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Prodrugs of 5-fluorouracil. IV. Hydrolysis kinetics, bioactivation and physicochemical properties of various N-acyloxymethyl derivatives of 5-fluorouracil *

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Summary

The kinetics and mechanism of hydrolysis of various 1-, 3- and 1,3-acyloxymethyl derivatives of 5-fluorouracil were studied to assess their potential as prodrugs with the aim of enhancing the delivery characteristics of the parent drug. All the derivatives hydrolyzed to yield 5-fluorouracil in quantitative amounts, passing through an unstable N-hydroxymethyl-5-fluorouracil intermediate. The pH–rate profiles obtained revealed the occurrence of specific acid and base catalysis as well as of a water-catalyzed reaction. The rates of hydrolysis were accelerated markedly in the presence of human plasma or rat liver homogenate, suggesting the utility of the derivatives as prodrugs. The derivatives were all more lipophilic than 5-fluorouracil as determined by partition experiments in octanol–aqueous buffer systems but the aqueous solubility was only slightly reduced or, for some derivatives, even greater than that of 5-fluorouracil. This behaviour was attributed to differences in the crystal lattice energy, and relationships between melting points, partition coefficients and water-solubilities for these and 11 other prodrug derivatives of 5-fluorouracil were established.

* This paper is part 37 of the series: Prodrugs as drug delivery systems. For part 36, see Bundgaard, H. and Falch, E., Allopurinol prodrugs. I. Synthesis, stability and physicochemical properties of various N_1-acyl allopurinol derivatives. Int. J. Pharm., in press.

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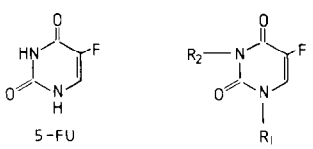
Introduction

5-Fluorouracil shows an incomplete and highly variable bioavailability following oral administration, largely due to a marked first-pass metabolism (Cohen et al., 1974; Christophidis et al., 1978; Finch et al., 1979; Fraile et al., 1980; Phillips et al., 1980; Almersjö et al., 1980), as well as a negligible absorption following rectal administration (Christophidis et al., 1978). Studies have been undertaken in this laboratory to overcome these delivery problems by the prodrug approach. It was thought that by bioreversible derivatization it may be possible to protect the drug against first-pass metabolism and to obtain prodrugs possessing a higher lipophilicity than the parent drug. The partition coefficient of 5-fluorouracil between octanol and water is only 0.15 (Buur and Bundgaard, 1984a) and this low lipophilicity may be a predominant factor for the poor biomembrane permeability of the drug. In previous studies (Buur and Bundgaard, 1984a, 1984b, 1985), various N-acyl, N-alkoxycarbonyl and 1-carbamoyl derivatives were evaluated as possible prodrug forms. In the present work a series of N-acyloxymethyl derivatives (Fig. 1) were prepared and assessed as potentially useful prodrugs. To this end, the chemical- and enzyme-mediated conversion of the derivatives to 5-fluorouracil was investigated and determinations of the aqueous solubility and lipophilicity of the compounds were performed. Furthermore, relationships between these properties of the prodrug derivatives including those studied previously are described.

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostatically controlled cell compartment and a Shimadzu U-135C recorder. One-cm quartz cells were used. ^{1}H-NMR spectra were run on a Varian 360 L instrument using tetramethylsilane as internal reference. The pH measurements were made at the temperature of study using a Radiometer



	R_1	R_2
I	$-CH_2OCOCH_3$	$-H$
II	$-CH_2OCOC_2H_5$	$-H$
III	$-CH_2OCOC_3H_7$	$-H$
IV	$-CH_2OCOC(CH_3)_3$	$-H$
V	$-CH_2OCOCH_3$	$-CH_2OCOCH_3$
VI	$-CH_2OCOC(CH_3)_3$	$-CH_2OCOC(CH_3)_3$
VII	$-H$	$-CH_2OCOCH_3$

Fig. 1. Chemical structure of 5-fluorouracil (5-FU) and various N-acyloxymethyl derivatives investigated in this study.

Type PHM 26 instrument. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was performed with a system consisting of a Waters pump model 6000 A, a variable wavelength UV-detector (Waters Type Lambda Max 480) and a 20-μl loop injection valve. The column used, 250 × 4 mm, was packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, F.R.G.). Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

5-Fluorouracil was purchased from Fluka AG, Switzerland and was used as received. Buffer substances and all other chemicals or solvents used were of reagent grade.

Preparation of N-acyloxymethyl derivatives of 5-fluorouracil (I–VII))

The 1-acyloxymethyl-5-fluorouracil derivatives (I–IV) were prepared by reacting 5-fluorouracil with the appropriate α-chloromethyl carboxylate in dimethyl sulfoxide and in the presence of potassium carbonate by the procedure previously described (Johansen et al., 1983). The 1,3-bis(acyloxymethyl) derivatives V and VI were obtained in a similar manner by using an excess of the α-chloromethyl carboxylate. 1,3-Bis(acetoxymethyl)-5-fluorouracil (V) was also prepared by the following method. 5-Fluorouracil (1.3 g, 0.01 mol) was dissolved in 4 ml of a 37% aqueous solution of formaldehyde with pH being adjusted to 7.0 with sodium hydroxide. After 5 h at room temperature the solution was lyophilized to give an oily residue of 1,3-dihydroxymethyl-5-fluorouracil. This was subsequently dissolved in 10 ml of dry pyridine and 3 ml (0.03 mol) of acetic anhydride was added while stirring. After standing at room temperature for 2 h the reaction solution was added to 100 ml of water. The mixture was extracted twice with ether and the extracts washed with diluted hydrochloric acid and water. The ether phase was dried over anhydrous sodium sulphate and evaporated in vacuo to give the title compound. It was recrystallized from ether–petroleum ether, yielding 0.62 g, m.p. 105–106°C.

