Efficient formation of H-addition radical and secondary alkyl radical in pentylthymines γ-irradiated at 77 K

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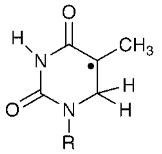
EPR detection of three pentylthymines γ-irradiated at 77 K showed the production of a 5,6-dihydro-5-thymyl radical (5-thymyl radical) as an H-addition radical and a secondary alkyl radical formed by C–H bond fission at the second carbon from the end of the pentyl group. The introduction of the pentyl group into thymine increased total radical yields at 77 K up to about twice that of thymine and considerably enhanced 5-thymyl radical yields. Several discussions on the radical formation suggest that the efficient production of 5-thymyl radical is related to the concomitant formation of the secondary radical.

Trapped free radicals are produced by ionized irradiation of organic compounds at 77 K and identified mostly by EPR spectroscopy. Ionized irradiation of thymine derivatives has been investigated as model systems for radiation damage induced in DNA[1] and the following radical formations were reported in several EPR studies on irradiated single crystals: the 5,6-dihydro-5-thymyl radical (5-thymyl radical) **1**,[2] the 7-thymyl radical **2** formed by loss of a hydrogen atom from the methyl group of the thymine ring,[3] the 5,6-dihydro-6-thymyl radical (6-thymyl radical) **3**,[4] the 1-thymyl radical **4** resulting from hydrogen dissociation from N_1,[5] the anion radical **5**[6] and its protonated form **6**.[7] Radiolysis of alkanes has also been studied for a long time. In γ-irradiated *n*-alkane solids, fission of all of the C–H bonds has been detected.[8] On the other hand, in γ-irradiated singly branched alkane glasses, selective C–H bond rupture giving secondary alkyl radicals of the type $RR'CH\text{-}\dot{C}HCH_3$ has been reported.[9,10] The selective C–H bond rupture in the singly branched alkanes may indicate alternative reaction paths caused by the introduction of a side chain into a carbon skeleton.



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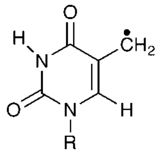

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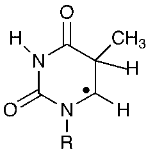

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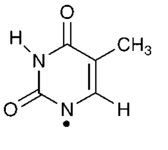

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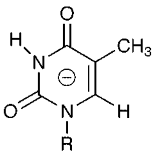

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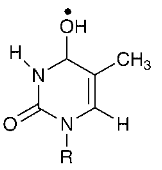

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Inaki and co-workers have extensively studied the adaptability of thymine derivatives having long alkyl chains and of polymers containing pendant thymine bases as photo and X-ray resists.[11] They have placed the focus on reactions of the thymine parts and hence the alkyl parts have been considered to be inert groups. On considering the selective C–H bond fission in the γ-irradiated singly branched alkane glasses, alkyl groups could take part in reactions induced by radiolysis of alkylthymines. In order to establish the adaptability of alkylthymines as X-ray resists it would be necessary to investigate radical formation in γ-irradiated alkylthymines. However, EPR studies on reactions caused by ionized irradiation of those compounds except for 1-methylthymine (MT) have not been reported to date as far as we know. Here we apply the EPR detection and the identification of free radicals produced by γ-irradiation of three pentylthymines at 77 K for the first time and discuss the free radical formation following radiolysis and what effects in the radical production are induced by the coexistence of alkyl and thymine groups.

Results and discussion

Octet signals produced by γ-irradiation at 77 K

γ-Radiolysis of 1-pentylthymine (PnT), 1-(1-methylbutyl)-thymine (MBT) and 1-(1-ethylpropyl)thymine (EPT) at 77 K gave the spectra as shown in Fig. 1(a), 1(b) and 1(c), respectively. The numbers on the right sides of the spectra indicate values of receiver gains. The low- and high-field sides of the spectra are displayed in ten-times enlarged scales. The sharp singlets in the center parts of the spectra mainly come from color centers produced in quartz sample tubes.[12] The color centers also overlap in the other parts marked by arrows. The spectra consist of eight broad lines similar to one another and the average separation (a_{AV}) of the eight lines was 2.02 mT for PnT, 2.06 mT for MBT and 2.05 mT for EPT. Almost identical spectra were obtained for brief (0.5 h) irradiation at 77 K. The similar eight lines irrespective of the thymine position on the pentyl skeleton might be attributed to the primary alkyl radical of $C_5H_5O_2N_2$–$C_3H_6CH_2\dot{C}H_2$. The eight lines, however, are not ascribable to the alkyl radical because the radical presumably gives six lines with an a_{AV} of about 2.2 mT.[13] Furthermore, the a_{AV} of 2 mT is rather small for alkyl radicals. Therefore, the eight lines could be assigned to free radicals, the unpaired electrons of which are mainly confined in the thymine rings. Deuterium exchange of the proton bonded to nitrogen nuclei of PnT did not induce observable change in the spectrum of PnT.

