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Prodrugs of 5-fluorouracil. I. Hydrolysis kinetics and physicochemical properties of various N-acyl derivatives of 5-fluorouracil

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Summary

The kinetics and mechanism of decomposition of nine N_1-acyl, N_3-acyl and N_1,N_3-diacyl derivatives of 5-fluorouracil in aqueous solution and in human plasma at 37 °C were studied to assess their suitability as prodrugs for the parent compound. All the derivatives hydrolyzed to yield 5-fluorouracil in quantitative amounts. N_1-Deacylation proceeded much faster than N_3-deacylation and showed a pH-dependence which could be ascribed to water-catalyzed and hydroxide ion-catalyzed reactions. The hydrolysis of the N_1,N_3-diacyl derivatives passed through the corresponding N_3-acyl compound. The pH–rate profiles obtained for the N_3-deacylation were accounted for in terms of spontaneous and hydroxide ion-catalyzed hydrolysis of undissociated N_3-acyl derivative ($pK_a \sim 7$) as well as hydroxide ion-catalyzed hydrolysis of anionic derivative. The rate of N_3-deacylation was accelerated in human plasma, the half-life of hydrolysis in 80% plasma solutions at 37 °C being 4.6, 20, 28 and 110 min for the N_3-acetyl, N_3-propionyl, N_3-butyryl and N_3-benzoyl derivatives, respectively. The N_3-acyl derivatives were more lipophilic than 5-fluorouracil as determined by partition experiments in octanol–aqueous buffer systems but their aqueous solubilities were even greater or only slightly reduced as compared with 5-fluorouracil. This behaviour was attributed to a decreased intermolecular hydrogen bonding in the crystal lattice achieved by blocking the 3-NH group by the acylation and manifested in a pronounced melting point decrease.

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The results suggested that N_3-acylation may be a promising means of obtaining prodrug forms of 5-fluorouracil with the aim of enhancing the delivery characteristics of the drug, e.g. by prodrugs exhibiting increased lipophilicity and/or affording protection against first-pass metabolism.

Introduction

5-Fluorouracil is one of the most widely used cytotoxic drugs in the palliative treatment of solid tumors in many organs. Although it has been used in clinical practice for more than 20 years and still remains an important antitumor agent it has a severe drawback in that it shows serious side-effects such as gastrointestinal toxicity and possesses properties (e.g. low lipophilicity) giving rise to various delivery problems. The bioavailability of 5-fluorouracil following oral administration is incomplete and shows a very high interindividual variability (Cohen et al., 1974; Christophidis et al., 1978; Finch et al., 1979; Fraile et al., 1980; Philips et al., 1980). This poor systemic bioavailability following oral administration, which may largely be due to a first-pass metabolism in the gastrointestinal tract and the liver (Christophidis et al., 1978; Collins et al., 1980; Almersjö et al., 1980), makes the oral route too unpredictable to be of clinical value (Myers, 1981; Chlebowski et al., 1981). The rectal route of administration appears to be of even less value than the oral one in that no detectable plasma levels of 5-fluorouracil were seen after giving the drug to four patients in the form of a rectal enema (Christophidis et al., 1978).

Several efforts have been made to overcome these delivery problems as well as to reduce the toxic side-effects by chemical modification of 5-fluorouracil. A particularly promising approach to improve the delivery characteristics of 5-fluorouracil may be development of transient derivatives (prodrugs) with enhanced physicochemical properties in terms of delivery from the site of administration to the site of action within the body. Several different derivatives have been described and tested as such masked forms of 5-fluorouracil, e.g. tetrahydro-2-furanyl derivatives (Yasumoto et al., 1978), N-acyl derivatives (Hoshi et al., 1974; Tada, 1975; Okada, 1979; Kametani et al., 1980), N-alkoxycarbonyl derivatives (Kametani et al., 1980; Yamashita et al., 1982), N-carbamoyl derivatives (Ozaki et al., 1977; Iigo et al., 1980; Kobari et al., 1981; Takada et al., 1983), special N-alkylated derivatives (Kundo and Schnitz, 1982), N-acyloxymethyl derivatives (Ozaki et al., 1981; Hoshi et al., 1982; Møllgaard et al., 1973) and various O-substituted compounds (Yamashita et al., 1982). However, only very sparse information is available on the converting efficiency of these derivatives to the parent 5-fluorouracil in vitro or in vivo, their bioavailability as well as on their physicochemical properties of relevance to bioavailability, such as lipophilicity and aqueous solubility.

Studies were undertaken to provide such information with the ultimate goal of obtaining prodrugs of 5-fluorouracil with improved delivery characteristics. In this paper the kinetics of conversion of a series of N-acyl derivatives of 5-fluorouracil (I–IX; Fig. 1) to the parent drug in aqueous solution and plasma solutions is

reported along with determinations of the lipophilicity and aqueous solubility of some of the prodrug candidates.