3-Acetoxymethyl-5-fluorouracil (VII) was prepared by alkaline hydrolysis of

TABLE 1

MELTING POINTS AND ELEMENTAL ANALYSES OF VARIOUS ACYLOXYMETHYL DERIVATIVES OF 5-FLUOROURACIL

Compound	m.p. (°)	Calculated			Found		
		C	H	N	C	H	N
I	122–123	41.59	3.49	13.86	41.73	3.61	13.79
II	100–102	44.45	4.20	12.96	44.55	4.27	12.95
III	92–93	46.96	4.82	12.17	47.11	4.92	12.12
IV	158–160	49.18	5.36	11.47	49.32	5.42	11.35
V	105–106	43.80	4.04	10.22	43.89	4.18	10.24
VI	102–104	54.63	6.47	7.81	53.91	6.59	7.74
VII	158–159	41.59	3.49	13.86	41.65	3.56	13.78

1,3-bis(acetoxymethyl)-5-fluorouracil (V). Compound V (1.0 g) was dissolved in a mixture of 35 ml of methanol and 35 ml of 0.1 M borate buffer of pH 10.0. The solution was kept at 22°C for 75 min at which time compound VII was formed in maximum yield as revealed by HPLC analysis (see later). Hydrochloric acid (2 M) was added to give a pH of 6 and the reaction solution was concentrated in vacuo to about 30 ml and extracted with ethyl acetate (3×50 ml). The extracts were dried over anhydrous sodium sulphate and evaporated in vacuo. After column chromatography (silica gel; eluents: toluene containing 1% of acetic acid with increasing amounts of ethyl acetate) of the residue pure VII was isolated and recrystallized from ethyl acetate–petroleum ether, yield 230 mg, m.p. 158–159°C.

The compounds showed a correct microanalysis (Table 1) and had spectroscopic properties (UV, NMR) in agreement with their structure. Ozaki et al. (1984) have recently reported the synthesis of the same compounds except IV and VII. The melting points (cf. Table 1) agreed with those reported by Ozaki et al. (1984). Compound IV has been described in an earlier study (Johansen et al., 1983).

Kinetic measurements

The hydrolysis of the 5-fluorouracil N-acyloxymethyl derivatives was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate, borate, carbonate and sodium hydroxide solutions were used as buffers; the total buffer concentration was 0.05 M except in experiments where buffer effects were studied specifically. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

At pH values higher than about 9 the reactions were generally followed by direct UV-spectrophotometry by recording the absorbance changes accompanying the hydrolysis at 300 nm where the absorption of substrate and 5-fluorouracil differed maximally. The reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 25 μl of stock solutions of the derivatives in acetonitrile to give a final concentration of $0.5–2 \times 10^{-4}$ M. Pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_\infty - A_t)$ against time where A_∞ and A_t are the absorbance readings at infinity and time, t, respectively. Rate constants for some slower reactions were determined by the method of Guggenheim (1926).

At pH values lower than about 9.5 the rates of hydrolysis were followed by using a reversed-phase HPLC procedure. Mobile phase systems of 25–55% v/v methanol in 0.01 M acetate buffer of pH 5.0 were used. The flow rate was 1.2 ml · min^{-1} and the column effluent was monitored at 266 nm. The compounds were quantified by measuring the peak heights in relation to those of standards chromatographed under the same conditions. The reactions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10.0 ml of buffer solution, pre-heated at 37°C, in screw-capped test tubes, the final concentration in the reaction mixture being $0.5–2 \times 10^{-4}$ M. The solutions were kept in a water-bath at 37.0 ± 0.2°C and at appropriate times samples were taken and chromatographed. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual 5-fluorouracil derivative against time.

The hydrolysis of the derivatives was also studied in 0.01 M phosphate buffer of pH 7.4 containing 80% human plasma or 20% rat liver homogenate (at 37°C). Preparation of the rat liver homogenate was done as previously described (Buur and Bundgaard, 1984b, 1985). Initial concentrations of the compounds were $1-5 \times 10^{-4}$ M. At appropriate times samples of 200 μl or 400 μl were withdrawn and added to 1000 μl of ethanol or 250 μl of 20% trichloroacetic acid, respectively, in order to deproteinize the biological media. After mixing and centrifugation for about 2 min, 20 μl of the clear supernatant was analyzed by HPLC as described above, using mobile phase systems of 10–65% v/v methanol in 0.01 M acetate buffer of pH 5.0.

For the determination of 5-fluorouracil by HPLC a solvent system of 5% v/v of methanol in 0.01 M acetate buffer of pH 5.0 was used.

Determination of aqueous solubility and partition coefficients

The aqueous solubility of the derivatives was determined at 22°C by adding excess amounts to 0.05 M acetate buffer of pH 4.0 as previously described (Buur and Bundgaard, 1984a). The apparent partition coefficients (P) were determined in an octanol–0.02 M acetate buffer (pH 4.0) system as previously described (Buur and Bundgaard, 1984a).

