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1,3-Bisalkylcarbonyl- and 3-Alkylcarbonyl-5-fluorouracil Prodrugs: Syntheses, Thermal, and Hydrolytic Stability

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ABSTRACT

Four homologous 1,3-bisalkylcarbonyl and the corresponding 3-alkylcarbonyl derivatives of 5-fluorouracil (1, 5-FU) have been synthesized and characterized by ^1H nuclear magnetic resonance, and infrared and ultraviolet spectroscopy. The 1,3-bisacetyl derivative hydrolyzed rapidly in pH 7.1 buffer at 32°C ($t_{1/2}$ = 0.8 min) to give the 3-acetyl derivative. The hydrolyses of 3-acetyl-, **6**, *and 3-propionyl-5-FU,* **7**, *were much slower ($t_{1/2}$ = 200 and 300 min, respectively) and they were biexponential. Both the 1,3- and the 3-derivatives hydrolyzed in the presence of formaldehyde to give significant, and approximately equal amounts of 1- and 3-alkylcarbonyloxymethyl-5-FU derivatives, in addition to 5-FU, which suggested that for both types of derivatives the hydrolyses of the 3-alkylcarbonyl group went through a symmetrical O^2-alkylcarbonyl intermediate. The 1,3-derivatives were thermally unstable above about 110°C, giving up to about 20% of the corresponding 1-alkylcarbonyl derivatives at 140°C. On the other hand, the 3-derivatives were thermally unstable even at 95°C, where complete decomposition to 1- and 1,3-derivatives as well as 5-FU was observed.*

INTRODUCTION

Several different types of 1-acyl prodrugs of 5-fluorouracil (5-FU) have recently been evaluated for their abilities to deliver 5-FU into (dermal) and through (transdermal) skin (1-4). These types of 1-acyl prodrugs exhibited a wide range of solubility properties that resulted in a correspondingly wide range of rates of delivery of total 5-FU species (5-FU and intact prodrug) into and through skin. In each of these series of more lipophilic prodrugs, the more water soluble member of each series was the most effective one at delivering total 5-FU species through skin. However, in the case of the 1-alkyloxycarbonyl prodrugs, mostly intact prodrug was delivered (3), while in the case of the 1-alkylcarbonyl prodrugs only 5-FU was delivered (4). The latter type of prodrug was also the most effective at delivering 5-FU into the skin (4). Both of these results were attributed to a much faster rate of hydrolysis of the 1-alkylcarbonyl prodrugs to 5-FU after they had partitioned into the skin (3,4). Thus, stability and solubility considerations are important for optimizing topical delivery, that is, delivery into and through skin.

Although previous studies have shown that 1-alkylcarbonyl derivatives of 5-FU hydrolyze rapidly to 5-FU, the same studies showed that they were thermally stable, so that they could be formulated in aprotic vehicles (5). Thus, it was proposed that 1-alkylcarbonyl derivatives of 5-FU were suitable prodrugs with which to enhance the topical delivery of 5-FU (5). On the other hand, 3-alkylcarbonyl derivatives of 5-FU were previously proposed as being more useful as prodrugs than 1-alkylcarbonyl or 1,3-bisalkylcarbonyl derivatives of 5-FU because they were hydrolytically more stable (6). However, the thermal stabilities of the 3-alkylcarbonyl and 1,3-bisalkylcarbonyl derivatives were not examined.

In this paper the syntheses and spectral characterization of a homologous series of 3-alkylcarbonyl and 1,3-bisalkylcarbonyl prodrugs of 5-FU and the evaluation of their hydrolytic and thermal stabilities are reported and compared with those of the corresponding 1-alkylcarbonyl prodrugs.

MATERIALS AND METHODS

Melting points (mp) were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab Incorporated (Norcross, GA). Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Infrared (IR) spectra were recorded with a Perkin–Elmer 1420 spectrophotometer. Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. The high-performance liquid chromatographic (HPLC) system consisted of a Beckman 110A pump, Rheodyne 7125 20 µl loop injector, Lichrosorb (250 × 4.6 mm) 10-µm RP-8 column, Beckman Model 153 fixed-wavelength detector, and a Hewlett Packard 3392A integrator. Differential scanning calorimetric (DSC) analyses in hermetically sealed pans were carried out using a Perkin–Elmer DSC-7 scanning calorimeter, and thermogravimetric analyses (TGA) were performed using a Perkin–Elmer TGA-7 thermogravimetric analyzer, both controlled by a Perkin–Elmer TAC-7 interface and IBM PS/2 Model 50Z microcomputer. 5-FU was purchased from Sigma Chemical Co. All other reagents were obtained from Aldrich Chemical Co.