Fig. 2(a) and 2(c) show the observed spectra of MT and thymine after γ-radiolysis at 77 K. The low- and high-field sides of the spectra are depicted in twenty-times enlarged scales. The sharp signals in the center parts are mainly attributable to color centers produced in quartz sample tubes.[12] In the high-gain spectra octet signals with a_{AV}'s of about 2 mT are scarcely discernible. MT and thymine upon storage for 2 h at room

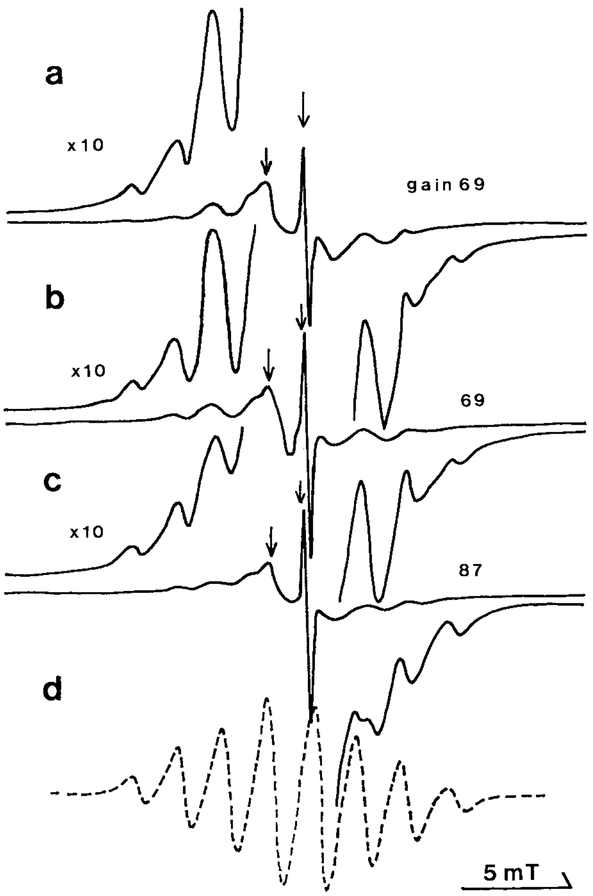


Fig. 1 EPR spectra of PnT (a), MBT (b) and EPT (c) γ-irradiated at 77 K and the calculated spectrum for the 5-thymyl radical produced in EPT (d). In the observed spectra the peaks indicated by arrows are due to signals of color centers produced in quartz EPR tubes. The proton hf couplings used in the calculation are shown in Table 1 and the linewidth was taken to be 0.72 mT.

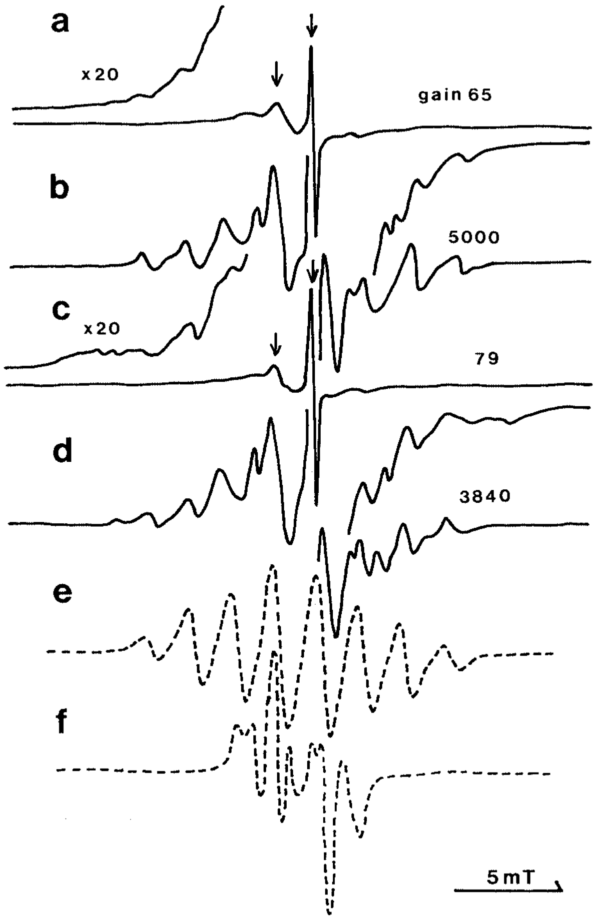


Fig. 2 EPR spectra of MT (a) and thymine (c) γ-irradiated at 77 K and those at RT after thermal annealing of γ-irradiated MT (b) and thymine (d). The calculated spectrum for the 5-thymyl radical in thymine at RT (e) and that for the 7-thymyl radical (f). In the observed spectra the peaks indicated by arrows are due to signals of color centers produced in quartz EPR tubes. The EPR parameters used in the calculation of (e) are shown in Table 1 and those in the calculation of (f) are in the text and the linewidths were taken to be 0.64 mT for (e) and 0.3 mT for (f).

temperature (RT) after irradiation at 77 K gave the spectra shown in Fig. 2(b) and 2(d) at RT. The octet signals are observable and the a_{AV} of the signal was 2.03 mT for MT and 1.94 mT for thymine. The central parts of the spectra are considerably overlapped by signals of color centers and are not shown to manifest the spectra. Hereafter, if color-center signals are strong, center parts of spectra are not displayed.