Determination of ionization constants

The ionization constants for the derivatives I–IV and VII were determined at 22°C and $\mu = 0.5$ by spectrophotometry according to Albert and Serjeant (1971). Upon dissociation of the 3-NH proton the UV-spectrum of the derivatives I–IV

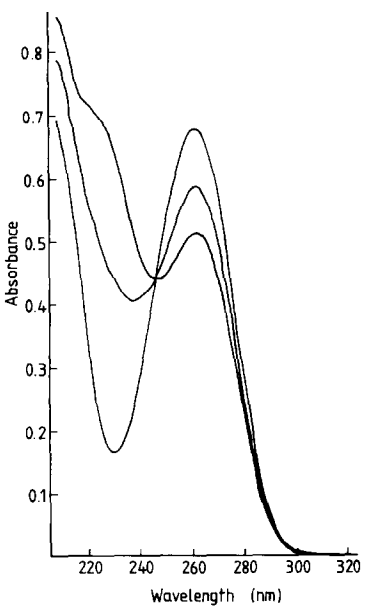


Fig. 2. Ultraviolet spectra of 1-acetoxymethyl-5-fluorouracil (I) in aqueous solution of various pH values.

changed profoundly with isosbestic points occurring at 248 nm (Fig. 2). The wavelength used for the determination of the pK_a values was 236 nm. The solute concentration was 1×10^{-4} M and the UV absorbances were measured at pH 2 and 10 and at 5 different pH values within the range pH 6–8. The pK_a value of the 3-substituted derivative VII was determined in a similar way, the measurements being made at 300 nm which is λ_{max} for the anionic species (due to ionization of the 1-NH proton).

Results and Discussion

Kinetics of hydrolysis

The kinetics of hydrolytic breakdown of the various 5-fluorouracil N-acyloxymethyl derivatives (Fig. 1) was studied in aqueous solution at 37°C over a wide range of pH values. Under the experimental conditions used the reactions displayed strict first-order kinetics for several half-lives and in all runs 5-fluorouracil was formed in stoichiometric amounts as evidenced by HPLC analysis. In some cases ($8.5 < pH < 11$) the rate of a given reaction was determined using both the direct UV-spectrophotometric method and the HPLC method and the values of the observed rate constants obtained therefrom agreed within $\pm 3\%$.

The rates of hydrolysis of the derivatives were subject to a slight catalysis by phosphate buffers whereas variation of the concentration (0.02–0.05 M) of the acetate, borate and carbonate buffers at a given pH produced no significant change in the rate constants. The buffer-independent pseudo-first-order rate constants (k) were determined by extrapolation of linear plots of the observed pseudo-first-order rate constants (k_{obs}) vs total phosphate concentration to zero concentration.

The influence of pH on the overall rates of degradation of the derivatives I–VI is shown in Fig. 3 where the logarithm of k is plotted against pH. The effect of pH

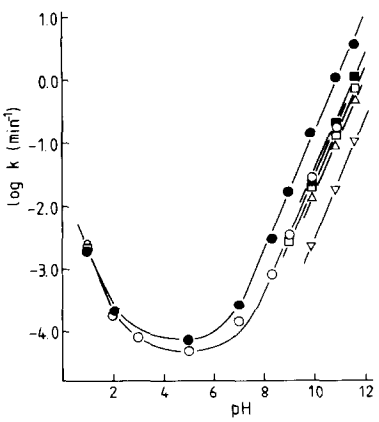


Fig. 3. The pH–rate profiles for the hydrolysis of N-acyloxymethyl-5-fluorouracil derivatives in aqueous solution ($\mu = 0.5$) at 37°C. Key: ○, I; □, II; △, III; ▽, IV; ●, V; ■, VI.

upon the hydrolysis of the 1-acetoxymethyl (I) and 1,3-bis(acetoxymethyl) (V) derivatives was examined in detail over the pH range 1–12. As seen from Fig. 3 the pH–rate profiles for these derivatives are U-shaped, indicating the occurrence of specific acid and base catalysis as well as a spontaneous or water-catalyzed reaction according to the following rate expression:

$$k = k_o + k_H a_H + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. The latter was calculated from the measured pH at 37°C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.62 \quad (2)$$

Values of the second-order rate constants for the specific acid (k_H) and specific base (k_{OH}) catalyzed hydrolysis were determined from the straight line portions of the pH–rate profiles at low and high pH values, respectively, whereas values of the first-order rate constant for spontaneous hydrolysis (k_o) were obtained on basis of Eqn. 1. The values of the rate constants derived are listed in Table 2. In Fig. 3 the solid curves or lines drawn were constructed from these constants and Eqn. 1.

The 1-acyloxymethyl derivatives I–IV are weak acids due to ionization of the 3-NH group, the pK_a values at 22°C and $\mu = 0.5$ being 7.3 (cf. Table 5). It is interesting to note that the neutral and anionic forms of the compounds appear to exhibit almost the same reactivity as evidenced by the lack of a significant curvature in the pH–rate profile for the 1-acetoxymethyl derivative at pH values near to the pK_a value. It has previously been found (Buur and Bundgaard, 1985) that in 1-carbamoyl derivatives ($pK_a \sim 6.7$) the neutral forms are considerably more reactive than the anionic species as is also the case for 1-alkoxycarbonyloxymethyl 5-fluorouracil derivatives (unpublished findings).