Materials and Methods

Apparatus

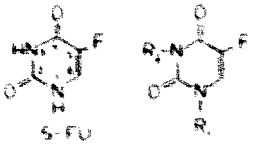
Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostatically controlled cell compartment, using 1-cm quartz cells. ^{1}H-NMR spectra were run on a JEOL C-60-HL instrument. Readings of pH were carried out on a Radiometer Type PHM26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was done with a system consisting of a Waters pump model 6000A, 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, Darmstadt).

Materials

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

Preparation of 5-fluorouracil N-acyl derivatives

The N_1,N_3-diacylated derivatives VI–VIII were prepared by reacting 5-fluorouracil with the appropriate acid chloride in a mixture of dioxane and triethylamine as described by Kametani et al. (1980). N_1-Acetyl-5-fluorouracil (V) was prepared by refluxing 5-fluorouracil in a mixture of acetic anhydride and pyridine as described by Hoffer (1962). N_1-Acetyl-N_3-propionyl-5-fluorouracil (IX) was made from com-



5-FU

I	R_1 = H	R_3 = $COCH_3$
II	R_1 = H	R_3 = $COCH_2CH_3$
III	R_1 = H	R_3 = $COCH_2CH_2CH_3$
IV	R_1 = H	R_3 = COC_6H_5
V	R_1 = $COCH_3$	R_3 = H
VI	R_1 = $COCH_3$	R_3 = $COCH_3$
VII	R_1 = $COCH_2CH_2CH_3$	R_3 = $COCH_2CH_2CH_3$
VIII	R_1 = COC_6H_5	R_3 = COC_6H_5
IX	R_1 = $COCH_3$	R_3 = $COCH_2CH_3$

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

pound V as described by Kametani et al. (1980) as was N_3-benzoyl-5-fluorouracil (IV). The N_3-acylated derivatives I–III were prepared by selective N_1-deacylation of the corresponding N_1,N_3-diacylated compounds: about 300 mg of these compounds were dissolved in 10–30 ml of ethanol and 1 ml of 1 M hydrochloric acid was added. Upon standing for 3–4 h at room temperature the N_1-deacylation was completed as revealed from UV spectral measurements on aliquots of the reaction mixtures. One ml of 1 M sodium hydroxide was added and the mixtures evaporated in vacuo. Extraction of the resulting residues with chloroform, followed by evaporation of the extracts, gave I–III which were finally recrystallized from chloroform–petroleum ether. The compounds prepared had spectroscopic properties (UV, NMR) in agreement with their structure and melting points agreeing with those reported (Kametani et al., 1980; Hoffer, 1962). The derivatives III and VII had not been reported before, their melting points being 132–134°C (III) and 47.5–48.5°C (VII) (recrystallized from petroleum ether).

Compound III: ^{1}H-NMR ($CDCl_3$) δ 0.65–0.95 (m, 3H, $C\underline{H}_3$), 1.4–1.8 (m, 2H, $C\underline{H}_2$), 3.00 (t, 3H, $C\underline{H}_2CO$ and $N\underline{H}$), 8.20 (d, 1H, C_6–$\underline{H}$). UV λ_{max} 268 nm (pH 2), 300 nm (pH 8 and 10).

Compound VII: ^{1}H-NMR ($CDCl_3$) δ 0.98 (t, 6H, $2C\underline{H}_3$), 1.50–1.90 (m, 4H, 2 $C\underline{H}_2$), 2.67–3.15 (m, 4H, 2 $C\underline{H}_2CO$), 8.32 (d, 1H, C_6–$\underline{H}$). UV λ_{max} 265 nm (pH 2), unstable (pH > 7).

Kinetic measurements

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

For some reactions the hydrolysis was followed spectrophotometrically by recording absorbance changes at a wavelength where the absorptions of substrate and products differed maximally: 300 nm (I–IV, absorbance decrease), 300 nm (V–IX, at pH about > 7, absorbance increase) and 266 nm (V–IX, at pH < 7, absorbance decrease). Reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 10–25 μl of stock solutions of the derivatives in acetonitrile or methanol to give a final concentration of 1.5×10^{-4} M. Pseudo-first-order rate constants were determined from linear plots of $\log(A_\infty - A_t)$ or $\log(A_t - A_\infty)$ vs time, where A_∞ and A_t are the absorbance readings at infinity and at time t, respectively, or by the method of Guggenheim (1926).

