P) using a 400-W high-pressure mercury arc lamp through a Pyrex filter. Prior to irradiation, the solution was flushed with nitrogen and nitrogen was bubbled through the solution constantly during irradiation. Column chromatography was carried out in silica gel (Mallinckrodt, 100 mesh) using chloroform–acetone (15:1) as eluent.

Irradiation of Methyl 7-Phenylacetamido-3-methyl-3-cephem-4-carboxylate (1a) in Methanol or Ethanol. A solution of 500 mg of **1a** in 500 mL of methanol (3×10^{-3} M) was irradiated for 8 h. During this period, **1a** completely disappeared (monitored by thin layer chromatography). After removal of the solvent under reduced pressure, the oily residue thus obtained was chromatographed on 50 g of silica gel. Elution with chloroform–acetone (15:1) gave 260 mg (50%) of 2-benzylthiazole-4-carboxamide derivative (**2a**). Recrystallization from ether–*n*-hexane gave colorless crystals of **2a**: mp 123–125 °C; IR (KBr) 3390 (NH), 1730 ($COOCH_3$), 1680 cm^{-1} (CONH); NMR ($CDCl_3$) δ 1.89 (3 H, broad s, $CH_2{=}CCH_3$), 3.34 (3 H, s, $-OCH_3$), 3.87 (3 H, s, $COOCH_3$), 4.37 (2 H, s, $C_6H_5CH_2$), 5.27 and 5.53 (each 1 H, m, isopropenyl vinyl protons), 7.40 (5 H, broad s, phenyl ring protons), 8.05 (1 H, s, thiazole ring proton), 8.65 (1 H, broad, NH, deuterium exchangeable); UV λ_{max} (MeOH) 228 nm (sh, ϵ 8×10^3); mass spectrum *m/e* 360 (M^+), 301 ($M^+ - 59$), 202 ($M^+ - 158$).

Anal. Calcd for $C_{18}H_{20}N_2O_4S$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.86; H, 5.66; N, 7.59.

Successive elution with the same solvent gave 10 mg (2%) of the isomeric compound (**3a**). Recrystallization from ether–*n*-hexane gave colorless crystals of **3a** melting at 111–113 °C; IR (KBr) 3350 (NH), 1700 ($COOCH_3$), 1660 cm^{-1} (CONH); NMR ($CDCl_3$) δ 1.96 (3 H, broad s, $=CCH_3$), 3.35 (3 H, s, OCH_3), 3.80 (3 H, s, $COOCH_3$), 4.34 (2 H, s, $C_6H_5CH_2$), 4.40 (2 H, broad s, CH_2OCH_3), 7.37 (5 H, broad s, phenyl ring protons), 8.06 (1 H, s, thiazole ring proton), 8.67 (1 H,

broad, NH, deuterium exchangeable); UV λ_{max} (MeOH) 240 nm (ϵ 8×10^3); mass spectrum *m/e* 360 (M^+), 301 ($M^+ - 59$), 202 ($M^+ - 158$).

Anal. Calcd for $C_{18}H_{20}N_2O_4S$: C, 59.99; H, 5.59; N, 7.77. Found: C, 60.16; H, 5.66; N, 7.47.

Analogously, a solution of 500 mg of **1a** in 500 mL of ethanol (3×10^{-3} M) was irradiated for 8 h. After removal of the solvent under reduced pressure, the oily residue thus obtained was submitted to silica gel chromatography to isolate 80 mg (15%) of 2-benzylthiazole-4-carboxamide derivative (**2b**) and 2.7 mg (0.5%) of its isomeric compound (**3b**). Recrystallization of **2b** from ether–*n*-hexane gave colorless crystals: mp 118–121 °C; IR (KBr) 3390 (NH), 1730 ($COOCH_3$), 1680 cm^{-1} ($CONH$); NMR ($CDCl_3$) δ 1.26 (3 H, t, OCH_2CH_3), 1.89 (3 H, broad s, $CH_2{=}CCH_3$), 3.54 (2 H, m, OCH_2CH_3), 3.87 (3 H, s, $COOCH_3$), 4.36 (2 H, s, $C_6H_5CH_2$), 5.25 and 5.54 (each 1 H, m, isopropenyl vinyl proton), 7.40 (5 H, broad s, phenyl ring protons), 8.40 (1 H, s, thiazole ring proton), 8.66 (1 H, broad, NH, deuterium exchangeable); UV λ_{max} (MeOH) 228 nm (sh, ϵ 8×10^3); mass spectrum *m/e* 374 (M^+), 315 ($m^+ - 59$), 202 ($M^+ - 172$).