The hydrolysis of the 1-acyloxymethyl derivatives most likely takes place via a two-step reaction as depicted in Scheme 1. Rate-determining cleavage of the ester grouping results in the formation of 1-hydroxymethyl-5-fluorouracil which is decom-

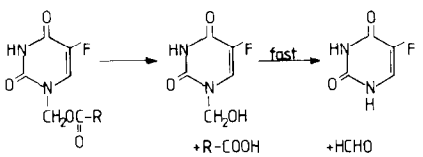
TABLE 2

RATE DATA FOR THE HYDROLYSIS OF VARIOUS N-ACYLOXYMETHYL-5-FLUOROURACIL DERIVATIVES IN AQUEOUS SOLUTION AT 37°C AND $\mu = 0.5$

Compound	k_H ($M^{-1} \cdot min^{-1}$)	k_o (min^{-1})	k_{OH} ($M^{-1} \cdot min^{-1}$)
I	0.020	4.97×10^{-5}	130
II	0.020	–	105
III	–	–	70
IV	–	–	12
V	0.020	6.58×10^{-5}	760
VI	–	–	125
VII	–	–	40

posed instantaneously into formaldehyde and 5-fluorouracil in accord with the behaviour of other similar N-hydroxymethyl derivatives (Johansen and Bundgaard, 1979, 1981; Bundgaard and Johansen, 1980, 1984; Bansal et al., 1981; Varia et al., 1984b). The suggested mechanism of hydrolysis is supported by several findings: (i) the rates of formation of formaldehyde upon degradation of 1-butyryloxymethyl-5-fluorouracil (III) in a pH 7.4 buffer or in plasma solutions are identical to those of formation of 5-fluorouracil and to those of 1-acyloxymethyl derivative disappearance (Møllgaard et al., 1982; Johansen et al., 1983); (ii) from the structure–reactivity relationship previously established (Bundgaard and Johansen, 1980) for the rate of hydrolysis of various N-hydroxymethyl derivatives of e.g. amides, imides and hydantoins it can be estimated that the half-life of hydrolysis of the proposed intermediate 1-hydroxymethyl-5-fluorouracil (Scheme 1) at pH 7.4 and 37°C may be

Scheme 1.



only about 0.4 s, using a pK_a value of 8.0 for the 1-NH group in 5-fluorouracil (Berens and Shugar, 1963); and (iii) no induction period in the formation of formaldehyde or 5-fluorouracil was observed in the above-mentioned studies. However, careful monitoring of the hydrolysis in basic solution by UV-spectrophotometry at 300 nm showed consistently an initial, albeit small, rapid decrease in absorption followed by a slower, first-order kinetically increase in absorption due to liberation of anionic 5-fluorouracil (Fig. 4). This absorbance change may be taken as

Fig. 4.

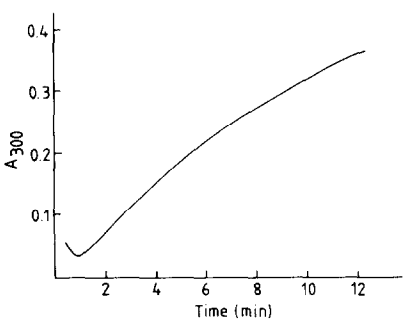


Fig. 5.

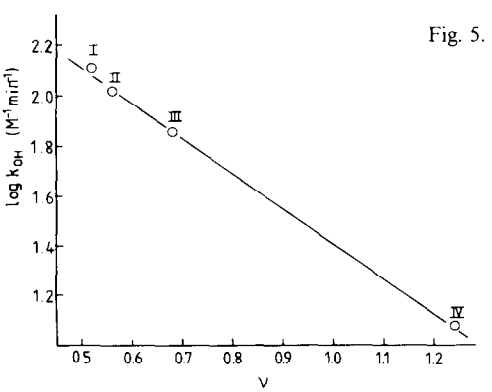


Fig. 4. UV-spectral changes at 300 nm following the degradation of compound III in aqueous solution at pH 10.85 and 37°C.

Fig. 5. Plot of log k_{OH} vs the steric parameter (ν) for various 1-acyloxymethyl-5-fluorouracil derivatives. The ν values refer to the alkyl moiety in the acyl groups, i.e. methyl, ethyl, propyl and tertiary butyl.

evidence for the existence of an intermediate in the reaction pathway.

Thus, the rate of 5-fluorouracil formation from the derivatives is solely dependent on the rate of the initial ester cleavage, which can be controlled by steric and electronic factors. The influence of the steric effect within the acyl substituents can be illustrated by the 1-acyloxymethyl derivatives I–IV. The polar effects of the acyl groups in these compounds are almost identical and the differences in reactivity in neutral and alkaline aqueous solution can solely be ascribed to differences in the steric properties as shown in Fig. 5, where log k_{OH} is plotted against Charton's steric parameter ν (Charton, 1977). The regression equation for the plot is given by Eqn. 3:

$$\log k_{OH} = -1.41(\pm 0.04)\nu + 2.82(\pm 0.03) \qquad (n = 4;\ r = 0.999) \qquad (3)$$

The hydrolysis of the 1,3-diacyloxymethylated derivatives V and VI to 5-fluorouracil is expected to proceed through the intermediate formation of the corresponding 1- and 3-monoacyloxymethyl derivatives, the overall rate of degradation being described by Eqn. 1, cf. the pH–rate profile for V in Fig. 3. The hydrolytic breakdown of derivative V at pH 9.90 was examined in detail using HPLC analysis. With a mobile phase system consisting of 25% v/v methanol in 0.01 M acetate buffer of pH 5.0 it was possible to monitor the disappearance of V and the time-course of hydrolysis products. In Fig. 6 are shown typical chromatograms of the reaction solution at various reaction times. The disappearance of V was found to

Fig. 6.