The octet spectra with a_{AV}'s of 2 mT in irradiated thymine and MT have been investigated by many researchers for a long time and are assigned to the 5-thymyl radical **1**.[1] Henriksen reported the weak octet spectrum due to the 5-thymyl radical produced in thymine exposed to X-rays at 77 K.[14] He observed the increase of the 5-thymyl radical signal by heat treatment up to 295 K after irradiation at 77 K. His observation is compatible with the results shown in Fig. 2. The octet spectra have been explained by the hyperfine (hf) interaction of an unpaired electron with two β-methylene and three methyl hydrogens of **1**. The hf coupling constant of the former (a(β-methylene-H)) has been found to be about 4 mT and that of the latter ($a(CH_3)$) about 2 mT.[1] Fig. 2(e) shows the calculated spectrum for the 5-thymyl radical in thymine at RT on using the values of a(β-methylene-H) and $a(CH_3)$ shown in Table 1. Although the calculated spectrum fairly well explains the outer four peaks of the observed spectra, the relative peak heights of the inner four peaks of the observed spectra are higher than those of the calculated spectrum and some extra peaks are present in the observed spectra. Hüttermann analyzed the EPR spectra of free radicals in single crystals of thymine monohydrate irradiated at RT and 77 K and proposed the presence of the 7-thymyl radical **2** in addition to the 5-thymyl radical.[3] Schmidt reported the formation of the thermally stable radical **2** in γ-irradiated single crystals of MT.[15] Fig. 2(f) shows the calculated spectrum for the radical **2** on using the EPR parameters reported by Schmidt.[15] Since the calculated spectrum gives several lines in the inner region of the octet spectra from the 5-thymyl radical, the discrepancy between the observed spectra at RT and the calculated spectrum for the 5-thymyl radical could result from the presence of the radical **2**.

Fig. 3(a), 3(b) and 3(c) show the observed spectra of PnT, MBT and EPT, respectively, at RT on keeping for 2 h at the same temperature after γ-irradiation at 77 K. The octet signals similar to those observed at RT in thymine and MT are clearly discernible. The a_{AV} value of the octet spectrum was 1.98 mT for PnT, 2.02 mT for MBT and 2.01 mT for EPT. On considering the explanation for the octet lines in thymine and MT the octet signals in pentylthymines may be interpreted by the hf interaction of three protons (a(3H)) and approximately twice that of two protons (a(2H)). The spectrum calculated on the basis of the above interpretation is shown in Fig. 3(d) for EPT; in the calculation the values of a(2H) and a(3H) were taken to be those summarized in Table 1. The calculated spectrum fairly well reproduces the outer four peaks of the observed spectra. In the inner four lines of both spectra, especially for PnT and EPT, some disagreement is recognized. The disagreement is explainable by the presence of the radical **2** because the radical could give the signal shown in Fig. 2(f) in the inner parts of the observed spectra.

Re-cooling of PnT, MBT and EPT heat-treated at RT to 77 K gave the spectra shown in Fig. 4(a), 4(b) and 4(c),

Table 1 Hyperfine coupling constants of 5,6-dihydro-5-thymyl radical

Compound	Temperature	a(β-methylene-H)/mT	a(CH$_3$)/mT	Remarks
1-Pentylthymine (PnT)	77 K	4.00 ± 0.06	2.04 ± 0.06	Powder^c
	RT	3.89 ± 0.06	2.04 ± 0.06	Powder^c
1-(1-Methylbutyl)thymine (MBT)	77 K	4.08 ± 0.09	2.09 ± 0.09	Powder^c
	RT	3.93 ± 0.09	2.09 ± 0.09	Powder^c
1-(1-Ethylpropyl)thymine (EPT)	77 K	4.02 ± 0.09	2.10 ± 0.09	Powder^c
	RT	3.90 ± 0.09	2.10 ± 0.09	Powder^c
Thymine	RT	3.77	2.08	Powder^d
	RT	3.45	2.05	Powder^e
	77 K	3.619, 4.375 (3.977)^a	2.17	Single crystal of monohydrate^f
	300 K	3.421, 4.079 (3.750)^a	2.17	
	77 K, RT	$A_{\parallel}$^{b} 3.68, 4.32 $A_{\perp}$^{b} 3.35, 4.06 (3.46)^a (4.15)^a (3.81)^a	$A_{\parallel}$ 2.16 $A_{\perp}$ 1.93 (2.01)^a	Single crystal of anhydrate^g
	77 K, RT	$A_{\parallel}$ 3.58, 4.44 $A_{\perp}$ 3.28, 4.06 (3.38)^a (4.19)^a (3.79)^a	$A_{\parallel}$ 2.16 $A_{\perp}$ 1.93 (2.01)^a	Single crystal of monohydrate^g
	RT	3.43 ± 1, 4.03 ± 1 (3.73)^a	2.05	Powder; H-bombardment^h
	77 K	3.83 ± 0.09	2.04 ± 0.09	Powder^c
	RT	3.75 ± 0.09	2.04 ± 0.09	Powder^c
1-Methylthymine	133 K	3.76, 4.31 (4.04)^a	1.99	Single crystal^i
	RT	3.68, 4.10 (3.89)^a	1.99	
	77 K	4.13 ± 0.04	2.08 ± 0.04	Powder^c
	RT	3.98 ± 0.04	2.08 ± 0.04	Powder^c
Thymidine	163 K	4.05	2.05	Single crystal^j
	RT	4.05	2.05	Single crystal^k
	RT	3.75	2.13	Powder^e