Anal. Calcd for $C_{19}H_{22}N_2O_4S$: C, 60.95; H, 5.92; N, 7.48; S, 8.54. Found: C, 61.06; H, 5.86; N, 7.26; S, 8.49.

Recrystallization of **3b** from ether–*n*-hexane gave colorless crystals melting at 93–95 °C: IR (KBr) 3350 (NH), 1700 ($COOCH_3$), 1660 cm^{-1} ($CONH$); NMR ($CDCl_3$) δ 1.22 (3 H, t, OCH_2CH_3), 1.98 (3 H, broad s, $=CCH_3$), 3.52 (2 H, q, OCH_2CH_3), 3.80 (3 H, s, $COOCH_3$), 4.34 (2 H, s, $C_6H_5CH_2$), 4.45 (2 H, broad s, $CH_2OCH_2CH_3$), 7.38 (5 H, broad s, phenyl ring protons), 8.07 (1 H, s, thiazole ring proton), 8.67 (1 H, broad, NH, deuterium exchangeable); UV λ_{max} (MeOH) 239 nm (8×10^3); mass spectrum *m/e* 374 (M^+), 315 ($M^+ - 59$), 202 ($M^+ - 172$).

Anal. Calcd for $C_{19}H_{22}N_2O_4S$: C, 60.95; H, 5.92; N, 7.48. Found: C, 60.82; H, 5.91; N, 7.42.

Synthesis of 2-Benzylthiazole-4-carboxamide Derivative (5). A mixture of 1.00 g (2.8×10^{-3} M) of methyl 6-phenylacetamidopenicillanate-1β-oxide (**8**) and 1.00 g (8×10^{-3} M) of trimethyl phosphite in 20 mL of benzene was heated under reflux for 28 h with stirring. After removal of the solvent under reduced pressure, the oily residue thus obtained was purified by silica gel chromatography and recrystallization from ether to isolate 756.5 mg (80%) of methyl α-isopropenyl-3-benzyl-1α,5α-4-thia-2,6-diaza[3.2.0]-2-heptene-6-acetate-7-one (**7**) as colorless crystals: mp 98–100 °C; IR (KBr) 1760 (β-lactam), 1730 cm^{-1} ($COOCH_3$); NMR ($CDCl_3$) δ 1.70 (3 H, broad s, $=CCH_3$), 3.86 (3 H, s, $COOCH_3$), 3.90 (2 H, broad s, $C_6H_5CH_2$), 4.85 (1 H, broad s, $CHCOOCH_3$), 4.91 and 5.07 (each 1 H, m, isopropenyl vinyl protons), 5.95 (2 H, q, β-lactam ring protons), 7.34 (5 H, broad s, phenyl ring protons); UV λ_{max} (MeOH) 242 nm (ϵ 1×10^3); mass spectrum *m/e* 331 ($M^+ + 1$), 271 ($M^+ - 59$), 175 ($M^+ - 155$); $[\alpha]_D^{15}$ −199.5° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.81; H, 5.49; N, 8.48; S, 9.68. Found: C, 61.73; H, 5.52; N, 8.29; S, 8.51.

