The stability of trifluorothymidine: hydrolysis in buffered aqueous solutions

M. F. JONES

Pharmacy Department, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, U.K.

The decomposition of trifluorothymidine in aqueous solution, and borate and phosphate buffers has been studied using an h.p.l.c. method. In aqueous solution accelerated studies demonstrate that a storage life, to 10% loss, of more than 30 years at 4 °C is predicted, but steam sterilization causes extensive breakdown. Considerable differences occur in the kinetics and routes of decomposition in borate and phosphate buffers in the pH range 4–8. Hydroxide ion attack producing 5-carboxy-2'-deoxyuridine is suppressed in borate buffers. Complex kinetics of decomposition in phosphate buffer are found and possible explanations suggested. Steam sterilization in buffered solution also leads to unacceptable breakdown.

Trifluorothymidine (5-trifluoromethyl-2-'deoxyuridine, F_3TdR) as well as showing early promise in the control of human neoplasias (Heidelberger et al 1964) has shown promise as an inhibitor of ocular herpetic keratitis, being superior to idoxuridine (5-iodo-2'-deoxyuridine) in this treatment (Wellings et al 1972). Idoxuridine is not stable and its major decomposition product, 5-iodouracil, is toxic at low concentration and inhibits the activity of the parent compound (Maloney & Kaufman 1963). The kinetics of idoxuridine breakdown (Garrett et al 1964, 1966) together with the use of F_3TdR as an ingredient of eyedrop formulations for ocular herpes simplex infection management has led to interest in the stability of the latter compound. Garrett et al (1966) showed that the pyrimidine deoxynucleosides were hydrolysed in acid solution to the respective sugar and pyrimidine, and at pH values closer to neutrality (3–7) this reaction has been shown to be independent of pH and of buffer composition (Shapiro & Kang 1969). At higher pH values the reaction rate decreases sharply. Superimposed on this degradation mechanism in the case of trifluorothymidine however is the hydroxide ion attack on the trifluoromethyl group investigated by Nestler & Garrett (1968). This proceeds via a difluorohydroxy and hypothetical monofluorocarbonyl intermediates before yielding 5-carboxy-2'-deoxyuridine (5-COOH-dR). The kinetics of this reaction in phosphate buffered 0·9% NaCl (saline) at pH values between 7 and 8 were measured by Clough et al (1978) using u.v. spectrophotometry and found to be first order and hydroxide ion catalysed. They also found that 5-COOH-dR had no antiviral activity against types 1 and 2 herpes simplex.

Lee (1979) used an h.p.l.c. method to study the decomposition of F_3TdR in aqueous solution and using the Arrhenius regression predicted storage lives at 4 ° and 20 °C from the first order reactions observed.

The present study attempts to define the conditions of pH control that would optimize the stability of F_3TdR in a pharmaceutically acceptable eyedrop formulation. The detailed kinetics of the degradation have been studied using an ion-pairing h.p.l.c. analytical method and the contrasting behaviour of different buffer constituents examined.

MATERIALS AND METHODS

Trifluorothymidine was supplied by International Enzymes Ltd. Other chemicals were of Analar quality unless otherwise specified.

H.p.l.c. assay

Sample solution (500 μl) was mixed with an internal standard solution (10·0 ml) containing 0·8 mg ml^{-1} of paracetamol B.P. in methanol and diluted to 50 ml with methanol. Samples (5μl) of the resulting mixture were chromatographed by direct injection onto a column (200 mm × 5 mm i.d.) of Spherisorb ODS (5μm). The eluting solvent was water–methanol (95:5) containing 0·1% w/v sodium lauryl sulphate (Biochemical reagent) and 0·3% v/v sulphuric acid, at a flow rate of 0·8 ml min^{-1}. Detection was normally by u.v. absorption at 265 nm. The instrument used was a Altex Model 110 reciprocating pump with a Cecil Instruments CE212 ultraviolet monitor. For quantitative studies the detector response was integrated using a Pye Unicam DP88 computing

integrator. Alternating injections of the initial solution which had been stored at 4 °C and treated similarly with internal standard were made. Peak area ratio calibration graphs for F_3TdR over the concentration range studied were linear.

F_3TdR is well resolved from the composite peak of 5-COOH-dR and 5-carboxyuracil (5-COOH-U, from hydroxide ion catalysed hydrolysis of trifluorom ethyluracil) and from trifluoromethyluracil itself which is the product of the acid-catalysed solvolysis of the glycoside bond of F_3 TdR (Fig. 1). The retention time of the paracetamol internal standard under these conditions is 11 min.
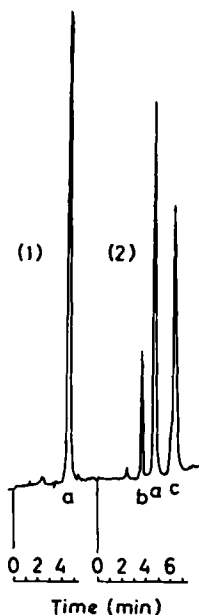

Fig. 1. Chromatograms of trifluorothymidine 4 °C sample (1), and a synthetic mixture with its decomposition products (2). Peaks a = F_3TdR, b = 5-COOH-dR + 5-COOH-U, c = F_3TMU. For conditions see text.

Thin layer chromatography

Aliquots (20 μl) of sample solutions were applied directly to silica gel 60$_{F_{254}}$ pre-coated t.l.c. plates (E Merck & Co, Darmstadt, supplied by BDH Chemicals Ltd) which were developed in either chloroform–propan-2-ol (1:1) (System 1), which allowed the rapid resolution of F_3TdR from trifluoromethyluracil and 2-deoxyribose but left strongly polar decomposition products as base-line zones or butan-2-ol–water–acetic acid (4:1:1) (System 2) which accomplished the separation of the more polar decomposition products formed by trifluoromethyl group attack.