Synthesis

Preparation of 1,3-Bisalkycarbonyl-5-FU (General Procedure)

To 1.31 g (0.01 mol) of 5-FU (**1**)* suspended in acetonitrile (20 ml) was added 1.01 g of triethylamine (0.01 mol) in acetronitrile (5 ml). The mixture was stirred continuously at 0°C while 0.011 mol of the appropriate acid chloride in acetonitrile (5 ml) was added dropwise over 5–10 min. The above sequence was repeated until 0.03 mol of triethylamine and 0.033 mol of acid chloride were added, then the mixture was stirred for an additional 30 min at 0°C. The mixture was filtered, and the residue was washed with acetonitrile (25 ml). The combined acetonitrile solutions were evaporated under reduced pressure, and the solid residue was crystallized from an appropriate solvent or solvent combination.

1,3-Bisacetyl-5-fluorouracil (2) Crystallization from ether gave 1.69 g of **2** (79%): mp 112–113°C [lit. (7) mp 111°–113°C]; IR (KBr) 1680, 1695, 1740, 1750, and 1795 cm^{-1} (C=O); ^{1}H NMR (CDCl$_3$) δ 2.58 (s, 3H, 3-C$\underline{H}_3$), 2.72 (s, 3H, 1-C$\underline{H}_3$), and 8.23 (d, J = 7 Hz, 1H, C^6-$\underline{H}$); UV$_{max}$ (CH$_3$CN) 262 nm (ε = 9.75 × 10^3).

1,3-Bispropionyl-5-fluorouracil (3) Crystallization from ether gave 1.82 g of **3** (75%): mp 100°–101°C;

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*See Tables 1 and 2 in the Results section.

IR (KBr) 1690, 1725, and 1795 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.28 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 2.85 (q, J = 7 Hz, 2H, 3-$COC\underline{H}_2$), 3.11 (q, J = 7 Hz, 2H, 1-$COC\underline{H}_2$), and 8.25 (d, J = 7 Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 262 nm (ε = 9.65 × 10^3).

Anal. calc. for $C_{10}H_{11}FN_2O_4$: C, 49.59; H, 4.58; N, 11.577. Found: C, 49.44; H, 4.59; N, 11.53.

1,3-Bisbutyryl-5-fluorouracil (4) Extraction of the residue with hot low-boiling petroleum ether gave a cloudy solution which cleared upon cooling to 0°C and produced a yellow resinous sediment. The mixture was allowed to warm to room temperature, and the clear solution was decanted from the sediment. The solution was cooled again to 0°C and crystallization gave 1.70 g of **4** (63%): mp 48°-49°C [lit (6) mp 47.5°-48.5°C]; IR (KBr) 1685, 1710, 1735, and 1795 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.00 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.03 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.6–1.9 (m, 4H, 3-$COCH_2C\underline{H}_2$), and 1-$COCH_2C\underline{H}_2$, 2.80 (t, J = 7 Hz, 2H, 3-$COC\underline{H}_2$), 3.07 (t, J = 7 Hz, 2H, 1-$COC\underline{H}_2$), and 8.23 (d, J = 6 Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 262 nm (ε = 1.051 × 10^4).

1,3-Bisvaleryl-5-fluorouracil (5) Extraction of the residue with hot low-boiling petroleum ether gave a cloudy solution which cleared upon cooling to 0°C and produced a brown resinous sediment. The mixture was allowed to warm to room temperature, and the clear solution was decanted from the sediment. The solution was cooled again to 0°C and crystallization gave 2.24 g of **5** (75%); mp 47°-48°C; IR (KBr) 1685, 1710, 1735, and 1795 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7 Hz, 6H, $C\underline{H}_3$), 1.3–1.8 (m, 8H, 3-$COCH_2C\underline{H}_2C\underline{H}_2$ and 1-$COCH_2C\underline{H}_2C\underline{H}_2$), 2.83 (t, J = 7 Hz, 2H, 3-$COC\underline{H}_2$), 3.08 (t, J = 7 Hz, 2H, 1-$COC\underline{H}_2$), and 8.22 (d, J = 7 Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 262 nm (ε = 1.071 × 10^4).