To a solution of 100 mg (3×10^{-4} M) of **7** in 30 mL of methanol was added 0.3 mL of concentrated HCl in one portion. The mixture was then heated under reflux for 2 h with stirring. The reaction mixture was poured into 100 mL of water, neutralized with 1 N NaOH solution, and extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried, and evaporated to dryness under reduced pressure. The oily residue thus obtained was recrystallized from ether-*n*-hexane to give 100 mg (100%) of **5** as colorless crystals: mp 62–63 °C; IR (KBr) 3400 (NH), 1720 ($COOCH_3$), 1670 cm^{-1} ($CONH$); NMR ($CDCl_3$) δ 1.87 (3 H, broad s, $=CCH_3$), 3.83 (3 H, s, $COOCH_3$), 4.35 (2 H, s, $C_6H_5CH_2$), 5.11 and 5.19 (each 1 H, broad s, isopropenyl vinyl protons), 5.26 (1 H, d, J = 8 Hz, $CHCOOCH_3$), 7.38 (5 H, broad s, phenyl ring protons), 8.03 (1 H, s, thiazole ring proton), 8.10 (1 H, broad s, J = 8 Hz, NH, deuterium exchangeable); UV λ_{max} (MeOH) 228 nm (ϵ 8×10^3); mass spectrum *m/e* 330 (M^+), 271 ($M^+ - 59$), 202 ($M^+ - 128$); $[\alpha]_D^{15}$ −51° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.67; H, 5.48; N, 8.22.

Conversion of Isopropenyl Derivative (2a) into Isopropylidene Derivative (3a). To a solution of 658.9 mg of **2a** in 30 mL of methanol (1.8×10^{-3} M) was added 0.5 mL of concentrated HCl in one portion. The mixture was then heated under reflux for 2 h. After removal of the solvent under reduced pressure, the residue was poured into 50 mL of water, neutralized with 1 N NaOH solution, and extracted with chloroform. The chloroform solution was washed with water, dried, and evaporated to dryness under reduced pressure. The oily residue thus obtained was submitted to silica gel chromatography to isolate 630.6 mg (95.7%) of **3a** which was identical in every respect with the compound isolated as a by-product upon irradiation of **1a** in methanol. Further elution gave 20 mg (4%) of methyl 2-benzylthiazole-**4**-carboxylate (**4a**) as an oil: NMR ($CDCl_3$) δ 3.91 (3 H, s, $COOCH_3$), 4.34 (2 H, s, $C_6H_5CH_2$), 7.32 (5 H, broad s, phenyl ring protons), 8.06 (1 H, s, thiazole ring proton).

Analogously, the reaction of **2a** with concentrated HCl in ethanol gave **3b** in 74% yield which was identical in every respect with the compound isolated as a by-product upon irradiation of **1a** in ethanol.

Conversion of Isopropenyl Derivative (2a) into Amide 4b and Lactone 4c. To a solution of 100 mg (2.8×10^{-4} M) of **2a** in 10 mL of dioxane and 10 mL of water was added 0.2 mL of concentrated HCl in one portion. The mixture was then heated under reflux for 2 h. After removal of the solvent under reduced pressure, the residue was poured into 50 mL of water, neutralized with 1 N NaOH solution, and extracted with chloroform. The chloroform solution was washed with water, dried, and evaporated to dryness under reduced pressure. The oily residue thus obtained was submitted to silica gel chromatography to isolate 20 mg (21%) of **4c** as an oil: IR (film) 3350 (NH), 1760 (lactone CO), 1670 cm^{-1} ($CONH$); NMR ($CDCl_3$) δ 2.30 (3 H, broad s, $=CCH_3$), 4.37 (2 H, s, $C_6H_5CH_2$), 4.84 (2 H, broad s, lactone ring protons), 7.39 (5 H, broad s, phenyl ring protons), 8.10 (1 H, s, thiazole ring proton), 9.02 (1 H, broad, NH, deuterium exchangeable); mass spectrum *m/e* 314 (M^+), 202 ($M^+ - 112$).

Further elution gave 47.7 mg (72%) of **4b**. Recrystallization of **4b** from ether gave colorless crystals melting at 195–196 °C: IR (KBr) 3350, 3200 (NH_2), 1680 ($CONH$), 1640 cm^{-1}; NMR ($CDCl_3$) δ 4.33 (2 H, s, $C_6H_5CH_2$), 7.37 (5 H, broad s, phenyl ring protons), 8.07 (1 H, s, thiazole ring proton); UV λ_{max} (MeOH) 230 nm (sh, ϵ 9×10^3); mass spectrum *m/e* 218 (M^+), 202 ($M^+ - 16$); 173 ($M^+ - 45$).

Anal. Calcd for $C_{11}H_{10}N_2OS$: C, 60.54; H, 4.62; N, 12.84. Found: C, 60.31; H, 4.60; N, 12.60.