[a] Average hf coupling constant. [b] $A_{\parallel}$ and $A_{\perp}$ show the parallel and perpendicular components of the hf coupling tensor. [c] This work. [d] P. S. Pershan, R. G. Shulman, B. J. Wyluda and J. Eisinger, *Physics*, 1964, **1**, 163. [e] M. G. Ormerad, *Int. J. Radiat. Biol.*, 1965, **9**, 291. [f] Reference 3. [g] A. Dulčić and J. N. Herak, *Radiat. Res.*, 1971, **47**, 573. [h] C. Heller and T. Cole, *Proc. Natl. Acad. Sci. U.S.A.*, 1965, **54**, 1486. [i] Reference 16. [j] J. N. Herak, *J. Chem. Phys.*, 1970, **52**, 6440. [k] Reference 2.

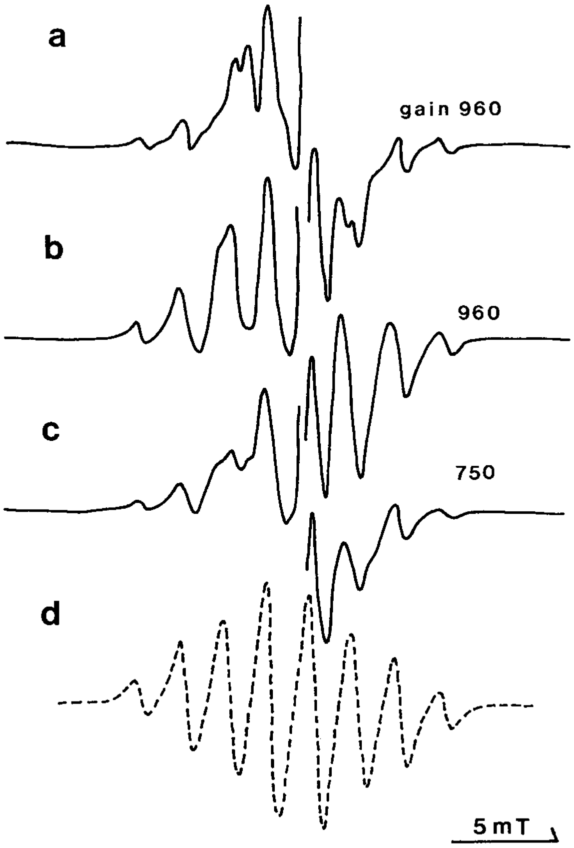


Fig. 3 EPR spectra at RT after thermal annealing of γ-irradiated PnT (a), MBT (b) and EPT (c) and the calculated spectrum for the 5-thymyl radical produced in EPT (d). The proton hf couplings used in the calculation are shown in Table 1 and the linewidth was taken to be 0.64 mT.

respectively. The spectra can be interpreted as broadening of the component lines of the spectra shown in Fig. 3(a), 3(b) and 3(c), however, the a_{AV}'s of the octet spectra at 77 K were slightly larger than those at RT; the a_{AV} was 2.02 mT for PnT, 2.06 mT for MBT and 2.05 mT for EPT. The calculated spectrum for the octet lines in EPT is shown in Fig. 4(d); the values of a(2H) and a(3H) used in the calculation were taken to be those shown in Table 1. The calculated spectrum reasonably explains the observed octet signals and also those in pentylthymines γ-irradiated at 77 K (Fig. 1).

In Table 1 the reported a(β-methylene-H) and a(CH$_3$) values of the 5-thymyl radicals in several irradiated thymine derivatives are summarized together with the values of a(2H) and a(3H) of the octet signals in pentylthymines. Taking into account slight scatter in the reported values, the a(2H) and a(3H) values agree fairly well with the reported values for the a(β-methylene-H) and a(CH$_3$) values of the 5-thymyl radicals. In pentylthymines the a(2H) values at 77 K were slightly larger than those at RT, while the a(3H) values were identical at both temperatures. Snipes and Schmidt reported that the a(β-methylene-H) value of the 5-thymyl radical produced in MT increased upon cooling, but the a(CH$_3$) value remained constant[16] as in the present results. These observations are supported by Hüttermann's results[3] for the 5-thymyl radical in a single crystal of thymine monohydrate (see Table 1). Furthermore, as described before, almost the same octet signals were observed in PnT, MBT and EPT and the deuterium exchange of PnT did not change the octet signal. All of the present results substantiate the assignment of the octet signals produced in pentylthymines γ-irradiated at 77 K to the 5-thymyl radical **1**.