Anal. calc. for $C_{14}H_{19}FN_2O_4$: C, 56.37; H, 6.42; N, 9.39. Found: C, 56.29; H, 6.48; N, 9.33.

Preparation of 3-Alkylcarbonyl-5-FU (General Procedure)

To a solution of the appropriate 1,3-bisalkycarbonyl-5-FU (0.01 mol) in ether (60–650 ml) at 0°C was added 0.73 g of *t*-butylamine (0.01 mol) in ether (25 ml) dropwise over 10 min with stirring. The solution was stirred for a total of 30 min at 0°C. The ether solution was evaporated under reduced pressure at room temperature until the volume was reduced to 25–50 ml. The solution was cooled again to 0°C until crystallization occurred.

3-Acetyl-5-fluorouracil (6) Crystallization gave 1.22 g of **6** (71%): mp 115°-117°C [lit. (7) mp 114°-117°C]; IR (KBr) 1650, 1685, 1720, and 1805 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, $C\underline{H}_3$) and 7.23 (bs, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 267 nm (ε = 6.57 × 10^3).

3-Propionyl-5-fluorouracil (7) Crystallization gave 0.89 g of **7** (48%): mp 102°-103°C [lit. (7) mp 99°-102°C]; IR (KBr) 1655, 1680, 1730, and 1815 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 2.86 (q, J = 7 Hz, 2H, $COC\underline{H}_2$), and 7.26 (bs, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 267 nm (ε = 6.66 × 10^3).

3-Butyryl-5-fluorouracil (8) Crystallization gave 1.22 g of **8** (61%): mp 111°-112°C [lit. (6) mp 132°-134°C]; IR (KBr) 1660, 1730, and 1810 cm^{-1} (C =O); ^{1}H NMR ($CDCl_3$) δ 1.02 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.6–2.0 (m, 2H, $COCH_2C\underline{H}_2$), 2.82 (t, J = 7 Hz, 2H, $COC\underline{H}_2$), and 7.26 (bs, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 267 nm (ε = 6.55 × 10^3).

Anal. calc. for $C_8H_9FN_2O_3$: C, 48.00; H, 4.53; N, 14.00. Found: C, 48.08; H, 4.55; N, 13.93.

3-Valeryl-5-fluorouracil (9) Crystallization gave 1.54 g of **9** (72%): mp 110°-111°C; IR (KBr) 1665, 1730, and 1815 cm^{-1} (C =O); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J = 7 Hz, 3H, $C\underline{H}_3$), 1.3–1.9 (m, 4H, $COCH_2C\underline{H}_2C\underline{H}_2$), 2.84 (t, J = 7 Hz, 2H, $COC\underline{H}_2$), and 7.23 (bs, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 267 nm (ε = 6.68 × 10^3).

Anal. calc. for $C_9H_{11}FN_2O_3$: C, 50.47; H, 5.18; N, 13.08. Found: C, 50.73; H, 5.24; N, 12.95.

Hydrolysis Experiments

Hydrolysis rates for the conversion of 1,3-bisacetyl-5-FU, **2**, to 3-acetyl-5-FU, **6**, were determined at 32°C in 0.05 M phosphate buffer (pH = 7.1, I = 0.12 M) and in the same buffer with 0.11% formaldehyde (3.6 × 10^{-2} M). The temperature was maintained with a Fisher model 80 water bath. The hydrolyses were followed by UV spectroscopy at 266 nm where the absorbance decrease accompanying conversion of the 1,3-bisacetyl derivative to the 3-acetyl derivative was maximized. Hydrolysis was initiated by adding 60 μl of a stock solution of the derivative in acetonitrile to 3 ml of buffer prewarmed to 32°C in a thermostated quartz cuvette to give final concentrations of about 1.8×10^4 M. Absorbances were recorded at appropriate intervals and pseudo-first-order rate constants were determined from the expression:

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - kt$$

where A_t is the absorbance at time $= t$, A_∞ is the absorbance at $t = \infty$, A_0 is the absorbance at $t = 0$, k is the pseudo-first-order rate constant, and t is the time. The hydrolyses were sufficiently fast and the 3-acetyl product was sufficiently stable to allow experimental determination of A_∞. The slopes, $-k$, of linear plots of $\ln(A_t - A_\infty)$ versus time were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from $t_{1/2} = 0.693/k$. Each hydrolysis reaction was run in triplicate and was followed for a minimum of eight half-lives. The correlation coefficients were ≥ 0.999.