Irradiation of Methyl 7-(Thiophene-2′-acetamido)-3-acetoxymethyl-3-cephem-4-carboxylate (1b). A solution of 500 mg of **1b** in 500 mL of methanol (2.4×10^{-3} M) was irradiated for 9 h. After removal of the solvent under reduced pressure, the residue was submitted to silica gel chromatography to isolate 112.9 mg of thiazole-4-carboxamide derivative (**2c**) as an oil: IR (film) 3380 (NH), 1730 ($COOCH_3$), 1680 cm^{-1} ($COCH$); NMR ($CDCl_3$) δ 2.11 (3 H, s, $OCOCH_3$), 3.35 (3 H, s, OCH_3), 3.88 (3 H, s, $COOCH_3$), 4.57 (2 H, s, CH_2), 4.81 (2 H, broad s, CH_2OCOCH_3), 5.58 and 5.81 (each 1 H, isopropenyl vinyl protons), 7.00–7.50 (3 H, m, thiophene ring protons), 8.09 (1 H, s, thiazole ring proton), 8.87 (1 H, broad, NH, deuterium exchangeable); UV λ_{max} (MeOH) 233 nm (ϵ 9×10^3); mass spectrum *m/e* 424 (M^+), 365 ($M^+ - 59$), 208 ($M^+ - 216$).

Irradiation of 1,3-Dihydrothiazinone Derivative (6). A solution of 60 mg of **6** in 30 mL of methanol (6×10^{-3} M) was irradiated for 1.5 h. After removal of the solvent under reduced pressure, the residue thus obtained was submitted to silica gel chromatography to isolate 23.3 mg (40%) of **2a** which was identical in every respect with the compound isolated as a major product upon irradiation of **1a** in methanol.

Methyl α-[4(Benzoxazol-2-yl)-dithio-3-phenylacetamido-2-oxoazetidin-1-yl]-α-isopropenyl Acetate (9a). A mixture of 200 mg (5.7×10^{-4} M) of methyl 6-phenylacetamidopenicillanate 1β-oxide (**8**) and 91.3 mg (6×10^{-4} M) of 2-mercaptobenzoxazole (**12a**) in 30 mL of dry toluene was heated under reflux for 4 h with stirring. After removal of the solvent under reduced pressure, the residue was purified by silica gel chromatography and recrystallization from ether-*n*-hexane to isolate 279.2 mg (98%) of **9a** as a colorless, amorphous solid: mp 47–49 °C; IR (KBr) 3300 (NH), 1770 (β-lactam), 1740 ($COOCH_3$), 1660 cm^{-1} ($CONH$); NMR ($CDCl_3$) δ 1.90 (3 H, broad s, $=CCH_3$), 3.69 (5 H, s, $COOCH_3$ and $C_6H_5CH_2$), 4.97 (1 H, s, $CHCOOCH_3$), 5.09 and 5.18 (each 1 H, m, isopropenyl vinyl protons), 5.54 (1 H, q, J = 4.5 and 8 Hz, C_3H), 5.62 (1 H, d, J = 4.5 Hz, C_4H), 7.37 (5 H, broad s, phenyl ring protons), 7.1–7.9 (5 H, m, NH and benzoxazole ring protons); UV λ_{max} (MeOH) 285 nm (ϵ 1×10^4), 278.5 (9×10^3), 245 (1.2×10^4); mass spectrum *m/e* 346 ($M^+ - 151$), 159, 151, 91.

Anal. Calcd for $C_{24}H_{23}N_3O_5S_2$: C, 57.94; H, 4.66; N, 8.45. Found: C, 58.22; H, 4.78; N, 8.22.

