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Reaction of 6-Substituted 1,3-Dimethyluracils with Benzoyl Peroxide

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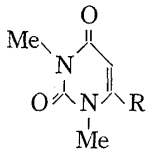
The reaction of 6-hydrazino-1,3-dimethyluracil (II) with the benzoyloxy radical gave a tricyclic pyridazine derivative, 1,3,6,8-tetramethyl-2,4,5,7-tetraoxo-1,2,3,4,5,6,7,8-octahydropyridazino[3,4-*d*: 6,5-*d'*]dipyrimidine (VII), while the reaction of 6-amino-1,3-dimethyluracil (I) with the benzoyloxy radical afforded 6-amino-5-benzoyloxy-1,3-dimethyluracil (VI). Treatment of VII with ammonia gave another tricyclic pyridazine derivative, 2,4,7,9-tetramethyl-1,3,8-trioxo-1,2,3,4,7,8,9-heptahydropyrimido[4,5-*c*]imidazo[4,5-*e*]pyridazine (VIII).

Keywords——benzoyloxy radical; pyridazino[3,4-*d*: 6,5-*d'*]dipyrimidine; pyrimido-[4,5-*c*]imidazo[4,5-*e*]pyridazine; paramagnetic shift; 6-amino-5-benzoyloxy-1,3-dimethyluracil

Many spectroscopic and synthetic investigations have recently appeared on the reactions of pyrimidine bases of nucleic acids with amino,[2,3] hydroxy,[2,4,5] and benzoyloxy[6] radicals, which appear to have mutagenic and carcinogenic effects.[7] These free radicals are electrophilic in nature, and hence they add predominantly to the 5-position of uracils when there is an electron-donating substituent at the 6-position, or even if there is no substituent at the 5-position.[5] On the other hand, it is well known that the benzoyloxy radical is formed on heating benzoyl peroxide[8] and adds to olefins to afford benzoyloxyalkyl adduct radicals.[9] Therefore, it was assumed that the benzoyloxy radical might add to the 5-position of 6-substituted 1,3-dimethyluracils to give various pyrimidine radical intermediates, in which the unpaired electron would be localized at the 6-position. Since little information is available on the free radical reactions of uracil residues bearing a nitrogen-functional group at the 6-position, the reaction of 6-substituted 1,3-dimethyluracil derivatives, shown in Chart 1, with the benzoyloxy radical was investigated as a model system in order to examine the reactivity of the intermediary pyrimidine radicals.



I: R=NH_2
II: R=$NHNH_2$
III: R=$NMeNH_2$
IV: R=$NHNHC_6H_5$
V: R=$NHNHCOCH_3$

Chart 1

6-Amino-1,3-dimethyluracil (I) was allowed to react with an equimolar amount of benzoyl peroxid by heating in acetic acid and chloroform for 2 hr on a boiling water bath. Removal of the solvent by evaporation left an oily mixture which was treated with chloroform to precipitate a colorless compound (VI). Mass and elemental analyses indicated a molecular formula of $C_{13}H_{13}N_3O_4$ for VI. The infrared (IR) spectrum exhibited C=O group absorption

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at 1730 cm^{-1}, as shown in Fig. 1, which presumably arises from the carboxylic ester. The nuclear magnetic resonance (NMR) spectrum showed two singlet signals for N-methyl protons at 3.13 and 3.34 ppm, broad signals for amino protons at 6.77 and 6.93 ppm, and multiplet signals for aromatic protons at 7.34 to 7.77 and 7.93 to 8.28 ppm. No olefinic proton signal was observed. On the basis of these data, VI was assumed to be 6-amino-5-benzoyloxy-1, 3-dimethyluracil. This structure was also supported by its ultraviolet (UV) spectrum, as shown in Fig. 2. Absorption maxima were observed at 242 and 273 nm, indicating the presence of a benzoyloxy group and a uracil residue, respectively. An attempt to hydrolyze VI with alkali was unsuccessful.