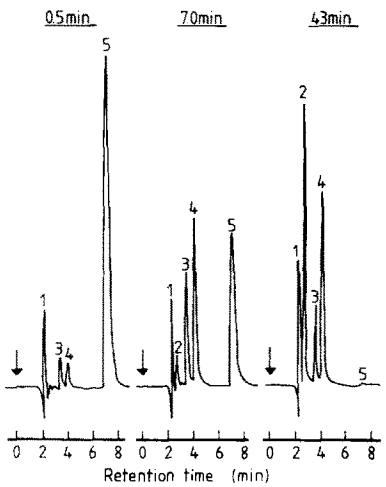


Fig. 6. HPLC chromatograms of a reaction solution of compound V of pH 9.90. Key: 1, solvent front; 2, 5-fluorouracil; 3, compound I; 4, compound VII; 5, compound V.

Fig. 7.

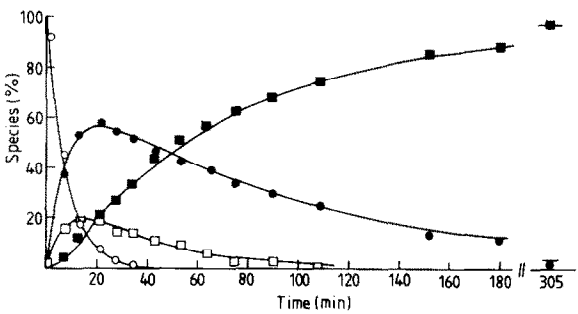
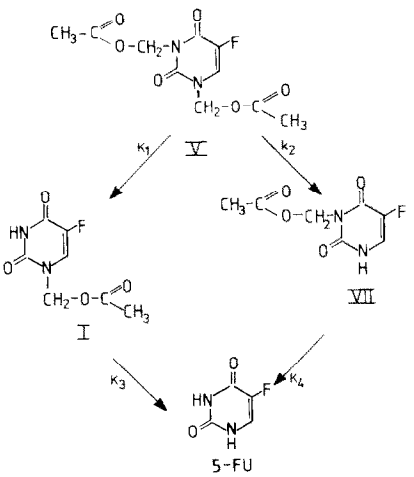


Fig. 7. Time-courses for compound I (□), compound V (○), compound VII (●) and 5-fluorouracil (■) in the degradation of compound V at pH 9.90 and 37°C. The concentrations at various times, expressed as percent of the initial V concentration were determined by HPLC (mobile phase: methanol–0.01 M acetate pH 5.0 (1 : 3 v/v)). The full curves are constructed from Eqns. 4–7.

be accompanied by the formation of 1-acetoxymethyl-5-fluorouracil (I), identified on the basis of its HPLC retention behaviour in comparison with that of authentic I, and of a compound (peak 4 in Fig. 6) which was isolated and determined to be 3-acetoxymethyl-5-fluorouracil (VII) as described in the experimental section. Ozaki et al. (1984) have previously shown that both a 1-acyloxymethyl derivative and the corresponding 3-acyloxymethyl derivative are formed upon alkaline hydrolysis of a 1,3-bis(acyloxymethyl)-5-fluorouracil derivative (1,3-bis(benzoyloxymethyl)-5-fluorouracil). Following their formation these products slowly degraded into 5-fluorouracil. This final product of hydrolysis was formed in quantitative amounts as evidenced by HPLC analysis of completed reaction solutions. The time-courses of the various species are shown in Fig. 7 and the proposed hydrolysis reactions taking place are depicted in Scheme 2, where k_1–k_4 are pseudo-first-order rate constants

Scheme 2.



for the depicted reactions. According to this scheme the time dependences of the concentrations of I and the products of hydrolysis are given by the following expressions:

$$V_t = V_o \cdot e^{-Kt} \tag{4}$$

$$I_t = V_o\left[\frac{k_1}{k_3 - K} \cdot e^{-Kt} + \frac{k_1}{K - k_3} \cdot e^{-k_3 t}\right] \tag{5}$$

$$VII_t = V_o\left[\frac{k_2}{k_4 - K} \cdot e^{-Kt} + \frac{k_2}{K - k_4} \cdot e^{-k_4 t}\right] \tag{6}$$

$$5\text{-}FU_t = V_o - (V_t + I_t + VII_t) \tag{7}$$

where V_o is the initial concentration of V and $K = k_1 + k_2$. The rate constant K was

TABLE 3

APPARENT FIRST-ORDER RATE CONSTANTS (in min^{-1}) FOR THE PROCESSES DEPICTED IN SCHEME 2 IN AQUEOUS SOLUTION AT pH 9.90 AND IN 80% HUMAN PLASMA SOLUTIONS AT 37°C

Rate constant	Buffer solution of pH 9.90	80% Human plasma (pH 7.4)
k_1	0.039	0.035
k_2	0.092	0.010
k_3	0.030	0.00077
k_4	0.008	0.0038

determined from a first-order plot of V vs time and k_3 and k_4 from first-order plots of the peak heights due to I and VII, respectively, vs time at reaction times where V has completely degraded (i.e. $t > 40$ min). The remaining rate constants (k_1 and k_2) were determined in the following way. When all V had disappeared Eqn. 5 is reduced to:

$$I_t/V_o = \frac{k_1}{K - k_3} \cdot e^{-k_3 t} \tag{8}$$

A plot of the logarithm of the percentage concentration of I against time produced a straight line as expressed by Eqn. 8. Extrapolation of the line to $t = 0$ gave an intercept from which k_1 was derived:

$$k_1 = (K - k_3) \cdot 100 \text{ (antilog intercept)} \tag{9}$$

Then, k_2 was obtained from the identity: $k_2 = K - k_1$. After a final curve-fitting the values of the various rate constants shown in Table 3 were derived (at pH 9.90 and 37°C).