The rates of hydrolysis of the N_3-acyl derivatives I–IV were also followed by using a reversed-phase HPLC procedure. Solvent systems of 20 or 45% v/v methanol in 0.01 M acetate buffer pH 5.0 were used. The flow rate was 1.2 or 1.6 ml · min^{-1} and the column effluent was monitored at 266 nm. Under these conditions the 3-acyl derivatives had elution times of 3–4 min while 5-fluorouracil eluted with the solvent front. Quantitation of the compounds was done 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 (about 2 mg · ml^{-1} in acetonitrile or methanol) to 10 ml of pre-heated buffer solution in screw-capped test tubes. The solutions were kept in a water-bath at 37 °C and at appropriate intervals 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.

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

The hydrolysis of the derivatives I–IV was also studied in 0.05 M phosphate buffer pH 7.40 containing 80% human plasma (at 37 °C). The initial concentrations of the compounds were about 0.01 mg · ml^{-1}. At appropriate intervals 200 μl samples were withdrawn and added to 1000 μl of ethanol in order to deproteinize the plasma. After mixing and centrifugation for 2 min, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Measurement of partition coefficients

The apparent partition coefficients (P) of the various N_3-acyl derivatives were determined in 1-octanol aqueous buffer systems. The aqueous phase was either a 0.02 M acetate buffer of pH 4.0 or a 0.02 M phosphate buffer of pH 7.4. The buffer solutions and octanol were mutually saturated at 20–25 °C before use. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could be measured by the aforementioned HPLC method. The partition coefficients were calculated from Eqn. 1:

$$P = \left(\frac{C_i - C_w}{C_w} \right) \frac{V_w}{V_0} \qquad (1)$$

where C_i and C_w represent the concentrations in the aqueous buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase and V_0 the volume of the octanol phase.

Determination of aqueous solubility

The solubility of some of the derivatives were determined in 0.05 M acetate buffer solutions (pH 4.0) at 22 °C. An excess amount of the compounds was added to the buffer solutions and the mixtures were placed in an ultrasonic water-bath for about 30 min and then rotated on a mechanical spindle for 1 h. It was ensured that solubility equilibrium was reached by this procedure. Upon filtration an aliquot of the filtrate was diluted with an appropriate amount of water and the mixture analyzed by HPLC. The concentration of the compounds in the saturated solutions was calculated from the measured peak heights by reference to those of standards chromatographed under the same conditions.

Determination of pK_a values

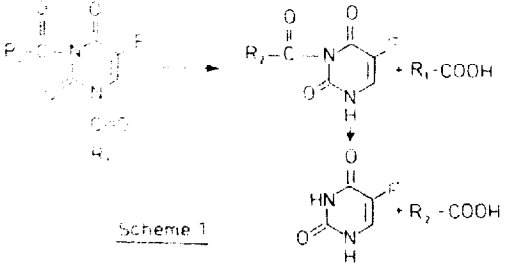
The ionization constants for the N_3-acyl derivatives I–IV were determined at 37 °C and $\mu = 0.5$ by spectrophotometry according to Albert and Serjeant (1971).

Upon dissociation of the 1-NH proton the UV-spectrum of the derivatives changed profoundly, λ_{max} shifting from 268 nm to 300 nm (I–III) or from 258 to 292 nm (IV). The wavelength used for the determination of the pK_a values was 300 nm for I–III and 292 nm for compound IV. 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. Clean isosbestic points were observed at 282 nm (I–III) and at 280 nm (IV).

Results and Discussion

Kinetics of hydrolysis

The kinetics of hydrolysis of the N-acyl 5-fluorouracil derivatives I–IX was studied in aqueous solution at 37 °C over a wide range of pH. The derivatives studied included various N_3-monoacylated compounds (I–IV), a N_1-monoacylated compound (V) and a number of N_1,N_3-diacylated derivatives (VI–IX) (Fig. 1). The end product arising from the hydrolytic decomposition of these derivatives was found to be 5-fluorouracil. It was formed in stoichiometric amounts at all pH values studied (pH 1–13) as evidenced by HPLC analysis of completed reaction solutions. Whereas the hydrolysis of the N_1- and N_3-monoacylated derivatives proceeded simply to give 5-fluorouracil the hydrolysis of the N_1,N_3-diacylated derivatives was found to take place as depicted in Scheme 1. The derivatives degraded initially to give the corresponding N_3-acylated compounds which subsequently at a slower step hydrolyzed to 5-fluorouracil. UV-spectral and HPLC analysis of reaction solutions of the compounds VI–IX showed that the corresponding N_3-acylated derivatives were formed in quantitative amounts, taking into account the slower hydrolysis of the derivatives once formed. The degradation course in basic solutions could conveniently be followed spectrophotometrically at 300 nm, the λ_{max} for the anionic forms of the N_3-acylated derivatives. As seen from the example shown in Fig. 2 an initial rapid rise in absorption at 300 nm due to the N_3-acylated species (I) occurred upon decomposition of compound VI, followed by a slower decrease in absorbance. The UV-spectrum of the reaction solution taken after 0.5 min was identical to that of compound I and, furthermore, the rate of hydrolysis of the intermediate formed corresponded exactly to that determined separately with an authentic sample of I under similar reaction conditions.