The reaction mixtures from the hydrolyses of 1,3-bisacetyl-5-FU, **2**, were also analyzed by HPLC using a mobile phase of 10% methanol, 90% acetate buffer, pH 5.0; a flow rate of 1.0 ml/min; $\lambda_{\text{anal}} = 254$ nm. The following retention times were found under those conditions: 5-FU, 4.5 min; 3-acetyl-5-FU, 9.1 min; 1-acetyloxymethyl-5-FU, 11.4 min; 1-acetyl-5-FU, 13.1 min; 3-acetyloxymethyl-5-FU, 15.7 min.

The experimental procedures for the hydrolyses of the 3-alkylcarbonyl-5-FU prodrugs have been previously reported (8).

Thermal Stability

Samples of 1,3-bisalkylcarbonyl and 1- and 3-alkylcarbonyl-5-FU were analyzed by ^{1}H NMR spectroscopy to assess their integrity and ensure their identity before they were heated. Mineral oil baths were heated with Corning PC-351 stirrer-hot plates in a hood until stable temperatures were obtained; this usually took about 2 hr. Different temperatures were obtained by incrementally changing the hot plate settings. The samples (about 20–50 mg) were placed in glass test tubes sealed with cork stoppers and heated in the oil baths at the indicated temperatures for 1 hr. The test tubes were removed from the oil baths, cooled to room temperature for 30 min, and analyzed by ^{1}H NMR spectroscopy using DMSO-d_6 or $CDCl_3$ as the solvent. Since 1-alkylcarbonyl groups hydrolyzed very rapidly in DMSO-d_6 containing even a small amount of water, the spectra were run immediately after DMSO-d_6 solutions were prepared and only freshly opened ampules of DMSO-d_6 were used.

For the analysis of the product from heating 3-acetyl-5-FU, the integrated areas of the following C^6–$\underline{H}$ absorptions were used to quantitate the components: (DMSO-d_6) δ 7.88 (3-acetyl-5-FU), 8.28 (1-acetyl-5-FU), 8.43 (1,3-bisacetyl-5-FU), and 7.67 (5-FU). For the analysis of the product from heating 1,3-bisbutyryl-5-FU alone or with 5-FU, the integrated areas of the following C^6–$\underline{H}$ and $C\underline{H}_2C{=}O$ absorptions, respectively, were used to quantitate the components: ($CDCl_3$) δ 8.29, 3.08 and 2.80 (1,3-bisbutyryl-5-FU), 8.28 and 3.08 (1-butyryl-5-FU), and 7.26 and 2.80 (3-butyryl-5-FU); (DMSO-d_6) δ 8.43 (1,3-bisbutyryl-5-FU), 8.28 (1-butyryl-5-FU), 7.88 (3-butyryl-5-FU), and 7.67 (5-FU). For the analysis of the product from heating 1-acetyl and 1-butyryl-5-FU, the integrated areas of the following C^6–$\underline{H}$, $C\underline{H}_2C{=}O$ and $C\underline{H}_3C{=}O$ absorptions, respectively, were used to quantitate the components: ($CDCl_3$:DMSO-d_6, 7:1) δ 8.28 (1-butyryl-5-FU), 3.08 (1-butyryl-5-FU); (DMSO-d_6) δ 8.28 (1-acetyl-5-FU) and 2.58 (1-acetyl-5-FU).

RESULTS AND DISCUSSION

Synthesis

The 1,3-bisalkylcarbonyl-5-FU prodrugs, **2–5**, were synthesized according to the general procedure of Kametani et al. (7) except that the acid chloride and triethylamine were each added in three portions (instead of one portion) at 0°C (instead of at room temperature) and in acetonitrile (instead of dioxane). Alternate addition of tertiary amine base and acid chloride was found to increase yield and decrease formation of colored side products that were difficult to remove by crystallization. In addition, no admixture of the reaction mixture with water was used in the workup due to the rapid hydrolysis of 1-alkylcarbonyl groups (5,6).

The 3-alkylcarbonyl-5-FU prodrugs, **6–9**, were synthesized from the corresponding 1,3-bisalkylcarbonyl-5-FU prodrugs by their reaction with *t*-butylamine in ether. A sterically hindered primary amine was used to take advantage of the greater steric hindrance to attack at the 3-position (8) to give preferential attack at the 1-position. Attempts to preferentially hydrolyze the 1-alkylcarbonyl group with ethanol (7) or acidic ethanol (6) did not give reproducible yields of pure products. Elemental analyses were obtained for all the new compounds (**3**, **5**, and **9**) and for **8**. All the analyses were within acceptable limits (±0.40%).