The full curves drawn in Fig. 7 were constructed on the basis of these values and Eqns. 4–7 and the good agreement observed between the calculated and experimental data demonstrates that Scheme 2 adequately describes the kinetics of hydrolysis of the 1,3-bis(acetoxymethyl)-5-fluorouracil derivative V. It should further be added that the values of the rate constants k_3 and k_4 derived agreed within 5% with the values obtained by following in separate experiments the rates of hydrolysis of compounds I and VII directly at the same reaction conditions.

Considering the values of the rate constants k_1–k_4 it is interesting to note that the 1-acyloxymethyl derivative is almost 4 times as susceptible to undergo alkaline hydrolysis as the corresponding 3-acyloxymethyl derivative. This parallels the hydrolytic behaviour of V in that the hydrolysis of the N_1-ester grouping (k_2) is 2.4 times as fast as that of the N_3-ester (k_1).

Enzymatic hydrolysis of the derivatives

The susceptibility of the N-acyloxymethyl derivatives to undergo a potential enzymatic hydrolysis was studied in vitro at 37°C in 0.01 M phosphate buffer

TABLE 4

HALF-LIVES FOR THE HYDROLYSIS OF THE N-ACYLOXYMETHYL DERIVATIVES OF 5-FLUOROURACIL (I–VII) IN AQUEOUS BUFFER OF pH 7.40, 80% HUMAN PLASMA AND 20% RAT LIVER HOMOGENATE AT 37°C

Compound	Half lives (h)		
	Buffer pH 7.40	80% Human plasma	20% rat liver homogenate
I	70	14.0	< 0.01
II	90 [a]	9.6	0.02
III	140 [a]	2.3	0.02
IV	700 [a]	40	0.40
V	20	0.29	< 0.01
VI	60 [a]	1.1	< 0.01
VII	–	3.0	–

[a] Estimated from the rate data obtained at pH > 8.

solutions (pH 7.40) containing 80% human plasma or in the supernatant fraction (diluted to 20% with the pH 7.4 buffer) of rat liver homogenate. The hydrolysis of the derivatives I–VII followed strict first-order kinetics for several half-lives and proceeded in all cases to give 5-fluorouracil in quantitative amounts. The observed half-lives for the hydrolysis in the biological media are listed in Table 4 along with the half-lives of hydrolysis in pure buffer solutions of pH 7.40. As appears from the rate data both plasma and rat liver enzymes markedly accelerate the rate of hydrolysis. The plasma-catalyzed hydrolysis is seen to increase with increasing alkyl chain length in the derivatives I–III. The low reactivity of compound IV may be ascribed to steric hindrance exhibited by the bulky pivaloyl group. The degradation course of the 1,3-bis(acetoxymethyl) derivative (V) in 80% human plasma was studied in a similar way as that described above for the run performed in aqueous

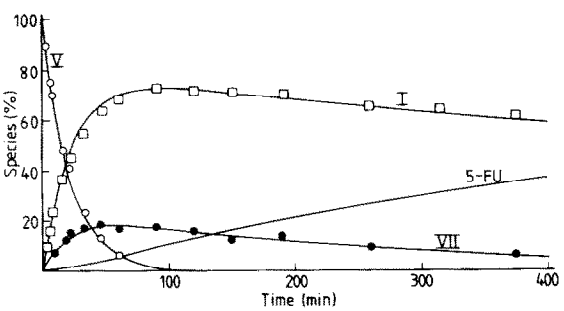


Fig. 8. Time-courses for compound I, V, VII and 5-fluorouracil in the degradation of compound V in 80% human plasma (pH 7.4) at 37°C. The concentrations at various times, expressed as percent of the initial V concentration, were determined by HPLC (mobile phase: methanol–0.01 M acetate pH 5.0 (1 : 9 v/v)). The full curves are constructed from Eqns. 4–7. At the completion of the reaction 5-fluorouracil was found to be formed in 100% yield.

solution at pH 9.90 and found to be as depicted in Scheme 2. Fig. 8 shows the time-courses of the various products and the values of the rate constants k_1–k_4 derived are listed in Table 3. Quite opposite to what is the case for alkaline hydrolysis the enzymatic hydrolysis of 3-acetoxymethyl-5-fluorouracil (VII) is more facile than that of the 1-acetoxymethyl derivative, the half-lives in 80% plasma being 3 and 14 h, respectively. Similarly, the enzyme-catalyzed hydrolysis of the N_3-ester grouping in V (k_1) is more facile than that of the N_1-ester grouping (k_2).