Scheme 1

At constant pH and temperature all reactions were shown to display strict first-order kinetics for several half-lives (cf. Fig. 3). In cases where the hydrolysis was followed using both direct UV-spectrophotometry and HPLC the rate constants obtained therefrom agreed within 5%.

The rates of hydrolysis were subject to catalysis by most of the buffer substances used to maintain constant pH. Plots of the observed pseudo-first-order rate constants (k_{obs}) at each pH value against the total buffer concentration were linear. From the intercepts of such plots values of the buffer-independent first-order rate constant (k) were obtained.

The influence of pH on the rates of hydrolysis of N_1-acetyl-5-fluorouracil (V) and of the N_1-deacylation of the diacylated derivatives VI–IX is shown in Fig. 4 where

Fig. 2.

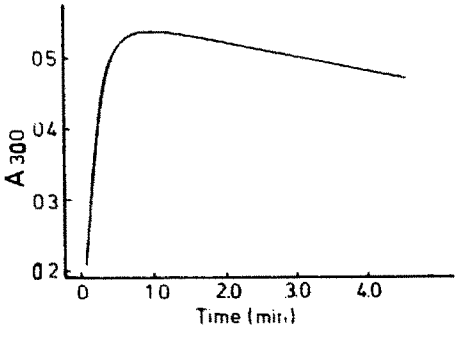


Fig. 3.

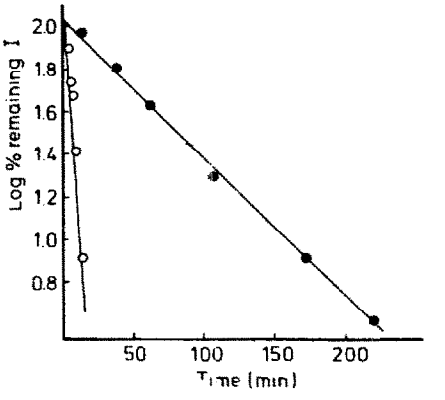


Fig. 2. Absorbance changes at 300 nm accompanying the hydrolysis of N_1,N_3-diacetyl-5-fluorouracil (VI) in a 0.05 M borate buffer solution of pH 9.00 at 37 °C.

Fig. 3. First-order plots for the hydrolysis of N_3-acetyl-5-fluorouracil (I) in 0.025 M phosphate buffer solution, pH 7.40 (●), and in 80% human plasma solution (○) at 37 °C. The residual concentrations of I were determined by the HPLC procedure described in the text.

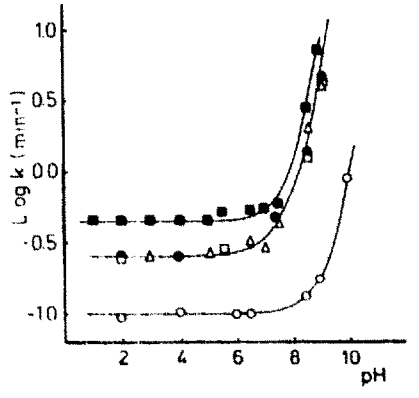


Fig. 4. The pH–rate profiles for the N_1-deacylation of various N-acyl derivatives of 5-fluorouracil (5-FU) in aqueous solution at 37 °C. ○, N_1-acetyl-5-FU (V); ●, N_1,N_3-diacetyl-5-FU (VI); △, N_1,N_3-dibutyryl-5-FU (VII); ■, N_1,N_3-dibenzoyl-5-FU (VIII); □, N_1-acetyl-N_3-propionyl-5-FU (IX).

log k is plotted against pH. At acidic and neutral pH values the rate is independent of pH whereas in alkaline solution the pH–rate profiles show straight lines with slopes of unity, revealing a first-order dependence in hydroxide ion. This rate–pH relationship indicates that the N_1-deacylation can be described in terms of a water-catalyzed or spontaneous reaction and a specific base-catalyzed reaction according to the following rate expression:

$$k = k_0 + k_{OH} a_{OH} \quad (2)$$

where a_{OH} refers to the hydroxide ion activity. This was calculated from the measured pH at 37 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.62 \quad (3)$$

Values of the second-order rate constants for the specific base-catalyzed hydrolysis (k_{OH}) and of those for spontaneous hydrolysis (k_0) of the compounds studied were obtained on basis of Eqn. 2 and are listed in Table 1.