6-Hydrazino-1,3-dimethyluracil (II) was similarly allowed to react with benzoyl peroxide, yielding an insoluble yellow compound (VII). Mass and elemental analyses indicated a molecular formula of $C_{12}H_{12}N_6O_4$ for VII. Its IR spectrum displayed absorption bands due to C=O at 1732 and 1730 cm^{-1}, as shown in Fig. 1. Its UV spectrum, shown in Fig. 2, exhibited absorption maxima at 258, 423, and 446 nm. The higher peaks at 423 and 446 nm suggest an extended conjugated system. These analytical and spectral data suggest that VII is a tricyclic pyridazine derivative, produced from two molecules of II by dehydrogenation and elimination of hydrazine. The NMR spectrum of VII showed two singlet signals for N-methyl protons at 4.13 and 3.74 ppm. These data show that VII is a symmetrical compound. Therefore, VII was assumed to be 1,3,6,8-tetramethyl-2,4,5,7-tetraoxo-1,2,3,4,5, 6,7,8-octahydropyridazino[3,4-*d*: 6,5-*d'*]dipyrimidine.

The reaction of 6-(1'-methylhydrazino)-1,3-dimethyluracil (III) with benzoyl peroxide also produced VII, although the yield was low compared with the reaction of II with benzoyl peroxide. The compounds IV and V did not give VII. These results showed that the 6-hydrazino group had no substituent in the terminal nitrogen atom for the formation of VII.

Treatment of VII with 28% ammonia solution yielded a colorless compound (VIII). Its mass and elemental analyses gave a molecular formula of $C_{11}H_{12}N_6O_3$ for VIII, indicating loss of a C=O moiety from one of the pyrimidine rings in the parent compound VII. The IR spectrum showed a change in the C=O absorption compared with that of the parent compound VII (Fig. 1). No absorption due to N–H bonding was observed. The UV spectrum exhibited absorption maxima at 244, 292, 302, and 358 nm, as shown in Fig. 2. The shift of the absorption maxima towards lower wavelength suggests alteration of the ring system in the parent compound. These analytical and spectral data indicated ring contraction in