The structure of the 1,3-bisalkylcarbonyl prodrugs is based on the published single-crystal x-ray diffraction data of 1,3-bisacetyl-5-FU, **2** (8), and on the fact that the ^1H NMR, IR, and UV spectra of **3–5** are essentially identical with those of **2** (see synthesis above) (1,8). Although no comparable single-crystal x-ray diffraction data are available for 3-acetyl-5-FU, **6**, the single-crystal x-ray diffraction data for 1,3-bisacetyl-5-FU shows that the 3-acetyl group is perpendicular to the plane of the 5-FU ring. Since the ^1H NMR spectrum of 1,3-diacetyl-5-FU is essentially a composite of the ^1H NMR spectra of 1- and 3-acetyl-5-FU (8), the acetyl group in 3-acetyl-5-FU must be in the same chemical and electronic environment as it is in 1,3-bisacetyl-5-FU and hence must also be perpendicular to the plane of the 5-FU ring on a time-averaged basis. The structure of the remaining 3-alkylcarbonyl-5-FU prodrugs, **7–9**, follows from the fact their ^1H NMR, IR, and UV spectra are essentially identical with those of **6** (see synthesis above) (1,8). In both the 1,3- and 3-series the conclusion that the ^1H NMR spectra are identical takes into account the fact that the $\underline{CH_2}$–C=O absorptions in **3–5** and **7–9** are shifted downfield from the $\underline{CH_3}$–C=O in **2** and **6**, respectively, by about 0.30–0.40 ppm.

The identity of the previously reported (6) 3-butyryl-5-FU prodrug, **8**, is questionable. The melting point that was reported (132°–134°C) is much higher than that reported here and more closely approximates that of the 1-butyryl prodrug (mp 145°–146°C) (5). In addition, the salient features of the reported (6) ^1H NMR spectrum [($CDCl_3$) δ 3.00 (t, 3H, $\underline{CH_2}$–C=O and 8.20 (d, 1H, C^6–$\underline{H}$)] more closely approximates that of the 1-butyryl prodrug [($CDCl_3$) δ 3.11 (t, 3H, $\underline{CH_2}$–C=O) and 8.25 (d, 1H, C^6–$\underline{H}$)] (5). On the other hand, the ^1H NMR and the melting points previously reported for 3-acetyl- and 3-propionyl-5-FU prodrugs (7) are essentially identical with those reported here, and the ^1H NMR and melting points of 1,3-diacetyl- (7) and 1,3-dibutyryl-5-FU (6) are also identical with those reported here.

Hydrolysis

The hydrolyses of 3-acetyl- (**6**) and 3-propionyl-5-FU (**7**) have previously been reported (6) and the interpretation of the results corrected (8). The important features of the latter results are: (*a*) the hydrolyses showed a biexponential relationship between ln concentration and time that supported an initial equilibrium of 3-acyl-5-FU with O^2-acyl-5-FU which subsequently hydrolyzed to 5-FU or rearranged to 1-acyl-5-FU and then rapidly hydrolyzed to 5-FU; (*b*) the terminal half-lives of **6** and **7** were much longer (about 200 and 300 min, respectively) than previously reported (6) (43 and 50 min, respectively) so that they were very similar to the half-lives of 1-alkyloxycarbonyl derivatives (9); (*c*) the O^2-acetyl intermediate was trapped with formaldehyde (3.6×10^{-2} M) and the rearrangement of the 1- and 3-hydroxymethyl-2-acetyl-5-FU that were formed gave 1- and 3-acetyloxymethyl-5-FU products (22% and 21%, respectively). Although authentic 1- and 3-acyloxymethyl-5-FU derivatives were not available to positively identify and quantitate the products from the hydrolysis of the other 3-acyl-5-FU derivatives (**7–9**) in the presence of formaldehyde, products with relative retention times and relative peak intensities similar to 1- and 3-acetyloxymethyl-5-FU were observed from the hydrolysis of the other 3-acyl derivatives in the presence of formaldehyde.