The results obtained show that the N_1-deacylation is a facile process, the half-times being only about 1–7 min at 37 °C and pH 1–7. It is also apparent that the mechanism for the N_1-deacylation is not influenced by the presence of an acyl group on the N_3-atom (cf. the similar shape of the pH–rate profiles for the compounds V and VI). On the other hand, N_3-acylation shows a significant influence on the lability of the N_1-acyl moiety toward hydrolysis. Thus, by comparing the rate data for the compounds V, VI and IX, it can be seen that the rate of removal of the N_1-acetyl group on diacyl derivatives is 3–50 times higher (dependent on pH) than when no N_3-acyl groups are present.

Fig. 5 shows the pH–rate profiles for the hydrolysis (i.e. N_3-deacylation) of the N_3-acylated 5-fluorouracil derivatives I–IV. At pH > 6 the pH–rate profiles show two linear segments with slopes of unity with a plateauing occurring between pH 8 and 10; at low pH the rate of hydrolysis becomes independent of pH. The derivatives I–IV are weak acids due to dissociation of the N_1-hydrogen atom, the pK_a values being about 7 (see later). The shape of the pH–rate profiles indicates

TABLE 1

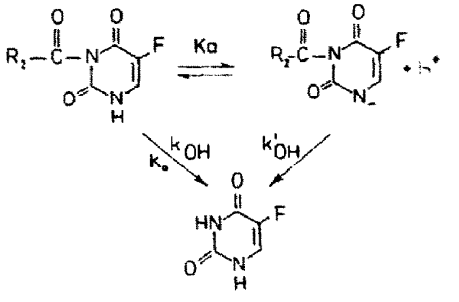
RATE CONSTANTS FOR THE N_1-DEACYLATION OF THE DERIVATIVES V–IX IN AQUEOUS SOLUTION ($\mu = 0.5$) at 37 °C

Compound	$k_{OH} \times 10^{-4}$ ($M^{-1} \cdot min^{-1}$)	k_0 (min^{-1})
V	0.36	0.10
VI	20.0	0.25
VII	20.0	0.25
VIII	26.3	0.45
IX	20.0	0.25

that the undissociated and the anionic forms of the derivatives undergo hydrolysis with different rates and that the overall hydrolysis can be described in terms of specific base-catalyzed reactions of these species along with a spontaneous (pH-independent) or water-catalyzed reaction of the undissociated form (Scheme 2):

$$k = k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (4)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of the compounds in the



Scheme 2

undissociated and anionic form, respectively, and K_a is the apparent ionization constant of the compounds. The rate constant k_0 refers to the pH-independent

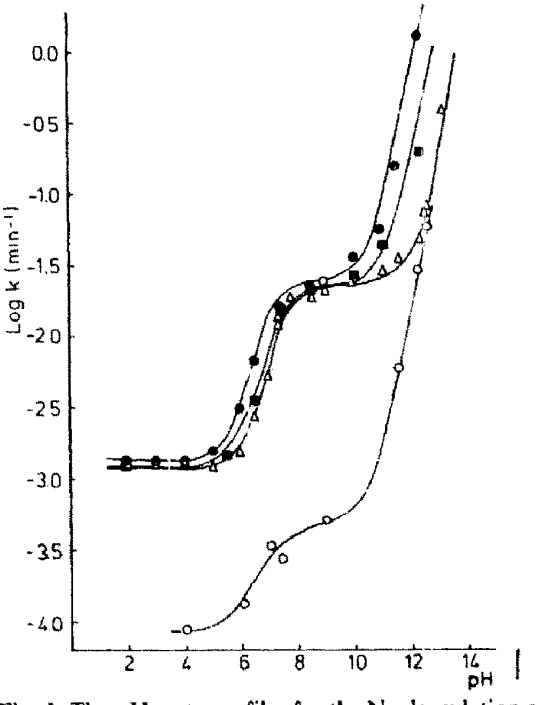
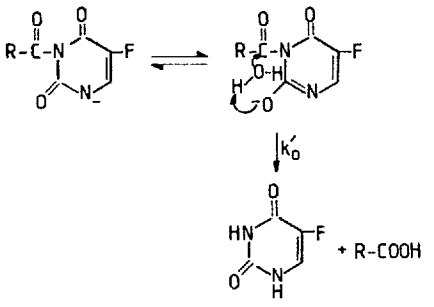


Fig. 5. The pH–rate profiles for the N_3-deacylation of various N_3-acyl derivatives of 5-fluorouracil (5-FU) in aqueous solution at 37 °C. ●, N_3-acetyl-5-FU (I); ■, N_3-propionyl-5-FU (II); △, N_3-butyryl-5-FU (III); ○, N_3-benzoyl-5-FU (IV).