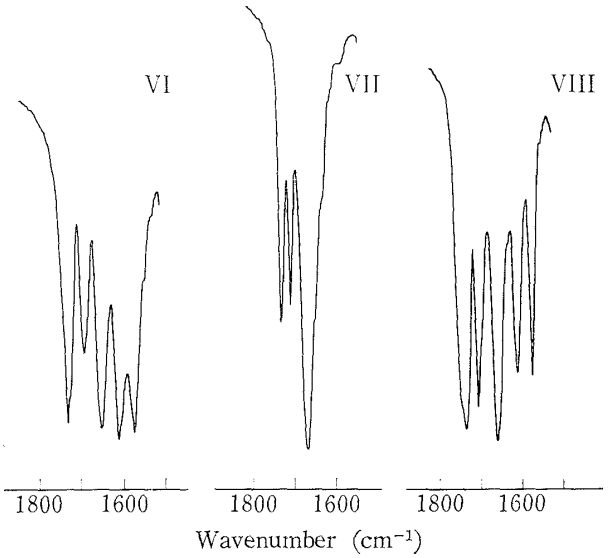


Fig. 1. IR Spectra of VI, VII, and VIII

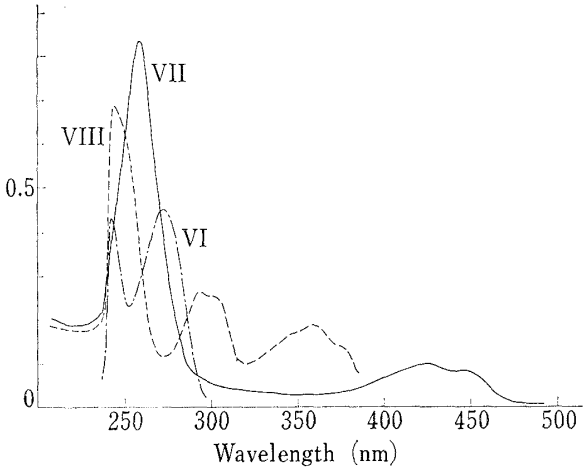
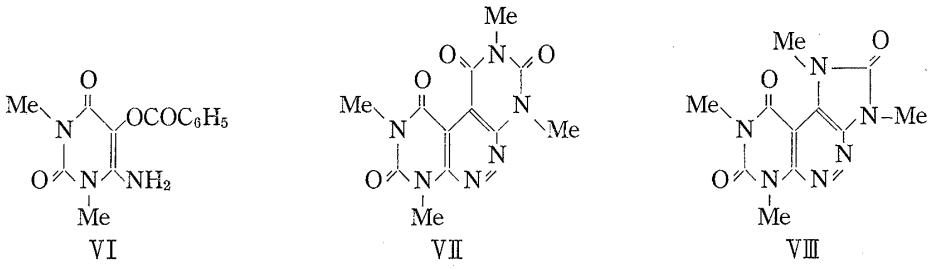


Fig. 2. UV Spectra of VI, VII, and VIII

VI (-·-·), VII (——), VIII (– – –).
All samples were dissolved in chloroform. The concentrations of VI, VII, and VIII were 2.30×10^{-5}, 2.78×10^{-5}, and 2.84×10^{-5} mol/l, respectively.

one of the pyrimidine rings of VII. Based on the above results, together with the findings of Taylor[10] and Yoneda,[11] VIII was assumed to be 2,4,7,9-tetramethyl-1,3,8-trioxo-1,2,3,4,7,8,9-heptahydropyrimido[4,5-*c*]imidazo[4,5-*e*]pyridazine. The NMR spectrum supported this structure. Four singlet signals were observed at 4.18 (4-methyl), 3.93 (9-methyl), 3.65 (2-methyl), and 3.60 (7-methyl) ppm. The signal of 9-methyl protons underwent a paramagnetic shift, presumably due to anisotropy or a steric compression effect arising from the 1-oxo group.[12] The observation of this effect confirmed the structure of VII, which is a symmetrical tricyclic pyridazinodipyrimidine derivative.



VI VII VIII

Chart 2

It was found that the yield of VII decreased when N,N-dimethylformamide or ethanol was used as a solvent. When ketones and triethylamine were employed, VII was not obtained. Triethylamine should promote the decomposition of benzoyl peroxide[13] and prevent the formation of the benzoyloxy radical. Ketones may produce hydrazones in the reaction with II or III, which does not lead to the formation of VII. The yields of VII in various solvents are listed in Table I. It appears that the variations in the yield of VII are not due to the polarity of the solvent or the reaction temperature. Since a mixture of acetic acid and chloroform favors its formation, the yield may depend on the pH of the solvent.

Table I. Effects of Solvent and Reaction Temperature on the Yield of VII from II[a]

Solvent	Reaction temperature	Yield (%)
AcOH–$CHCl_3$ (1:1)	Water bath	45
AcOH	Reflux[b]	9
DMF	Water bath	11
	Reflux	9
EtOH	Reflux	12
$CHCl_3$	Reflux	33
MeCOEt	Reflux	—
$(CH_3)_2CO$	Reflux	—
Et_3N	Reflux	—

a) Reaction time, 2 hr.
b) bp_{760}: AcOH-118°, DMF-153°, MeCOEt-73.4°, acetone-56.5°, and Et_3N-89°.