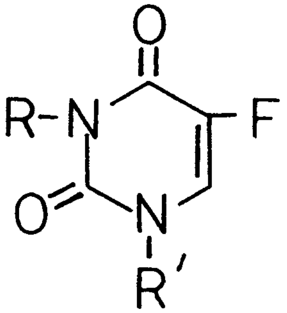
The hydrolyses of 1,3-bisacetyl- and 1,3-bisbutyryl-5-FU have been previously reported (6) to give identical k_{OH} and identical k_0 values, and to hydrolyze quantitatively to the corresponding 3-acyl derivatives. The k_{obs} value for the hydrolysis of 1,3-bisacetyl- to 3-acetyl-5-FU obtained in this study at pH 7.1 and 32°C (k_{obs} = 0.80 min^{-1}) is similar to that estimated from the literature (6) (k_{obs} = 0.32 min^{-1}) at pH 7.4 and 37°. The hydrolysis of 1,3-bisacetyl-5-FU at pH 7.1 in the presence of formaldehyde gave the same result as that obtained from the hydrolysis of 3-acetyl-5-FU in the presence of formaldehyde: formation of 5-FU and of approximately equal amounts of 1- and 3-acetyloxymethyl-5-FU (27% and 30%, respectively). The hydrolyses of the other 1,3-bisacyl derivatives, **3–5**, in the presence of formaldehyde gave the same products, with the same retention times and relative peak intensities as obtained from the hydrolyses of the corresponding 3-acyl derivatives. This result supports the fact that the hydrolysis of the 1,3-bisacyl derivatives proceeds through the formation of the 3-acyl derivative (and hence the O^2-acyl intermediate) since the 1-acyl derivatives do not form 1- and 3-acyloxymethyl-5-FU upon their hydrolysis in the presence of formaldehyde.

Thermal Stability

The capillary melting points of the 3-acyl derivatives were difficult to determine reproducibly because of their dependence on the rate at which the temperature was increased. The melting points given in Table 1 were each obtained using the same melting point apparatus set at the same voltage so they are reproducible relative to each other. Immediately after melting, each derivative

Table 1

Melting Point, DSC, and TGA Data for 3-Alkylcarbonyl-5-FU



R′ = H, R =	mp, °C[a]	DSC[b] °C	TGA[c] Onset, °C	First Phase, °C (% Loss)	Complete, °C
6 CH_3CO	115–117	114	164	194 (72)	260
7 C_2H_5CO	102–103	105	171	198 (83)	250
8 C_3H_7CO	111–112	106	169	196 (81)	251
9 C_4H_9CO	110–111	119	181	213 (88)	249

[a] After melting, samples resolidify and slowly remelt as temperature increases.

[b] Temperature at which peak in endotherm occurs, run at 5°C/min. Endotherm followed immediately by large exotherm; no other endotherm observed as temperature increase continues.

[c] Temperatures at which changes in mass loss occurs; run at 5°C/min.

resolidified and then very slowly remelted (**6** and **7**) or only partially remelted (**8** and **9**) up to 250°C.

The differential scanning calorimetric (DSC) analyses of the 3-acyl derivatives were as expected based on the behavior of the 3-acyl derivatives in the capillary apparatus. The derivatives all exhibited a sharp endotherm at a temperature that was dependent on the rate at which the temperature was increased, and was followed immediately by an exotherm of approximately the same peak area. The temperatures at which the peak in the endotherm occurred were very close to the observed capillary melting points. The endotherms reported in Table 1 were from analyses run at 5°C/min, but the range of temperatures for the endotherms for analyses run at from 2°C/min to 20°C/min were from 16°C for **8** to 24°C for **6**. A sample of the 3-acetyl derivative was also held at 150°C for 5 min and at 175°C for 5 min, and then cooled to 40°C. The DSC analysis was then rerun at 5°C/min. It gave an endotherm at 126°C, which was at the same temperature as the endotherm observed from the DSC analysis of a sample of 1-acetyl-5-FU which had previously been held at 135°C for 20 min and then cooled before being rerun. This suggests that the 3-acetyl derivative rearranges to the 1-acetyl derivative when heated.

Onset of mass loss during the thermogravimetric analysis (TGA) of the 3-acyl derivatives started at between 164° and 181°C (Table 1). Smooth loss of sample mass continued as the temperature was increased at 5°C/min until temperatures of 194° (72% mass loss), 198° (83%), 196° (81%), and 213°C (88%) for **6**, **7**, **8**, and **9**, respectively, were reached. At that point, there was an abrupt decrease in rate of mass loss until complete mass loss occurred at 260°, 250°, 251°, and 249°C, respectively.

