side was added. The mixture was allowed to stir for 15–20 hr at ambient temperature, then quenched with ca. 20 ml of water and concentrated in vacuo to 10 ml. For those less soluble 5′-chloronucleoside products, the aqueous solution was neutralized to pH 7–8 with 2 *N* aqueous ammonia. The resulting solution was cooled and crystals collected by filtration and washed thoroughly with ice water. The combined filtrates were applied to ca. 10 ml of ion exchange resin (Dowex 50W-X4, 50–100 mesh, H^+ form). The resin was washed well with water, then with 1 or 2 *N* NH_4OH to remove the product. The ammonia solutions were concentrated to produce a second crop of crystals. For those samples which did not crystallize on neutralization, the entire original acidic solution was passed through a large column (100–150 ml) of Dowex 50W-X4. The product was again removed by elution with NH_4OH, after first washing the column thoroughly with water. Yields averaged in the 75–100% range. The products were identified by their ir and NMR spectra and their chromatographic properties.

General Reaction Procedure for the Preparation of *S*-Adenosylhomocysteine Analogues from the 5′-Chloro-5′-deoxynucleoside and L-Homocystine. A 0.75-mmol sample (1.4 equiv) of L-homocystine was dissolved in 25 ml of liquid ammonia and treated with sodium until the solution remained blue for at least 15 min. A stirring bar was introduced and enough NH_4Cl was added to discharge the color. Then 1.05 mmol (1.0 equiv) of the appropriate 5′-chloro-5′-deoxynucleoside was added and stirring was continued for approximately 12 hr. After the ammonia had evaporated and the last traces were removed in vacuo, 5 ml of H_2O was added and the solution neutralized to pH 6 with 5% HCl. The crude mixture was then applied to an ion exchange column (30 ml of Dowex 50W-X4, 50–100 mesh, NH_4^+ form) and eluted slowly with water. The first fractions contained the inorganic ions and homocystine. The desired product along with some starting 5′-chloro-5′-deoxynucleoside was removed either by further elution with water or by elution of the column with 1 *N* and/or 2 *N* NH_4OH. The fractions containing product were concentrated in vacuo and lyophilized. Where appropriate, impure samples were further purified by using preparative TLC [Avicel, 3:2 EtOH–H_2O, or silica gel, 9 (20EtOH:2H_2O:2HOAc) + 1 (0.5 *M* phosphate buffer pH 7.0)]. Yields averaged from 40 to 75% for this condensation step, giving overall yields of 30–60% of the products from the corresponding nucleoside. The *S*-adenosylhomocysteine analogues were characterized by their ir, NMR, and uv spectra, their chromatographic properties against standard samples, and chemical analyses.

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Registry No.—**1**, 979-92-0; **2**, 53228-06-1; **3**, 53199-58-9; **4**, 57344-98-6; **5**, 57274-11-0; **6**, 57274-12-1; L-homocystine, 626-72-2; D-homocystine, 6027-15-2.

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Synthesis and Characterization of 5-Hydroperoxymethyluracil (Thy$^{\alpha}$OOH)

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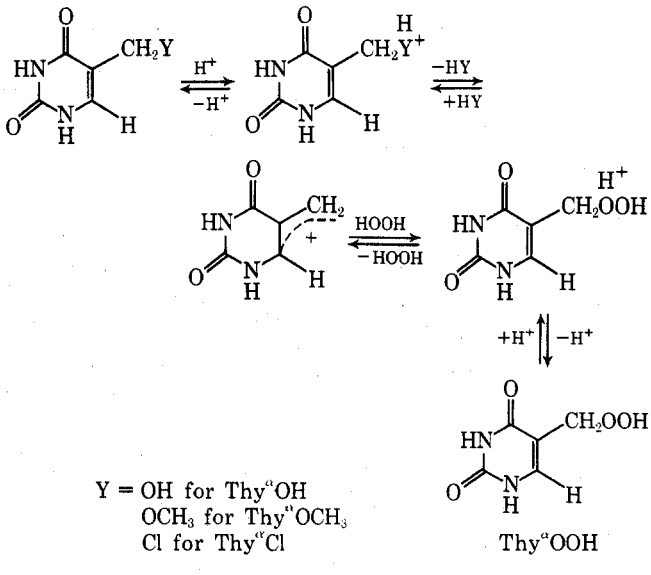
Cadet and Teoule[1] have shown that radiolysis of thymine (Thy)[2] and thymidine (dThd) in pH 1.7–7 aerated aqueous solutions results in the formation of Thy$^{\alpha}$OOH. Swinehart et al.[3] studied the γ-ray-induced production of [^{3}H]H_2O from [^{3}H]Thy in single- and double-stranded DNA and suggested the formation of Thy·$^{\alpha}$, a probable intermediate for the formation of Thy$^{\alpha}$OOH and other analogous products, to explain the observed [^{3}H] release. Earlier, the same suggestion was made by Wang and Alcantara[4] about the photooxidation of Thy in aqueous solutions. These findings indicate the possible importance of Thy$^{\alpha}$OOH in radiobiology and photobiology, and thus the study of the effects of Thy$^{\alpha}$OOH on biological compounds and systems seems to be warranted, especially in view of the effects of *cis*-5,6-dihydro-6-hydroperoxy-5-hydroxythymine (ho^{5}ho$_2$^{6}hThy, 6-TOOH)[5] on neighboring bases, cells, chromosomes, etc.[6]