Scheme 3

hydrolysis of the undissociated form (equal to k at pH 2–4) while k_{OH} and k'_{OH} are the second-order rate constants for the hydroxide ion-catalyzed hydrolysis of the undissociated and anionic species, respectively. Values of the latter constants were determined from the straight line portions of the pH–rate profiles and those of K_a on basis of Eqn. 4. The various rate and dissociation constants derived are listed in Table 2. In Fig. 5 the solid curves drawn were constructed from these constants and Eqn. 4 and the good agreement observed between the calculated and experimental data demonstrates that Eqn. 4 and, accordingly, Scheme 2 adequately describes the N_3-deacylation kinetics. As appears from Table 2 the kinetically derived pK_a values agreed satisfactorily with those determined by spectrophotometry. Concerning the k_{OH}-reaction it should be noted, however, that it is not possible on the basis of the present data to distinguish it from the kinetically equivalent reaction involving water-catalyzed hydrolysis of the anionic species. Such a reaction can be envisioned to take place via an intramolecular general base-catalyzed mechanism as depicted in Scheme 3. The rate constant for this alternative reaction (k'_0) is related to k_{OH} by the following equation: $k'_0 = k_{OH}k_w/K_a$ where K_w is the ionization constant of water.

Considering the structural factors influencing the stability of the N_3-acylated derivatives it may be noted that the N_3-benzoyl derivative is considerably more stable than those containing an aliphatic N_3-acyl group in neutral and weakly basic solution. The reactivity of the latter derivatives differ as expected only slightly.

TABLE 2

IONIZATION CONSTANTS AND RATE DATA FOR THE HYDROLYSIS OF VARIOUS N_3-ACYL 5-FLUOROURACIL DERIVATIVES ($\mu = 0.5$; 37 °C)

Compound	k_0 (min^{-1})	k_{OH} ($M^{-1} \cdot min^{-1}$)	k'_{OH} ($M^{-1} \cdot min^{-1}$)	pK_a
N_3-acetyl-5-fluorouracil (I)	1.32×10^{-3}	1.06×10^5	19.5	7.0 [a]; 7.1 [b]
N_3-propionyl-5-fluorouracil (II)	1.20×10^{-3}	5.73×10^4	7.2	7.2 [a]; 7.2 [b]
N_3-butyryl-5-fluorouracil (III)	1.23×10^{-3}	4.97×10^4	1.1	7.3 [a]; 7.2 [b]
N_3-benzoyl-5-fluorouracil (IV)	8.91×10^{-5}	3.00×10^3	0.56	6.8 [a]; 6.9 [b]

[a] Kinetically determined value.

[b] Determined by UV-spectrophotometry at 37 °C ($\mu = 0.5$).

By comparing the rate data obtained for the N_1-deacylation (Table 1) and N_3-deacylation (Table 2) it is evident that the former process occurs much more easily. Thus, by considering N_1-acetyl-5-fluorouracil (V) and N_3-acetyl-5-fluorouracil (I) it can be calculated that the rate of N_1-deacetylation exceeds that of N_3-deacetylation by factors of 180 at pH > 10, 6 at pH 7.4 and 80 at pH < 5. For the diacetylated derivative (VI) the difference is even greater. This difference in lability toward hydrolysis, which has been recognized before and formed the basis for the synthesis of N_3-acyl derivatives from diacyl derivatives (Kametani et al., 1980), may most likely be ascribed to the difference in the leaving ability of the 5-fluorouracil moiety of the derivatives. The pK_a values for 5-fluorouracil are 8.0 (1-NH) and about 13 (3-NH) (Berens and Shugar, 1963), thus indicating the better leaving ability of the N_1-anion.

Hydrolysis in plasma

For the evaluation of the N-acyl derivatives as being potential prodrugs of 5-fluorouracil it is important to ascertain whether plasma enzymes would be able to catalyze the conversion of the derivatives to the parent drug. Therefore, the hydrolysis of the N_3-acyl derivatives I–IV was studied in 80% human plasma (pH 7.4) at 37 °C and compared to that occurring in pure aqueous buffer solution. Due to their great lability in aqueous solution the N_1-acylated derivatives were not included in the investigation.

Under the given reaction conditions strict first-order kinetics (cf. Fig. 3) were observed and the reactions proceeded to give 5-fluorouracil in quantitative amounts. As appears from the rate data obtained (Table 3), plasma accelerated the rate of hydrolysis of the derivatives although to a varying extent. The benzoyl derivative (IV) showed the highest rate acceleration while the acetyl derivative (I) showed the most rapid hydrolysis, the half-life in 80% plasma being only 4.6 min. The rate of the enzymatic hydrolysis is seen to decrease with increasing alkyl chain length and the difference in the case of enzymatic hydrolysis of the compounds I–III can be accounted for in terms of the steric effects of the acyl groups.