Experimental[14]

6-Amino-5-benzoyloxy-1,3-dimethyluracil (VI)——A mixture of 500 mg of 6-amino-1,3-dimethyluracil and 774 mg of benzoyl peroxide was placed in a flask, and 200 ml of AcOH–$CHCl_3$ (1:1) was added. The

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14) All melting points are uncorrected. Absorption spectra were measured with a Hitachi 200-20 Recording spectrophotometer in a cell of 10 mm optical length, IR spectra with a JASCO IRA-1 spectrophotometer, mass spectra with a JEOL KMS-01S mass spectrometer, and NMR spectra with a JEOL JNM-PS-100 spectrometer at 60 MHz, using tetramethylsilane as an internal standard.

solution was heated for 2 hr on a boiling water bath. Removal of the solvent *in vacuo* left an oily residue, which was dissolved in a small amount of $CHCl_3$ by heating in order to remove benzoic acid, unreacted starting materials, and other products. The $CHCl_3$ solution was cooled to precipitate VI, which was recrystallized from $CHCl_3$–EtOH to give colorless needles, mp 233—234°. Yield, 113 mg (13%). MS *m/e*: 275 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1730 (C=O). NMR ppm (DMSO-d_6): 3.13 (3H, s, N–Me), 3.34 (3H, s, N–Me), 6.77—6.93 (2H, br.s, NH_2) 7.34—7.77 (3H, m, aromatic), 7.93—8.28 (2H, m, aromatic). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 242 (4.18), 272 (4.20). *Anal.* Calcd. for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.27. Found: C, 56.72; H, 4.68; N, 15.27.

1,3,6,8-Tetramethyl-2,4,5,7-tetraoxo-1,2,3,4,5,6,7,8-octahydropyridazino[3,4-*d*: 6,5-*d'*]dipyrimidine (VII) ——A mixture of 500 mg of 6-hydrazino-1,3-dimethyluracil and 730 mg of benzoyl peroxide was placed in a flask, and 200 ml of AcOH–$CHCl_3$ (1: 1) was added. The solution was heated for 2 hr on a boiling water bath. Removal of the solvent *in vacuo* left an oily mixture, which was dissolved in a small amount of $CHCl_3$ by heating in order to remove benzoic acid, starting materials, and other minor products. The $CHCl_3$ solution was cooled to precipitate VII, which was recrystallized from AcOH to afford yellow prisms, mp>360°. Yield, 200 mg (45%). MS *m/e*: 304 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1732 and 1710 (C=O). NMR ppm (CF_3COOH): 3.74 (6H, 3- and 6-Me), 4.13 (6H, s, 1- and 8-Me). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 258 (4.56), 423 (3.58), 446 (3.51). *Anal.* Calcd. for $C_{12}H_{12}N_6O_4$: C, 47.37; H, 3.98; N, 27.62. Found: C, 47.45; H, 3.85; N, 27.84.

When 6-(1′-methylhydrazino)-1,3-dimethyluracil was allowed to react with benzoyl peroxide, the yield of VII was 1.5%.

2,4,7,9-Tetramethyl-1,3,8-trioxo-1,2,3,4,7,8,9-heptahydropyrimido[4,5-*c*]imidazo[4,5-*e*]pyridazine (VIII) ——Compound VII (100 mg) was placed in a flask, and 50 ml of DMF and 100 ml of 28% ammonia were added. The solution was heated for 8 hr on a boiling water bath. After neutralization with 10% HCl, the reaction product was extracted with $CHCl_3$. The $CHCl_3$ solution was dried over Na_2SO_4, then removal of the solvent left a colorless compound VIII, which was recrystallized from $CHCl_3$-EtOH to provide colorless needles, mp 291—293°. Yield, 42 mg (47%). MS *m/e*: 276 (M^+). IR ν_{max}^{KBr} cm^{-1}: 1732 and 1703 (C=O). NMR ppm (CF_3COOH): 3.60 (3H, s, 7-Me), 3.65 (3H, s, 2-Me), 3.93 (3H, s, 9-Me), 4.18 (3H, s, 4-Me). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244 (4.39), 292 (4.15), 302 (4.14), 358 (4.03). *Anal.* Calcd. for $C_{11}H_{12}N_6O_3$: C, 47.82; H, 4.38; N, 30.42. Found: C, 47.71; H, 4.40; N, 30.58.

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