Thermally unstable species

The thermal annealing of pentylthymines at RT after γ-irradiation at 77 K made the 5-thymyl radical signals prominent (see Fig. 1 and 4). This result suggests that thermally unstable species giving broad signals decayed during the heat treatment and the signal resolution could be improved. Fig. 5(a), 5(c) and 5(e) show the difference spectra of PnT, MBT and EPT, respectively, before and after thermal annealing at RT. The difference spectra consist of eight lines for PnT and MBT

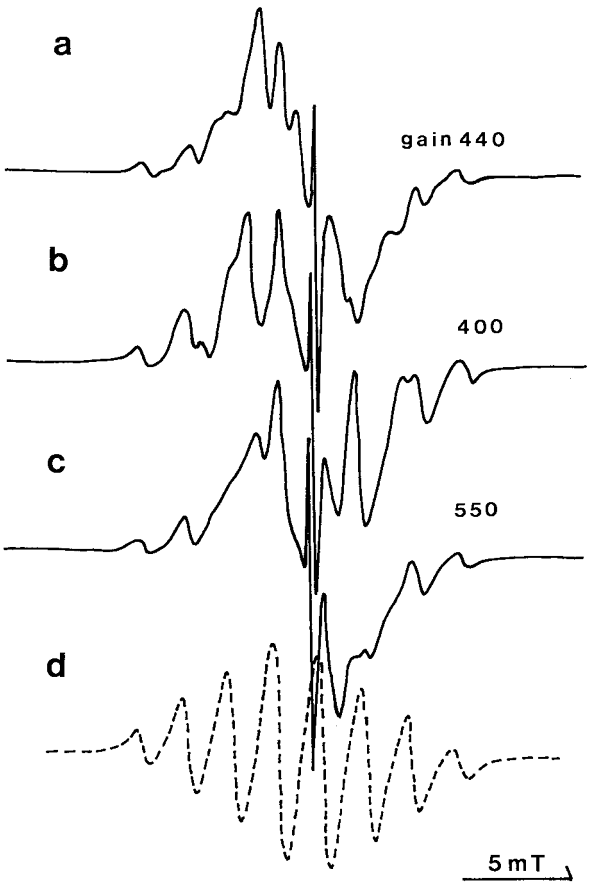


Fig. 4 EPR spectra at 77 K after thermal annealing of γ-irradiated PnT (a), MBT (b) and EPT (c) and the calculated spectrum for the 5-thymyl radical produced in EPT (d). The proton hf couplings used in the calculation are shown in Table 1 and the linewidth was taken to be 0.72 mT.

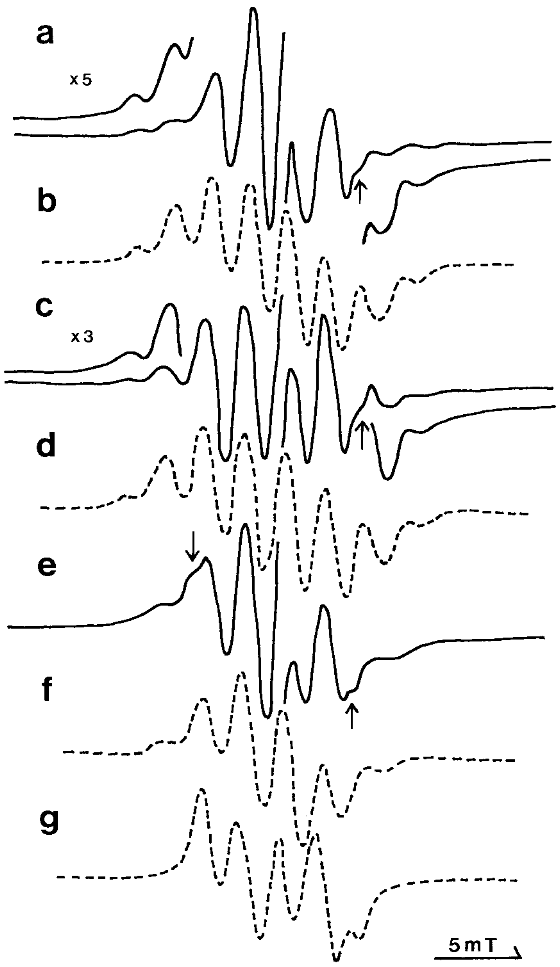


Fig. 5 The difference spectra of the observed spectra at 77 K in γ-irradiated PnT (a), MBT (c) and EPT (e) before and after thermal annealing at RT. The calculated spectra for the secondary alkyl radicals produced in PnT (b), MBT (d) and EPT (f) and the calculated spectrum for the 6-thymyl radical (g). In the observed spectra the peaks assignable to the 6-thymyl radical are indicated by arrows. The EPR parameters used in the calculations are shown in the text and the linewidths were taken to be 1.60 mT for (b), 1.70 mT for (d) and (f) and 0.6 mT for (g).