Methyl α-[4-(Benzothiazol-2-yl)dithio-3-phenylacetamido-2-oxoazetididin-1-yl]-α-isopropenyl Acetate (9b). **9b** was prepared in a

similar manner to the above case by the reaction of **8** with 2-mercaptobenzothiazole (**12b**). Recrystallization from ether gave **9b** as colorless crystals in 96% yield: mp 138–141 °C; IR (KBr) 3260 (NH), 1780 (β-lactam), 1740 (COOCH$_3$), 1660 cm^{-1} (CONH); NMR (CDCl$_3$) δ 1.95 (3 H, broad s, =CCH$_3$), 3.73 (5 H, s, COOCH$_3$ and C$_6$H$_5$CH_2), 4.92 (1 H, s, CHCOOCH$_3$), 5.06–5.22 (each 1 H, n3H isopropenyl vinyl protons), 5.40 (1 H, q, J = 4.5 and 8 Hz, C$_3$H), 5.53 (1 H, d, J = 4.5 Hz, C$_4$ H), 6.51 (1 H, broad d, J = 8 Hz, deuterium exchangeable), 7.39 (5 H, broad s, phenyl ring protons), 7.3–8.0 (4 H, m, benzothiazole ring protons); UV λ_{max} (MeOH) 298 nm (sh, ε 7 × 10^3), 287 (sh, 8 × 10^3), 267 (1.2 × 10^4); mass spectrum *m/e* 346 (M$^+$ − 167), 315, 287, 256, 167.

Anal. Calcd for $C_{24}H_{23}N_3O_4S_3$: C, 56.14; H, 4.52; N, 8.18; S, 18.70. Found: C, 55.91; H, 4.35; N, 8.11; S, 19.30.

Methyl α-[4-(Pyridin-2-yl)-dithio-3-phenylacetamido-2-oxoazetidin-1-yl]-α-isopropenyl Acetate (9c). In a similar manner to the above case, **9c** was obtained by the reaction of **8** with 2,2′-pyridyl disulfide as an oil in 24% yield: IR (film) 3300 (NH), 1770 (β-lactam), 1740 (COOCH$_3$), 1660 cm^{-1} (CONH); NMR (CDCl$_3$) δ 1.94 (3 H, broad s, =CCH$_3$), 3.70 (2 H, s, C$_6$H$_5$CH_2), 3.77 (3 H, s, COOCH$_3$), 4.89 (1 H, s, CHCOOCH$_3$), 5.09 and 5.23 (each 1 H, m, isopropenyl protons), 5.34 (1 H, d, J = 4.5 Hz, C$_4$ H), 5.56 (1 H, q, J = 4.5 and 8 Hz, C$_3$ H), 7.40 (5 H, broad s, phenyl ring protons), 7.0–8.6 (5 H, m, NH and pyridine ring protons); UV λ_{max} (MeOH) 283 nm (ε 4.8 × 10^3), 235 (1 × 10^4); mass spectrum *m/e* 346 (M$^+$ − 111), 220, 156, 111.

Irradiation of Dithioazetidinones (9). A solution of 200 mg of **9a** in 200 mL of acetonitrile (2 × 10^{-3} M) was irradiated for 1 h. After removal of the solvent under reduced pressure, the residue was dissolved in 200 mL of chloroform and washed twice with 100 mL of 5% sodium bicarbonate solution to remove 2-mercaptobenzoxyazole (**12a**). The chloroform solution was washed repeatedly with water, dried over anhydrous sodium sulfate, and chromatographed on 50 g of silica gel with chloroform–acetone (15:1) to separate two major products. The first product to be eluted was 22 mg (15.8%) of methyl 7-phenylacetamido-3-methyl-2-cephem-4-carboxylate (**11**) which was identical in every respect with an authentic sample prepared by the reaction of **1a** with triethylamine.

The second product eluted was 88 mg (63%) of methyl 7-phenylacetamido-3-methylenecepham-4-carboxylate (**10**). Recrystallization of **10** thus obtained from a mixture of chloroform, ether, and *n*-hexane gave colorless crystals melting at 108–109 °C: IR (KBr) 3300 (NH), 1770 (β-lactam), 1740 (COOCH$_3$), 1660 cm^{-1} (CONH); NMR (CDCl$_3$) δ 3.17 (1 H, broad d, J = 14 Hz, C$_2$ H), 3.67 (1 H, broad d, J = 14 Hz, C$_2$ H), 3.65 (2 H, s, C$_6$H$_5$CH_2), 3.80 (3 H, s, COOCH$_3$), 5.10 (1 H, s, C$_4$ H), 5.23 (2 H, broad d, exomethylene protons), 5.38 (1 H, d, J = 4.5 Hz, C$_6$ H), 5.68 (1 H, q, J = 4.5 and 9 Hz, C$_7$ H), 6.73 (1 H, broad d, J = 9 Hz, NH, deuterium exchangeable), 7.35 (5 H, broad s, phenyl ring protons); UV λ_{max} (MeOH) 264 nm (sh, ε 6 × 10^2), 258 (sh, 8 × 10^2), 251 (1 × 10^3); mass spectrum *m/e* 346 (M$^+$), 172, 91.