Lipophilicity and aqueous solubility of the N-acyl derivatives

Partition coefficients (P) for the N_3-acyl 5-fluorouracil derivatives as determined

TABLE 3

RATE DATA FOR THE HYDROLYSIS OF N_3-ACYL DERIVATIVES OF 5-FLUOROURACIL IN 0.05 M PHOSPHATE BUFFER OF pH 7.40 AND IN 80% HUMAN PLASMA AT 37 °C

Compound	Buffer		80% plasma		k_{obs} (plasma) / k_{obs} (buffer)
	k_{obs} (min^{-1})	$t_{1/2}$ (min)	k_{obs} (min^{-1})	$t_{1/2}$ (min)	
I	1.6×10^{-2}	43	0.15	4.6	9.3
II	1.4×10^{-2}	50	3.4×10^{-2}	20	2.4
III	1.2×10^{-2}	58	2.5×10^{-2}	28	2.1
IV	2.5×10^{-4}	2.9×10^{3}	6.4×10^{-3}	110	25

using the widely used 1-octanol–water system are listed in Table 4 along with data for the aqueous solubility. Since the compounds are partly ionized at pH 7.4 the log P values determined using a pH 7.4 buffer as the aqueous phase (apparent partition coefficients, P_{app}) are lower than those determined using a pH 4 buffer. The log P values determined at the latter pH value represent true partition coefficients since the compounds are totally unionized at pH 4. The values actually determined at pH 7.4 were found to be in good agreement with those calculated on basis of the true log P values and the relationship:

$$\log P_{app} = \log P + \log(a_H/(a_H + K_a)). \quad (5)$$

The results obtained show that the derivatives are all more lipophilic than the parent 5-fluorouracil. The increase in log P on going from the acetyl derivative to the propionyl derivative and from this to the butyryl derivative amounts to 0.53 and 0.48, respectively, which agrees with the *Π* value of 0.5 for a methylene group (Hansch and Leo, 1979).

The lipophilicity of the derivatives was also evaluated by means of reversed-phase HPLC (e.g. Brent et al., 1983; Hafkenscheid and Tomlinson, 1983). In this method the capacity factor (k′) of a solute is taken as a measure for the relative lipophilicity:

$$k' = (t_r - t_0)/t_0 \quad (6)$$

where t_r is the retention time of the solute and t_0 is the elution time of the solvent. With methanol–acetate buffer pH 5.0 (20 : 80 v/v) as mobile phase the derivatives I–IV showed the k′ values given in Table 4. These data also demonstrate the higher lipophilicity of the derivatives in comparison with 5-fluorouracil. As has been observed for other compounds (Hafkenscheid and Tomlinson, 1983; and references cited therein) a linear relationship existed between log k′ and log P (at pH 4.0) for

TABLE 4

PARTITION COEFFICIENTS (P) (OCTANOL/BUFFER), CAPACITY FACTORS (k′) AND AQUEOUS SOLUBILITIES (S) OF 5-FLUOROURACIL AND VARIOUS N_1-ACYL DERIVATIVES AT 22 °C

Compound	log P [a]		k′	S (mg/ml) pH 4.0
	pH 4.0	pH 7.4		
5-Fluorouracil	−0.83	−0.96	< 0.1	11.1
I	−0.34	−0.68	0.64	42.8
II	0.19	−0.21	1.46	35.3
III	0.67		3.59	
IV	0.80	0.16	4.42	1.3

[a] The values determined using the pH 4.0 buffer are true partition coefficients whereas those obtained at pH 7.4 are apparent partition coefficients due to partial ionization of the compounds at this pH.

compounds I–IV:

$$\log k' = 0.75\ (\pm 0.02)\ \log P + 0.04\ (\pm 0.01)\ \ (n = 4;\ r = 0.999) \qquad (7)$$

where n and *r* are number of compounds and correlation coefficient, respectively.