Because access to sufficient quantities of Thy$^{\alpha}$OOH is necessary for similar studies, improved methods have been developed with analogous starting materials, two of which are novel for the preparation of Thy$^{\alpha}$OOH. These syntheses give Thy$^{\alpha}$OOH in yields of ~90%, which is considerably greater than in the previous method.[7] In addition, Thy$^{\alpha}$OOH exhibits some interesting photochemical and chemical behavior. Furthermore, purified Thy$^{\alpha}$OOH is in fact rather stable, contrary to the early belief,[1] and that is convenient for our intended studies.

Results and Discussion

These syntheses are straightforward and give excellent yields of Thy$^{\alpha}$OOH. Considering the ease of reaction and the requirement of concentrated HCl, acid-catalyzed formation of an electrophilic center is probably involved as shown in the following scheme.



Because Cl is a much better leaving group than OH and OCH_3 under the present reaction condition, acid catalysis is necessary for the reactions of Thy$^{\alpha}$OH and Thy$^{\alpha}$$OCH_3$

but not for Thy$^{\alpha}$Cl. Also, the synthesis goes more readily and provides somewhat better yields when Thy$^{\alpha}$Cl is used as the reactant. However, whether these reactions proceed in a stepwise manner as shown has not yet been studied.

Our structural assignment of Thy$^{\alpha}$OOH is corroborated by the spectral data. The ir spectrum shows bands of νO–O (875 cm^{-1}) and νCH$_2$–O (973, 1033, and 1071 cm^{-1}). The last two bands also appear in the ir spectrum of CH$_2$OH-containing analogue, Thy$^{\alpha}$OH, but are in greatly reduced intensities. This reduction is to be expected[8,9] for C–OOH as compared to analogous C–OH compounds. In the NMR spectrum, the assignments are rather straightforward; however, the signal for OOH is absent. Similarly, the signal for OH in Thy$^{\alpha}$OH is also absent. On the other hand, we observed[9] the OOH signal as a sharp singlet in a similar compound, 6-TOOH. When Thy$^{\alpha}$OOH was allowed to stand in (CD$_3$)$_2$SO, it was reduced to the corresponding alcohol, Thy$^{\alpha}$OH [NMR in (CD$_3$)$_2$SO: δ 4.17 (s, 2, CH$_2$), 7.32 (d, 1, J = 6 Hz, C$_6$H)]. However, in spite of its allylic hydroperoxide function, Thy$^{\alpha}$OOH is surprisingly stable in Me$_2$SO with an apparent half-life of approximately 14 days, whereas, 6-TOOH has an apparent half-life of 27 min at 35°C. No appreciable change can be detected when Thy$^{\alpha}$OOH is stored for 1 week at room temperature; however, in solution it is gradually converted to 5-formyluracil (5CHO-Ura). Because Thy$^{\alpha}$OOH and 5CHO-Ura are reactive species, they may interact with biomolecules and thus affect biological systems when they are formed directly or indirectly by radiation.

Furthermore, when Thy$^{\alpha}$OOH (0.1 mM) was irradiated with 254-nm light, it was quantitatively converted to 5CHO-Ura within 220 sec at a light intensity of 198 ergs/mm^2 sec^{-1} (without filter) with ϕ = 0.47 (when Corning filter No. 954 is used, ϕ = 0.27). This efficacious photoconversion may have relevance in the study of radiobiology. In addition, 5CHO-Ura has been identified as a photoproduct of Thy.[4]

Thy$^{\alpha}$OOH can be easily reduced to Thy in H$_2$O by hydrogenation in the presence of 10% Pd/C at room temperature.

In short, the characteristics of Thy$^{\alpha}$OOH make it an interesting compound to be considered in the study of radiation effects of biological systems.