and six lines for EPT and their a_{AV} values were about 2.3 mT. The a_{AV} values indicate that the difference spectra are mostly attributable to alkyl radicals. The eight-line spectra of PnT and MBT are similar to those of secondary alkyl radicals of the type $RCH_2\dot{C}HCH_3$. The observed structure is accounted for[13] by assuming that one α- and one β-methylene proton and three methyl protons have almost equal hf coupling constants of about one-half that of the other β-methylene proton. Fig. 5(b) and 5(d) show the calculated spectra for the eight-line spectra on taking the *a*(α-proton) of 2.13 mT, the *a*(CH_3) of 2.26 mT and the *a*(β-methylene proton)s of 1.55 mT and 4.22 mT for PnT and those of 1.83 mT and 4.81 mT for MBT. Component line shape was assumed to be Gaussian. The six lines in EPT show spectral features close to that of 3-methylpentyl radical ($C_2H_5(CH_3)CH\dot{C}HCH_3$), the spectrum of which has been explained by the nearly equal hf couplings of one α- and one β-methine proton and three methyl protons.[9] Fig. 5(f) shows the spectrum calculated using the *a*(α-proton) of 2.13 mT, the *a*(CH_3) of 2.26 mT and the *a*(β-methine proton) of 2.07 mT. Component line shape was assumed to be Gaussian. Although the difference spectra are rather broad and might be composed of signals from several species, the calculated spectra agree reasonably well with the difference spectra. Some disagreement of the spectra could be responsible for the presence of some other species except for color centers produced in quartz EPR tubes.

Flossmann *et al.* reported the formation of the 6-thymyl radical in MT single crystals exposed to X-rays between 77 and 300 K.[1d] They observed the conversion of the 6-thymyl radical into the 5-thymyl radical by heat treatment. Bernhard and Snipes also found the same radical conversion upon storage at room temperature in γ-irradiated dihydrothymine.[17] The calculation corresponding to the 6-thymyl radical gave the spectrum as shown in Fig. 5(g); the calculation was performed using the EPR parameters reported by Henriksen and Snipes[4] for the 6-thymyl radical produced in irradiated dihydrothymine single crystals at RT. The signals assignable possibly to the 6-thymyl radical are indicated by arrows in Fig. 5.

The thymine anion and/or its protonated form might participate in the thermally unstable species. Sevilla observed a thermally unstable doublet spectrum of a separation of 1.6 mT produced in photoirradiated alkaline D_2O glasses containing thymine and $K_4Fe(CN)_6$ at 77 K and assigned the spectrum to the thymine anion.[18] Sagstuen *et al.* reported that the thymine anion produced in thymidine single crystals after X-irradiation at 10 K was present as the protonated form **6** and decayed into a diamagnetic product at about 40 K.[7] In pentylthymines positive evidence for the presence of the thymine anion or its protonated form was not obtained.

Another candidate for thermally unstable species is radical pairs which could give signals spread over about 30 mT in the field at $g = 2$.[19–21] Bergene and Melø observed EPR signals of radical pairs due mainly to two cation radicals in single crystals of thymine anhydrate exposed to 4 MeV electrons at 77 K.[19] Flossmann *et al.*[20] and Dulčić and Herak[21] reported the production of radical pairs composed of a hydrogen-abstraction

Table 2 Relative radical yields and percent contents of thermally unstable species in pentylthymines, 1-methylthymine and thymine γ-irradiated at 77 K

Compound	Relative yield[a]	Percent content
1-Pentylthymine (PnT)	2.14 ± 0.14	59.7 ± 1.2
1-(1-Methylbutyl)thymine (MBT)	2.32 ± 0.18	50.4 ± 1.0
1-(1-Ethylpropyl)thymine (EPT)	1.89 ± 0.09	55.2 ± 3.8
1-Methylthymine (MT)	1.19 ± 0.04	64.4 ± 2.2
Thymine	1.0	67.3 ± 1.7

[a] The values include the contributions of signals from color centers produced in quartz sample tubes. If the contributions[12] are taken into account in the radical yields, the relative yields of pentylthymines may increase about 0.04 unit.

radical and the hydrogen-addition radicals **1** and **3** in single crystals of 1-methyluracil irradiated with γ- or X-rays at 77 K. These studies stated that the radical pairs decayed when the single crystals were heated to RT. In the spectra of pentylthymines γ-irradiated at 77 K, however, the splittings ascribable to radical pairs were hardly found as shown in Fig. 1. Furthermore, the observation of forbidden transitions characterized by $\Delta Ms = 2$ of radical pairs was unsuccessful.

Radical yields and reaction mechanisms

Table 2 shows the relative radical yields obtained by the double integration of the spectra of thymine, 1-methyl- and pentylthymines γ-irradiated at 77 K. The values given in the table are the average values of three sets of the double-integrated values relative to those of thymine. The results show that the introduction of the pentyl group into thymine increased the radical yields up to about twice that of thymine. On considering the efficient formation of 5-thymyl radical in pentylthymines γ-irradiated at 77 K, as described before, the increases of the radical yields indicate the enhancement of the 5-thymyl radical formation. In the table the percent contents of the thermally unstable species are also shown. The contents were estimated by the double integration of the difference spectra before and after heat-treatment at RT. Since the difference spectra of pentylthymines are mostly assigned to the secondary alkyl radicals, the percent content of about 50% implies considerable production of the alkyl radicals at 77 K.