Lipophilicity and aqueous solubility of the N-acyloxymethyl derivatives and other 5-fluorouracil prodrugs

Partition coefficients (P) for the the N-acyloxymethyl derivatives as determined using an octanol–aqueous buffer system (pH 4.0) are listed in Table 5 along with melting points and solubilities in water at pH 4.0. The results obtained show that the derivatives are all more lipophilic than the parent 5-fluorouracil. The difference in

TABLE 5

MELTING POINTS, PARTITION COEFFICIENTS (P), AQUEOUS SOLUBILITIES (S) AND pK_a VALUES OF 5-FLUOROURACIL AND VARIOUS PRODRUG DERIVATIVES

Compound	m.p. °C	log P [a]	S [b] (mg ml^{-1})	pK_a
5-Fluorouracil (5-FU) [c]	280–284	−0.83	11.1	8.0, 13.0
1-Acetoxymethyl-5-FU (I)	122–123	−0.67	43.1	7.3
1-Propionyloxymethyl-5-FU (II)	100–102	−0.11	33.6	7.3
1-Butyryloxymethyl-5-FU (III)	92–93	0.47	9.6	7.3
1-Pivaloyloxymethyl-5-FU (IV)	158–160	0.90	2.3	7.3
1,3-Bis(acetoxymethyl)-5-FU (V)	105–106	−0.37	4.3	–
1,3-Bis(pivaloyloxymethyl)-5-FU (VI)	102–104	2.54	0.045	–
3-Acetoxymethyl-5-FU (VII)	158–159	−0.42	20.0	8.0
1-Acetoxymethyl-3-benzoyl-5-FU (VIIa) [d]	127–128	0.92	0.14	–
1-Butyryloxymethyl-3-benzoyl-5-FU (VIII) [d]	81–82	1.91	0.062	–
3-Acetyl-5-FU (IX) [c]	115–116	−0.34	42.8	7.1 [g]
3-Propionyl-5-FU (X) [c]	113–114	0.19	35.3	7.2 [g]
3-Benzoyl-5-FU (XI) [c]	169–170	0.80	1.3	6.9 [g]
3-Ethyloxycarbonyl-5-FU (XII) [e]	126–128	0.11	72.0	8.6
3-Phenyloxycarbonyl-5-FU (XIII) [e]	169–170	1.73	0.15	6.6
1-Methylcarbamoyl-5-FU (XIV) [f]	225–228	−0.20	0.62	6.7
1-Ethylcarbamoyl-5-FU (XV) [f]	190–196	0.35	1.5	6.7
1-Butylcarbamoyl-5-FU (XVI) [f]	136	1.44	0.82	6.8
1-Dimethylcarbamoyl-5-FU (XVII) [f]	226–227	−0.37	6.0	6.7

[a] Determined in octanol–acetate buffer of pH 4.0 at 22°C.
[b] Solubility in acetate buffer of pH 4.0 at 22°C.
[c] From Buur and Bundgaard (1984a).
[d] Unpublished data.
[e] From Buur and Bundgaard (1984b).
[f] from Buur and Bundgaard (1985).
[g] These values are at 37°C, the other pK_a values being determined at 22°C ($\mu = 0.5$).

the log P values for the derivatives I–IV is as expected on the basis of the π substituent values (Hansch and Leo, 1979). Despite the increased lipophilicity some of the derivatives (I, II and VII) possess also a higher aqueous solubility relative to 5-fluorouracil (Table 5). As discussed previously (Bansal et al., 1971; Buur and Bundgaard, 1984a) the relatively poor solubility of 5-fluorouracil and uracil itself in water and other solvents like octanol may largely be a result of the high crystal lattice energy in the molecules due to intermolecular hydrogen bonds formed between NH-protons in one molecule and a carbonyl group in another molecule. Consequently, disruption or decrease of such hydrogen bonding by replacement of the N-1 or N-3 protons by acyloxymethyl or other groups should lead to derivatives with decreased crystal lattice energy (as manifested in a melting point decrease, cf. Table 5) and thus, with higher solubilities. If the N_1- or N_3-substituents introduced into 5-fluorouracil are relatively non-lipophilic as such, e.g. an acetoxymethyl group, the net result may be both enhanced water solubility and an increased octanol–water partition coefficient. That crystal lattice energy and thus melting points play a major role in the relationship between aqueous solubility and octanol–water partition coefficients of crystalline solutes is well recognized (Valvani and Yalkowsky, 1980; Yalkowsky and Valvani, 1980; Yalkowsky, 1981; Yalkowsky et al., 1983). As shown by these authors the relationship between the aqueous solubility (S, in molar concentration) and octanol–water partition coefficient of crystalline organic compounds contains a term for melting point:

$$\log S = -\underline{a} \cdot \log P - \underline{b} \cdot \text{m.p.} + \underline{c} \qquad (10)$$

where $\underline{a}$, $\underline{b}$ and $\underline{c}$ are constants which may vary somewhat for different types of chemical structures, $\underline{a}$ usually being around unity and $\underline{b}$ around 0.01. It is thus seen

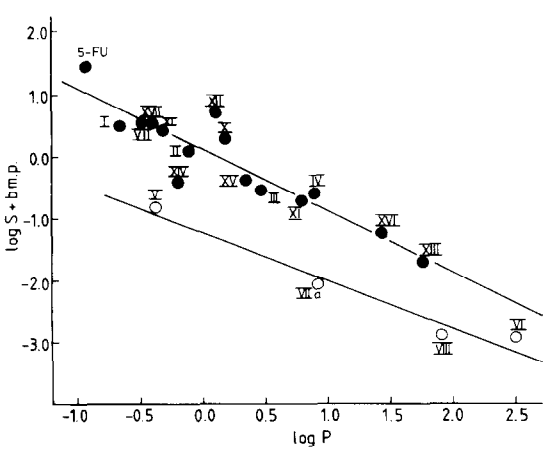


Fig. 9. Plots showing the relationships between melting point, aqueous solubility (S) and octanol–aqueous buffer (pH 4.0) partition coefficient (P) for various derivatives of 5-fluorouracil. The numbers refer to the derivatives listed in Table 5. For the monosubstituted derivatives (upper line) a 'b' value of 0.009 was used; for the disubstituted derivatives (lower line) a 'b' value of 0.010 was used.

that a 100°C decrease in melting point may result in approximately a 10-fold higher aqueous solubility for the same log P value.