On the other hand, the capillary melting points for the 1,3-bisacyl derivatives were well defined and easily reproducible (Table 2). The 1,3-bisacyl derivatives exhibited peaks in their endotherms at approximately the same temperatures as their melting points when the DSC analyses were run at 5°C/min (Table 2). In contrast to the 3-acyl derivatives, there was not much variation in the temperature at which the peak in the endotherm occurred for the 1,3-bisacyl derivatives when the rate of temperature increase was varied from 2°C/min to 20°C/min (e.g., 107° and 112°C, respectively, for 1,3-bis-

Table 2

Melting Points, DSC, and TGA Data for 1,3-Bisalkylcarbonyl-5-FU

			TGA[b]		
R = R′ =	mp, °C	DSC,[a] °C	Onset, °C	First Phase, °C (% Loss)	Complete, °C
2 CH_3CO	112–113	108	154	201 (92)	242
3 C_2H_5CO	100–101	102	163	196 (97)	210
4 C_3H_7CO	48–49	41	177	209 (99)	215
5 C_4H_9CO	47–48	37	176	209 (97)	215

[a] Temperature at which peak in endotherm occurs; run at 5°C/min.

[b] Temperatures at which changes in mass loss occur; run at 5°C/min.

acetyl-5-FU). Onset of mass loss during the TGA of the 1,3-bisacyl derivatives occurred at somewhat lower temperatures (154° to 177°C) than those for the 3-acyl derivatives. In contrast to the 3-acyl derivatives, the first phase of vaporization resulted in almost complete mass loss for **2**, **3**, **4**, and **5** at 201° (92% mass loss), 196° (97%), 209° (99%) and 209°C (97%), respectively, before an abrupt decrease in the rate of mass loss occurred.

In order to better understand the thermochemistry of the 3-acyl and 1,3-bisacyl derivatives, the effect of different temperatures on samples (20–50 mg) of 3-acetyl- and 1,3-bisbutyryl-5-FU was examined using ^1H NMR spectroscopy to identify and quantitate the various products. The results of that study are given in Table 3 with the results from heating 1-acetyl- (5) and 1-butyryl-5-FU under similar conditions.

The results for the thermal degradation of 1,3-bisbutyryl-5-FU were straightforward. At temperatures above 110°C, the 3-butyryl group was lost and, as the temperature was increased, gave progressively higher yields of 1-butyryl-5-FU. The formation of 1-acetyl-5-FU was observed when 1,3-bisacetyl-5-FU was heated under similar conditions (10). 1-Acetyl (5), 1-butyryl-, 1-hexanoyl- (10), and 1-decanoyl-5-FU (10) were all thermally stable up to 145°C. The formation of 1-butyryl-5-FU by heating 1,3-bisbutyryl-5-FU appears to be similar to the previously reported thermal degradation of 7-acyltheophyllines to theophylline (11), 1-acetyl-5-FU to 5-FU at 205°C (5), and 1-alkylaminocarbonyl-5-FU to 5-FU (12), where losses of ketenes and alkylisocyanates have been proposed.

The results for the thermal degradation of 3-acetyl-5-FU were more complicated. In addition to 5-FU,

Table 3

Product Compositions After Heating Samples of 1- and 3-Alkylcarbonyl-5-FU and 1,3-Bisalkylcarbonyl-5-FU

	% Composition after Heating, t = 1 hr					
	t = 0	140°C	126°C	110°C	95°C	65°C
R = R′ = C_3H_7CO	100	78	89	97		
R = H, R′ = C_3H_7CO	0	22	11	3		
R′ = H, R = CH_3CO	100		0	0	0	68
R′ = CH_3CO, R = H	0		65	58	48	10
R′ = R = H	0		27	28	30	10
R′ = R = CH_3CO	0		8	14	22	12
R′ = CH_3CO, R = H	100	100[a]				
R′ = C_3H_7CO, R = H	100	100[a]				

[a] Run at 145°C.

which would be expected from the results for 1,3-bisbutyryl-5-FU, 1,3-bisacetyl-5-FU, and 1-acetyl-5-FU were observed. Similar results were obtained when other 3-acyl-5-FU derivatives were heated under similar conditions (10). The amount of 5-FU formed by loss of the 3-acetyl group remained essentially constant at about 30% as the temperature was increased from 95° to 126°C, while the amount of 1-acetyl-5-FU increased and the amount of 1,3-bisacetyl-5-FU decreased. Onset of loss of the 3-acetyl group to give 5-FU occurred at less than 65°C where 10% 5-FU was formed from the thermal degradation of 3-acetyl-5-FU. Thus, loss of the 3-acyl group from a 3-acyl derivative occurred more readily than loss of the 3-acyl group from a 1,3-bisacyl derivative.