Many studies[1] on radical formation in irradiated thymine derivatives suggest possible reactions in γ-irradiated pentylthymines as shown in eqns. (1)–(4).

$$\mathrm{TH{-}RH} \xrightarrow{\gamma} \mathrm{TH^{+}{-}RH} + \mathrm{e^{-}} \qquad (1)$$

$$\mathrm{TH^{+}{-}RH} \longrightarrow \mathrm{T^{\bullet}{-}RH} + \mathrm{H^{+}} \qquad (2)$$

$$\mathrm{TH{-}RH} + \mathrm{e^{-}} \longrightarrow \mathrm{TH^{-}{-}RH} \qquad (3)$$

$$\mathrm{TH^{-}{-}RH} + \mathrm{H^{+}} \longrightarrow \mathrm{TH_2{-}RH} \qquad (4)$$

The pentylthymine is abbreviated as TH–RH where TH and RH represent thymine and pentyl moieties. Electron ejection by γ-rays at the thymine portion may produce a cation ($\mathrm{TH^{+}{-}RH}$) of which an unpaired electron is mainly located at the thymine ring [reaction (1)]. The cation may split off a proton and generate a deprotonated radical ($\mathrm{T^{\bullet}{-}RH}$) assignable to the radical **2** [reaction (2)]. The electron produced by ionization of TH–RH might be captured by another TH–RH and a TH–RH anion radical could be generated [reaction (3)]. Since the electron affinity of thymine is possibly greater than that of pentane, the unpaired electron of the anion radical may be confined to the thymine ring and thus the anion radical is represented as $\mathrm{TH^{-}{-}RH}$. Protonation of the anion radical may give hydrogen-addition radicals ($\mathrm{TH_2{-}RH}$) assigned to the radicals **1** and possibly **3** [reaction (4)]. The reaction (4) might require thermal excitation[1] and hence the yields of the hydrogen-addition radicals at 77 K are predicted to be low as the results of thymine and MT. Therefore, the high yields of the 5-thymyl radicals in pentylthymines at 77 K are quite unexpected. Furthermore, the reactions (1)–(4) cannot explain the high radical yields and the efficient formation of the alkyl radicals of the type $\mathrm{RR'CH\dot{C}HCH_3}$ in pentylthymines at 77 K.

The proton generated in reaction (2) might recombine with an electron and produce a hydrogen atom [reaction (5)].

$$\mathrm{H^{+}} + \mathrm{e^{-}} \longrightarrow \mathrm{H} \qquad (5)$$

The hydrogen atom may abstract a hydrogen from the pentyl group and add to the thymine ring giving an alkyl radical ($\mathrm{TH{-}R^{\bullet}}$) and the hydrogen-addition radicals [reactions (6) and (7)].

$$\mathrm{TH{-}RH} + \mathrm{H} \longrightarrow \mathrm{TH{-}R^{\bullet}} + \mathrm{H_2} \qquad (6)$$

$$\mathrm{TH{-}RH} + \mathrm{H} \longrightarrow \mathrm{TH_2{-}RH} \qquad (7)$$

If hydrogen production by reaction (5) occurs to a major extent in the present systems, the following results would be expected: 1) total radical yields of pentylthymines comparable to those of MT and thymine, 2) a greater yield of 5-thymyl radical in thymine than in MT and pentylthymines and 3) a smaller percent content of thermally unstable species in thymine than in pentylthymines. The last expectation is based on the observation that $\mathrm{TH_2{-}RH}$ was more thermally stable than $\mathrm{TH{-}R^{\bullet}}$. All of those expectations are contrary to the experimental results, therefore, reaction (5) may not proceed significantly in γ-irradiated pentylthymines.

γ-Rays can attack the pentyl groups equally for the thymine moiety and eject an electron from the pentyl part [reaction (8)].

$$\mathrm{TH{-}RH} \xrightarrow{\gamma} \mathrm{TH{-}RH^{+}} + \mathrm{e^{-}} \qquad (8)$$

The recombination of $\mathrm{TH{-}RH^{+}}$ and the ejected electron might produce an excited molecule ($\mathrm{TH{-}RH^{*}}$) which may split off a hydrogen atom giving $\mathrm{TH{-}R^{\bullet}}$ [reaction (9)].

$$\mathrm{TH{-}RH^{+}} + \mathrm{e^{-}} \longrightarrow \mathrm{TH{-}RH^{*}} \longrightarrow \mathrm{TH{-}R^{\bullet}} + \mathrm{H^{\bullet}} \qquad (9)$$