Anal. Calcd for $C_{17}H_{18}N_2O_4S$: C, 58.95; H, 5.24; N, 8.09. Found: C, 58.75; H, 5.47; N, 7.85.

Irradiation of **9b** in a similar manner to the above case gave **10** and **11** in 45 and 11% yields, respectively. Analogously, **10** and **11** were isolated from the irradiated solution of **9c** in 18 and 4% yields, respectively.

Preparation of Methyl 7-Phenylacetamido-3-methyl-2-cephem-4-carboxylate (11). To a solution of 150 mg of **1a** in 30 mL of chloroform was added 10 mL of triethylamine in one portion. The solution was then heated under reflux for 8 h with stirring. After cooling, the reaction mixture was washed with 0.5 N HCl solution, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. (The ratio of **1a** and its isomeric compound **11** in the reaction mixture was 1:1 by NMR.)

A solution of the residue in 200 mL of acetonitrile was irradiated until the disappearance of **1a** was complete (monitored by TLC, for 10 h). The reaction mixture was evaporated to dryness under reduced pressure and submitted to silica gel chromatography to isolate **11.** The 2-cephem derivative (**11**) thus obtained was recrystallized from ether to give 70 mg (47%) of colorless crystals melting at 153–154 °C; IR (KBr) 3290 (NH), 1770 (β-lactam), 1740 (COOCH$_3$), 1660 cm^{-1} (CONH); NMR (CDCl$_3$) δ 1.87 (3 H, broad s, C$_3$ CH$_3$), 3.67 (2 H, s, C$_6$H$_5$CH_2), 3.82 (3 H, s, COOCH$_3$), 4.74 (1 H, m, C$_4$ H), 5.26 (1 H, d, J = 4 Hz, C$_6$ H), 5.66 (1 H, q, J = 4 and 9 Hz, C$_7$ H), 5.94 (1 H, m, C$_2$ H), 6.52 (1 H, broad d, J = 9 Hz, NH, deuterium exchangeable), 7.40 (5 H, broad s, phenyl ring protons); UV λ_{max} (MeOH) 246 nm (sh, ε 5 × 10^3); mass spectrum *m/e* 346 (M$^+$), 287, 172.

Anal. Calcd for $C_{17}H_{18}N_2O_4S$: C, 58.95; H, 5.24; N, 8.09. Found: C, 58.77; H, 5.28; N, 8.05.

Methyl α-[4-(Benzothiazole-2-yl)-dithio-3-phenylacetamido-2-oxoazetidin-1-yl]-α-isopropylidene Acetate (13). A solution of 200 mg (4 × 10^{-4} M) of **9a** in 6 mL of dry pyridine was stirred at room temperature for 24 h. The reaction mixture was poured into a cold 1 N HCl solution and extracted with chloroform. The chloroform solution was washed with water, dried, and evaporated under reduced pressure. The residue was purified by silica gel chromatography and recrystallization from ether–*n*-hexane to obtain 193.1 mg (96%) of **13** as a colorless, amorphous solid: mp 64–68 °C; IR (KBr) 3300 (NH), 1780 (β-lactam), 1720, 1700 (COOCH$_3$), 1670 cm^{-1} (CONH); NMR (CDCl$_3$) δ 2.15 (3 H, broad s, =CCH$_3$), 2.17 (3 H, broad s, =CCH$_3$), 3.63 (3 H, s, COOCH$_3$), 3.71 (2 H, s, C$_6$H$_5$CH_2), 5.11 (1 H, q, J = 4.5 and 8 Hz, C$_3$ H), 5.56 (1 H, d, J = 4.5 Hz, C$_4$ H), 7.04 (1 H, broad d, J = 8 Hz, NH, deuterium exchangeable), 7.38 (5 H, broad s, phenyl ring protons), 7.3–8.0 (4 H, m. benzothiazole ring protons); UV λ_{max} (MeOH) 297 nm (sh ε 2.5 × 10^3), 287 (sh, 7 × 10^3), 266 (1 × 10^4); mass spectrum *m/e* 346 (M$^+$ − 167), 332, 167, 91.