In addition to the presently described N-acyloxymethyl derivatives Table 5 contains data for solubility, partition coefficient and melting point of various other potential 5-fluorouracil prodrugs described previously (Buur and Bundgaard, 1984a, 1984b, 1985). Multiple-regression analysis of this series of 5-fluorouracil derivatives, covering a wide variety of melting points, partition coefficients and aqueous solubilities, yielded the relationships shown in Fig. 9. As can be seen the coefficients a and b in Eqn. 10 for the N-monosubstituted derivatives are significantly different from those for the N_1, N_3-disubstituted derivatives. The regression equations obtained are given in Eqn. 11 (monosubstituted derivatives) and Eqn. 12 (disubstituted derivatives):

$$\log S = -0.98(\pm 0.13) \log P - 0.009(\pm 0.002)\text{m.p.} + 0.12(\pm 0.33) \quad (11)$$

$(n = 15;\ r = 0.914)$

$$\log S = -0.77(\pm 0.17) \log P - 0.010(\pm 0.011)\text{m.p.} - 1.23(\pm 1.27) \quad (12)$$

$(n = 4;\ r = 0.975)$

It is seen that the coefficient for the log P term is close to -1 and that for the melting point term close to 0.01, the theoretical values (Valvani and Yalkowsky, 1980). These relationships may be valuable for the prediction of aqueous solubilities of 5-fluorouracil prodrug derivatives from a knowledge of the melting point and partition coefficient, the latter being generally estimated a priori from the group contribution approach (Hansch and Leo, 1979; Chou and Jurs, 1980; Yalkowsky and Morozowich, 1980).

It should be noted that the log P and S values given in Table 4 refer to the undissociated forms of the derivatives. Several of the compounds are weak acids with pK_a values around 7 (Table 5) and therefore, the water-solubilities will be somewhat higher and the partition coefficients somewhat lower at physiological pH of 7.4 as has also actually been confirmed experimentally for some of the compounds (Buur and Bundgaard, 1984a). The values at e.g. pH 7.4 can be calculated from the following relationships:

$$\log P_{pH} = \log P + \log(a_H/(a_H + K_a)) \quad (13)$$

$$\log S_{pH} = \log S + \log((a_H + K_a)/a_H) \quad (14)$$

where P and S refer to the properties of the undissociated species as given in Table 5.

N-Acyloxymethyl derivatives as potential prodrug candidates for 5-fluorouracil

The results of the present study suggest that N-acyloxymethylation is a potentially useful approach to obtain prodrugs of 5-fluorouracil. The usefulness of this approach which in the past has also been applied to various other NH-acidic drugs

(Bodor, 1979, 1981; Sloan and Bodor, 1982; Sloan et al., 1983; Pitman, 1981; Bundgaard, 1982; Varia et al., 1984a and b) stems from the fact that by varying the acyl portion of the derivatives it is possible to control the rate of regeneration of the drug and to obtain prodrugs with varying physicochemical properties of primary importance for drug delivery such as water-solubility or lipophilicity. For 5-fluorouracil, N-acyloxymethylation provides derivatives with a much reduced melting point relative to that of the parent drug and as shown above this enables the preparation of prodrugs possessing both higher partition coefficients and higher or only slightly reduced aqueous solubilities. Whereas the derivatives show good stability in aqueous solution at pH 4–6, they are subject to marked enzyme-mediated hydrolysis. Thus, whereas the half-life of the 1-butyryloxymethyl derivative is about 6 days in phosphate buffer of pH 7.4 at 37°C it is only 2.3 h in 80% human plasma. Compared with other similar N-acyloxymethyl derivatives such as those of theophylline (Johansen et al., 1983) and phenytoin (Yamaoka et al., 1983), however, the 5-fluorouracil acyloxymethyl derivatives are not particularly susceptible to undergoing hydrolysis in the presence of plasma. Apparently, although not surprising, the nature of the NH-acidic drug can have a profound effect upon the reactivity. It should be added that the rates of hydrolysis as measured in vitro in plasma solutions may most likely be lower than those in full blood or those attained in vivo as has been shown for O-acyloxymethyl derivatives of ampicillin (Daehne et al., 1970, 1971), cf. also the very strong catalysis by liver enzymes described above.

Some 1-acyloxymethyl derivatives of 5-fluorouracil have recently been shown to possess strong antitumor activity in mice (Hoshi et al., 1982). In view of the present results this activity may certainly be due to 5-fluorouracil formed upon enzymatic hydrolysis in vivo. In a previous paper, we have shown the utility of 1-butyryloxymethyl-5-fluorouracil to enhance the topical delivery of the parent drug (Møllgaard et al., 1982). The derivative permeated more readily through human skin in vitro than 5-fluorouracil and at the same time was delivered in the form of parent drug due to highly efficient cutaneous metabolism as mediated by hydrolytic skin enzymes. The potential utility of the N-acyloxymethyl prodrugs to improve the oral and rectal delivery of 5-fluorouracil is under current examination.

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