If reaction (9) takes place efficiently in pentylthymines, the produced hydrogen atom could abstract hydrogen from the pentyl group and add to the thymine ring [reactions (6) and (7)]. Since reactions (8) and (9) may occur also in MT, the results for MT are expected to be similar to those for pentylthymines contrary to the experimental results. The lack of agreement of the results for MT with those for pentylthymines may relate to the formation of secondary alkyl radicals of the type $\mathrm{RR'CH\dot{C}HCH_3}$. Fig. 6(a) and 6(b) show the observed spectra of 1-ethylthymine (ET) and 1-propylthymine (PrT) γ-irradiated at 77 K. The low- and high-field sides of the spectra are displayed in twenty-times enlarged scales. The octet signal similar to that of the 5-thymyl radical is clearly discernible in the spectrum of PrT, while the signal is hardly found in the spectrum of ET. Since the formation of a secondary alkyl radical of the type $\mathrm{RR'CH\dot{C}HCH_3}$ is possible in the case of PrT and not in the case of ET, the production of the secondary alkyl radical could relate to the efficient formation of 5-thymyl radicals in pentylthymines γ-irradiated at 77 K. The present results suggest that for discussions on radiolysis and X-ray resists of alkylthymines reactions caused by radiation damage in the alkyl part should be considered in addition to those in the thymine moiety.

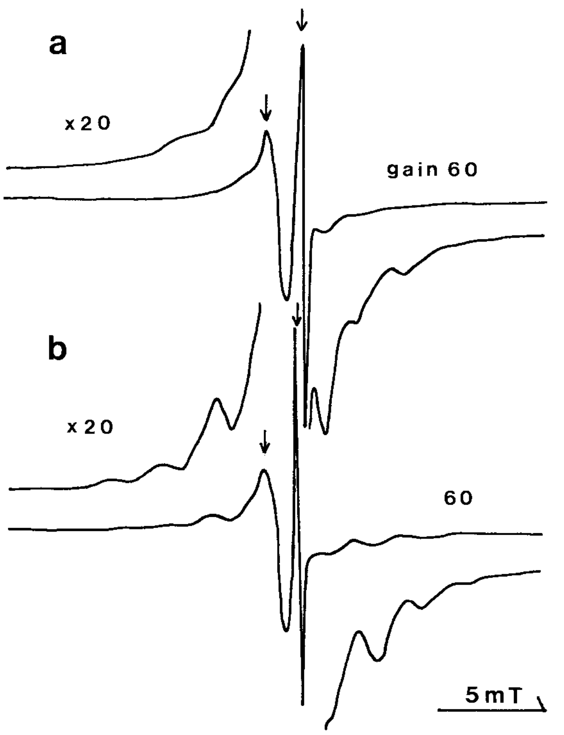


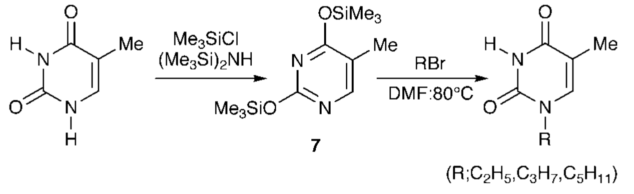
Fig. 6 EPR spectra of ET (a) and PrT (b) γ-irradiated at 77 K. The peaks indicated by arrows are due to signals of color centers produced in quartz EPR tubes.

Conclusion

Ionized irradiation of pentylthymines at 77 K efficiently produces the 5-thymyl radical and secondary radicals of the type $RR'CH\dot{C}HCH_3$ in addition to the hydrogen-abstraction radical **2** and possibly the hydrogen-addition radical **3**. The efficient production of 5-thymyl radicals in pentylthymines is discussed and concluded to be related to the formation of secondary alkyl radicals.

Experimental

Thymine was supplied by Aldrich Chemicals and recrystallized from water. 1-Methylthymine from Sigma Chemicals was used as received. The other alkylthymines were synthesized from thymine by the procedure[22] adopted in the preparation of bromoalkylthymines; thymine was converted to its bis(trimethylsilyl) ether derivative **7** and **7** was alkylated with the appropriate bromoalkane to afford the alkylthymines (Scheme 1). The reagents used in the syntheses of the alkylthymines were all commercially available from Aldrich Chemicals, Shinetsu Silicone and Tokyo Kasei except for 2- and 3-bromopentanes which were prepared by bromination of the corresponding alcohols. The synthesized alkylthymines were purified by repeated recrystallizations from *n*-hexane–methanol or –ethanol.



Scheme 1

Deuterium exchange of PnT was carried out by the dissolution of PnT in CD_3OD.

Quartz EPR tubes (4 mm i.d., Suprasil) containing weighed powder samples were evacuated and sealed. EPR samples were γ-irradiated with a ^{60}Co source at 77 K for 3 h at a dose rate of 0.96 kGy min^{-1}. EPR measurements were carried out with an FE-1X JEOL spectrometer with 100 kHz field modulation at 77 K unless otherwise noted. EPR signals obtained at an interval of (50/8191) mT were digitized by a 12-bit A/D converter and fed to an NEC PC-9801 microcomputer. To monitor changes in EPR sensitivity and field spacing a capillary tube containing Mn^{2+} dispersed in MgO was inserted into an EPR cavity and fixed. All of the spectra in the text are shown as those eliminated Mn^{2+} signals from observed spectra. Difference spectra and double integrals of the spectra were taken by the microcomputer at the standardization of the peak heights of the second peak of Mn^{2+}. EPR spectral simulation was performed by use of the Lefebvre and Maruani program.[23] Component line shape was taken to be Lorentzian unless otherwise indicated.

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