Experimental Section

Preparation of 5-Hydroperoxymethyluracil (Thy$^{\alpha}$OOH). From 5-Methoxymethyluracil (Thy$^{\alpha}$OCH$_3$). The starting material was prepared according to the method of Santi and Pogolotti.[10] First, Thy$^{\alpha}$OCH$_3$ (62 mg, 40 mmol) was dissolved in 10 ml of 15% H$_2$O$_2$, then, dropwise, 50 μl of concentrated HCl in 5 ml of H$_2$O$_2$ was added. After standing at room temperature for 24 hr with stirring, the reaction solution was lyophilized. The residue was washed three times with cold water and the purified product (56 mg, 89%) was obtained by recrystallization from 10% methanol solution.

From 5-Hydroxymethyluracil (Thy$^{\alpha}$OH). The procedure is analogous to that described above. In this case, 57 mg (40 mmol) of Thy$^{\alpha}$OH was used and 57 mg (90%) of the purified Thy$^{\alpha}$OOH was obtained.

From 5-Chloromethyluracil (Thy$^{\alpha}$Cl). The starting material was synthesized according to the method of Giner-Sorolla and Medrek.[11] Thy$^{\alpha}$Cl (50 mg, 32 mmol) was added portionwise to 1 ml of 50% H$_2$O$_2$ solution with stirring. The product began to appear as fine crystals at the completion of the addition; however, the stirring was continued for an additional 30 min at room temperature. The product was collected by filtration and washed with 50% methanol until the washings gave a negative AgNO$_3$ test for Cl$^-$. Again, recrystallization was carried out in 10% MeOH solution and 45 mg (92%) of the purified product was obtained: mp >230° dec; λ_{max} (H$_2$O) 261 nm (ϵ 7500); ir (KBr film) 11.43 μ for –O–O–, 10.28, 9.68, and 9.34 μ for C–OOH, respectively; NMR [(CD$_3$)$_2$SO] δ 4.52 (s, 2, CH$_2$), 7.52 (d, 1, J = 6 Hz, C$_6$H), 9.47 (d, 1, J = 6 Hz, N$_1$H), and 9.67 (b, 1, N$_3$H); mass spectrum m/e 142 (M − 16). Anal. Calcd for C$_5$H$_6$N$_2$O$_4$: C, 38.00; H, 3.80; N, 17.71. Found: C, 37.88; H, 3.76; N, 17.79.

Reduction of Thy$^{\alpha}$OOH to Thy. Thy$^{\alpha}$OOH (31.7 mg, 20 mmol) and 10 mg of 10% Pd/C were suspended in 10 ml of water. The solution was shaken with H$_2$ at room temperature. A theoretical amount (9.6 ml, 2 molar equiv) of H$_2$ was taken up at the end of 4 hr. The catalyst was removed by filtration and the filtrate was evaporated until dry. The residue, after recrystallization from 20% methanol, gave 23.4 mg of thymine (~93% yield).

Formation of 5CHO-Ura from Thy$^{\alpha}$OOH by Irradiation (254 nm). Thy$^{\alpha}$OOH (5 mg, 36 μmol) was dissolved in 40 ml of water. The solution in a quartz tube was irradiated (254 nm) for 220 sec and evaporated until dry. The residue, after recrystallization from absolute methanol, gave 4.0 mg of 5CHO-Ura (88% yield), mp >300° dec, λ_{max} (H$_2$O) 278 nm (ϵ 11850).[12]

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Registry No.—Thy$^{\alpha}$OOH, 33499-50-2; Thy$^{\alpha}$OCH$_3$, 57346-43-7; Thy$^{\alpha}$OH, 4433-40-3; Thy$^{\alpha}$Cl, 3590-48-5; 5CHO-Ura, 1195-08-0.

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Allylic Rearrangement from O^6 to N-3 and N-7 of Guanine Blocked at C-8

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It was recently reported[2,3] from this laboratory that the displacement reaction of 2-amino-6-chloropurine (1) with the sodium salts of allylic alcohols proceeds through an O^6 ether to yield an 8-substituted guanine. The O^6 to C-8 rearrangement occurs intramolecularly and is judged to proceed by two anionic [3,3] sigmatropic shifts via C-5 (Scheme I). By blocking the 8 position of the purine ring with a methyl group, we sought to trap the C-5 intermediate or to redirect the migrating group. Thereby, another allylic rearrangement has been revealed in which the overall migration, with allylic retention, is from O^6 to the N-3 and N-7 positions of the guanine ring (Scheme II), with corresponding mechanistic implications.