An increase in lipophilicity is generally accompanied by a decrease in water solubility. Inspection of the data in Table 4 reveals, however, that the aqueous solubility of the derivatives I and II is in fact increased in comparison to that of 5-fluorouracil despite the much higher log P values of the compounds. As discussed by Bansal et al. (1981) the relatively poor water solubility of uracil (3 mg · ml^{-1}) is largely a result of the high crystal lattice energy in the molecule due to intermolecular hydrogen bonds formed between NH-protons in one molecule and a carbonyl group in another molecule. Disruption or decrease of such hydrogen bonding by replacement of the N-1 or N-3 protons in uracil by methyl groups results in greatly increased solubility (Bansal et al., 1981). The behaviour of 5-fluorouracil and its N_3-acyl derivatives may be similarly explained. Support for the suggestion that replacement of the N-3 proton in 5-fluorouracil by acyl groups results in a decreased crystal lattice energy comes from comparison of the melting point of 5-fluorouracil (280–284°C) with those of the N_3-acyl derivatives (I: 116–118°C; II: 113–114°C; III: 132–134°C; IV: 172–174°C). That 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 et al., 1983). According to 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:

$$\log S = -a \log P - 0.01\ \text{m.p.} + b \qquad (8)$$

where a and b are constants which may vary somewhat for different types of chemical structures. In Fig. 6 the solubility and partition data for 5-fluorouracil and the N_3-acyl derivatives I, II and IV have been plotted according to this equation. The

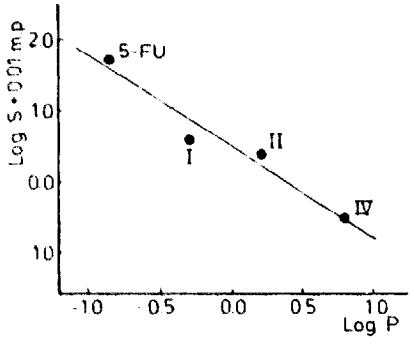


Fig. 6. Plot of the solubility and partition data for 5-fluorouracil and the derivatives I, II and IV according to Eqn. 8.

following relationship was derived from the plot:

$$\log S = -1.29(\pm 0.26)\log P - 0.01\ \text{m.p.} + 0.47(\pm 0.16)\ \ (n = 4;\ r = 0.962) \qquad (9)$$

where S is the molar solubility in aqueous buffer of pH 4.0 and P is the partition coefficient between octanol and aqueous buffer of pH 4.0. This relationship might be of value for the prediction of the aqueous solubility of various N_3-acyl derivatives from a knowledge of the melting point and partition coefficient, the latter being predictable from the present data and Π substituent values.

Consideration of N-acyl derivatives of 5-fluorouracil as prodrugs

The results of the present study show that N-acylation may be a potentially useful approach to obtain prodrug forms of 5-fluorouracil. The derivatives examined undergo a quantitative conversion to the parent 5-fluorouracil in aqueous solution with appreciable rates at physiological conditions of pH and temperature.

Due to their high lability N_1-acyl derivatives appear less promising as prodrugs than N_3-acyl derivatives. Thus, the half-lives of hydrolysis of the derivatives V, VI, VII, VIII and IX are only 6.9, 2.7, 2.7, 2.7 and 1.6 min, respectively, at pH 7.4 and 37 °C and only slightly more at acidic pH values. Therefore, degradation in the gastrointestinal tract may certainly be of such an extent that their oral absorption behaviour would not differ from that of 5-fluorouracil. From a number of N-acyl derivatives N_1-acetyl-N_3-*o*-toluyl-5-fluorouracil has been selected as a promising antitumor agent (Okada, 1979; Kametani et al., 1980). However, the great lability of the N_1-acetyl group does not appear to have been recognized and the effects observed might certainly have been due to the N_3-*o*-toluyl derivative formed upon N_1-deacetylation.

In contrast to the N_1-acyl derivatives, the N_3-acylated derivatives possess stabilities which make them suitable prodrug candidates. The hydrolysis of these N-imido acylated compounds is catalyzed by human plasma and it should be feasible to obtain N_3-acylated prodrugs with a varying degree of lability in vivo by appropriate selection of the acyl group. The above-mentioned N_1-acetyl-N_3-*o*-toluyl derivative has been given orally to mice (Kametani et al., 1980). Analysis of blood samples showed the in vivo formation of 5-fluorouracil but most of the compound was present in the blood in the form of N_3-*o*-toluyl-5-fluorouracil. The bulky *o*-toluyl group in this derivative may certainly hinder a facile enzymatic hydrolysis and based on the rate data presented here, an aliphatic acyl moiety should be preferred in order to obtain a quantitative conversion to the active parent drug in vivo.

Besides cleavage rates, such physicochemical properties as aqueous solubility and lipophilicity can be modified by appropriate selection of the acyl group in N_3-acylated derivatives. As has been demonstrated it is possible to choose an N_3-acylated derivative which is both more lipophilic than 5-fluorouracil and possesses a higher water-solubility. Therefore, in conclusion, N_3-acylation of 5-fluorouracil appears to be a potentially useful means to obtain prodrug forms with better delivery properties, e.g. increased lipophilicity and maintenance of adequate water-solubility, than the parent drug. Studies are in progress to determine the delivery characteristics of some of the derivatives.

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