Anal. Calcd for $C_{24}H_{23}N_3O_4S_3$: C, 56.14; H, 4.52; N, 8.18. Found: C, 56.91; H, 4.59; N, 7.67.

Irradiation of Dithioazetidinone (13). A solution of 200 mg of **13** in 200 mL of acetonitrile (2 × 10^{-3} M) was irradiated for 1 h. After removal of the solvent under reduced pressure, the residue was submitted to silica gel chromatography and recrystallization (ether–*n*-hexane) to isolate 100.5 mg (37%) of disulfide **14** as a colorless, amorphous solid; mp 97–90 °C; IR (KBr) 3300 (NH), 1770 (β-lactam), 1720 (COOCH$_3$), 1670 cm^{-1} (CONH); NMR (CDCl$_3$) δ 2.02 (6 H, broad s, =CCH$_3$), 2.28 (6 H, broad s, =CCH$_3$), 3.68 (4 H, s, C$_6$H$_5$CH_2), 3.82 (6 H, s, COOCH$_3$), 4.87 (2 H, q, J = 4.5 and 9 Hz, C$_3$ H), 5.06 (2 H, d, J = 4.5 Hz, C$_4$ H), 6.75 (2 H, broad d, J = 9 Hz, NH, deuterium exchangeable), 7.40 (10 H, broad s, phenyl ring protons); UV λ_{max} (MeOH) 336 nm (ε 2.3 × 10^3); mass spectrum *m/e* 346 (M$^+$/2 − 1), 228, 169, 91.

Anal. Calcd for $C_{34}H_{38}N_4O_8S_2$: C, 58.78; H, 5.51; N, 8.07; S. 9.21. Found: C, 58.30; H, 5.40; N, 8.04; S, 10.06.

Preparation and Irradiation of Deuterated Compounds of 9b-d. A solution of 500 mg (1.4 × 10^{-3} M) of **8** in 15 mL of dry toluene and 7 mg of deuterated water was heated under reflux for 4 h. After removal of the solvent under reduced pressure, the residue was submitted to silica gel chromatography to isolate 338.5 mg (67.7%) of **8-d.** NMR spectral analysis showed that the deuterium content of **8-d** was ca. 40%.

A mixture of 200 mg (5.7 × 10^{-4} M) of **8-d** and 100.9 mg (6 × 10^{-4} M) of **12b** in 15 mL of dry toluene was heated under reflux for 4 h. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel to isolate 249.4 mg (85%) of **9b-d.** The deuterium content of **9b-d** thus obtained was estimated to be 30% by NMR spectroscopy.

A solution of 200 mg of **9b-d** in 200 mL of acetonitrile (2 × 10^{-3} M) was irradiated for 1 h. After removal of the solvent under reduced pressure, the residue was submitted to silica gel chromatography to isolate 63.3 mg (47%) of a mixture of **10-d** and **11-d.** The NMR spectra of these products showed that the deuterium contents were ca. 30%, respectively.

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References and Notes

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(2) (a) Author to whom correspondence should be addressed. (b) On leave from Toyo Jozo Co. Ltd., Mifuku, Ohito, Tagata, Shizuoka, 410-23, Japan.
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Kinetics and Mechanism of Carbon-8 Methylation of Purine Bases and Nucleosides by Methyl Radical[1]

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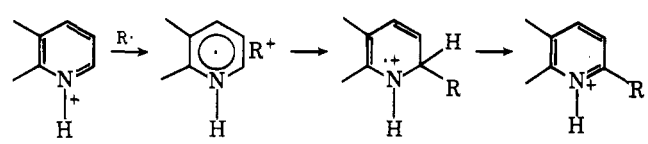
Abstract: The kinetics of homolytic methylation of the model purine compound caffeine at carbon-8 were determined as a function of several reaction variables. The methyl radical was generated from *tert*-butyl peracetate (BPA) either thermally (65–95 °C) or photochemically (>300 nm, 25 °C). The thermal 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