If the mechanism for the loss of the 3-acyl group from the 3-acyl and 1,3-bisacyl derivatives is assumed to be the same in both cases, the greater thermal stability of the 3-acyl group in the 1,3-bisacyl derivatives must be due to the 1-acyl group. In the mechanism proposed (8) for the loss of the 3-acetyl group from 3-acetyl-5-FU, the oxygen of the $C^2=O$ group functioned as an intramolecular base to remove a proton from the α-carbon in the acetyl group. Thus, electron delocalization into the carbonyl oxygen (–I) of the planar (conjugated) 1-acyl group should reduce electron density at the oxygen of the $C^2=O$ group in comparison with the electron density present when there is no 1-acyl group. This decrease in electron density would make it more difficult for the oxygen in the $C^2=O$ group to remove a proton from the α-carbon in the 3-acyl group of 1,3-bisacyl-5-FU derivatives.

The formation of 1,3-bisacetyl-5-FU requires an intermolecular mechanism. Two possibilities are that either the acetyl group on one 3-acetyl-5-FU is transferred directly to the 1-position of another molecule of 3-acetyl-5-FU or the acetyl group is lost as ketene which reacts with another molecule of 3-acetyl-5-FU to give 1,3-bisacetyl-5-FU before it can escape the crystal lattice or melt. The fact that the amount of 1,3-bisacetyl that was formed decreased as the temperature increased suggests that the latter may be more reasonable.

The formation of 1-acetyl-5-FU can take place by an intramolecular mechanism involving an O^2-acetyl intermediate which has previously been described (8), or it can take place by an intermolecular mechanism which involves loss of ketene from 3-acetyl-5-FU to give 5-FU followed by recombination of ketene with 5-FU to give 1-acetyl-5-FU. There is good precedent for the intramolecular mechanism from hydrolysis experiments with 3-acetyl-5-FU where the O^2-acetyl intermediate was trapped with formaldehyde as 1- and 3-acetyloxymethyl-5-FU (8). However, in a separate experiment, 20% of 1,3-bisbutyryl-5-FU, 50% of 1-butyryl-5-FU, and 30% of 5-FU were obtained when equimolar amounts of 1,3-bisbutyryl-5-FU and 5-FU were heated at 140°C for 1 hr. Similar results were obtained when 1,3-bisacetyl-5-FU and 5-FU were heated (8). The fact that much greater than 50% of the molecules contain a 1-acyl group in both experiments demands that at least part of the 1,3-bisacyl molecules rearrange by an intermolecular mechanism.

CONCLUSIONS

The results of the present study show that, although the 3-alkylcarbonyl derivatives are more stable hydrolytically, they are much less stable thermally than either the 1-alkylcarbonyl- or the 1,3-bisalkylcarbonyl-5-FU derivatives. After 1 hr at 65°C, 32% of a sample of 3-acetyl-5-FU had decomposed, while after 1 hr at 95°C, 100% of the sample had decomposed. Thus, 3-acetyl-5-FU and the other 3-alkylcarbonyl derivatives would not be amenable to commercial formulation in aprotic vehicles for topical use and are not practical prodrug candidates for enhancing the topical delivery of 5-FU.

On the other hand, although 1-3,bisbutyryl-5-FU was not as stable hydrolytically as 1-butyryl-5-FU, it was reasonably stable at 110°C and 1 hr (97% intact), so that it and other 1,3-bisalkylcarbonyl derivatives could be formulated in aprotic, nonhygroscopic vehicles for topical use. However, the 1,3-bisalkylcarbonyl derivatives hydrolyze rapidly to give the 3-alkylcarbonyl derivatives, which are likely to remain intact during permeation. This fact suggests that the 1,3-bisalkylcarbonyl derivatives are also not practical prodrugs for enhancing the dermal delivery of 5-FU but may be useful for enhancing the transdermal delivery of 3-alkylcarbonyl-5-FU. Subsequent hydrolysis of 3-alkylcarbonyl-5-FU in vivo to 5-FU would then give enhanced transdermal delivery of 5-FU.

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