Visualization by u.v. irradiation at 254 nm followed by spraying with sulphuric acid and heating for 5 min at 100 °C allowed discrimination between molecules having the sugar moiety present (charring after H_2SO_4 treatment) and the pyrimidine nucleus (u.v. absorption), or both.

Kinetic experiments

A 1% w/v solution of F_3TdR in either water or the requisite buffer system was distributed into ampoules, sealed, and stored either at room temperature (20 °C) or in incubators equilibrated at the required elevated temperature. Decomposition in water at 80 °C was in a thermostatically controlled water-bath. The phosphate buffer solutions were mixtures of 0·066 M disodium monohydrogen phosphate and sodium dihydrogen phosphate solutions, relative quantities being based on the phosphate vehicle of the United States Pharmacopeia, (USP XIX, p. 703). The borate buffer solution was that of Palitzsch, described in Britton (1955).

RESULTS

Hydrolysis in aqueous solution

Using h.p.l.c. assay, comparison of the decomposition occurring in ampoules sealed under nitrogen with that in ampoules sealed under oxygen, both stored at 60 °C, confirmed that no oxidative degradation occurred. Pseudo-first order kinetics were observed under all temperature conditions tested, the reactions being followed for at least two half-lives. Table 1 shows the results obtained.

Table 1. First order rate constants for the hydrolysis of F_3TdR in water.

Temperature °C	k(days^{-1})	Standard error of k (days^{-1})
50	0·0321	0·0008
60	0·1423	—
70	0·5330	0·0135
80	2·323	0·0663

The Arrhenius regression plot of these data is shown in Fig. 2. Extrapolation to 20 ° and 4 °C give k values of 1·882 × 10^{-4} and 7·828 × 10^{-6} days^{-1} respectively, corresponding to $t_{0.9}$ storage lives of 569 days (95% confidence limits 494–644) and 37·8 years (31·0–44·7) at these temperatures. The steep temperature dependence that this represents (ΔH_A = 32·1 kcal mol^{-1}, 134 kJmol^{-1}) and the pre-exponential factor (log$_{10}$A = 15·264) are in good agreement with the values of Garrett et al (1966).
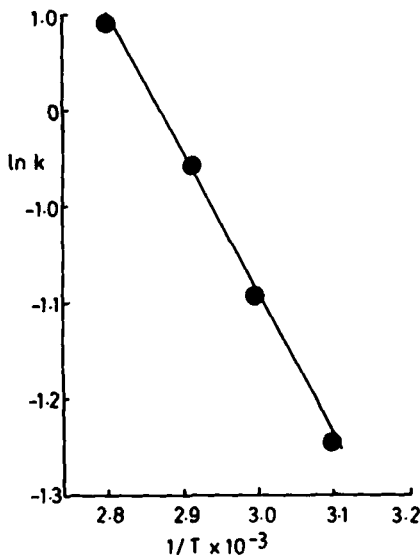
FIG. 2. Arrhenius plot for the decomposition of tri-
fluorothymidine in water solution.

T.l.c. and h.p.l.c. confirmed that the only major
products of decomposition were trifluoromethyl-
uracil (F_3TMU) and 2-deoxyribose from the H_2O-
mediated solvolysis of the glycosidic bond.

Hydrolysis in borate buffers

Table 2 shows the observed pseudo-first order rate
constants obtained in borate buffers over the pH
range 5·7–7·85 at 60 °C. In all cases except that of
pH 7·85 the first order regressions were linear from
zero reaction. At pH 7·85 the linear regression was
preceded by a short period of rapid rate increase.
T.l.c. examination of the samples decomposed for 8
days showed that F_3TMU and 2-deoxyribose were
the principal degradation products, except in the
case of pH 7·85 where a small proportion of 5-
carboxyuracil (5-COOH-U) was present presumably
from hydroxide ion attack on F_3TMU.

Hydrolysis in phosphate buffers

In contrast to the borate buffer experiments simple
first order kinetics were not observed at 60 °C (over
the pH range 3–8·05) except at pH 3·0. Divergence
from linearity increased with increasing pH. Fig. 3
shows the kinetic data obtained at 0·066 M buffer
concentration.

At pH 3·0 t.l.c. showed that F_3TMU and 2-
deoxyribose were the only major decomposition
products, in marked contrast to the situation at
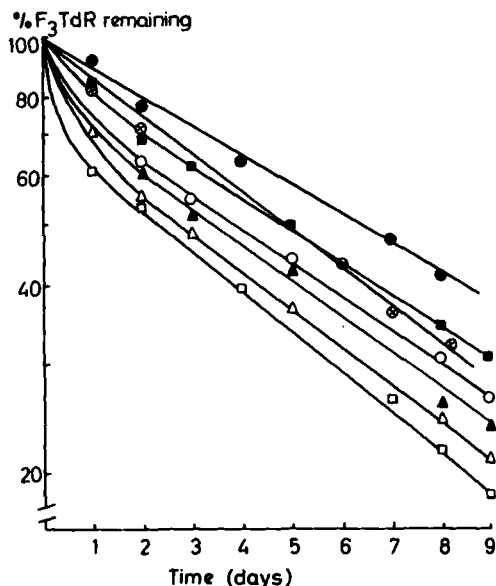higher pH where the production of 5-COOH-dR and

FIG. 3. Kinetics of the decomposition of trifluoro-
thymidine in 0·066 M phosphate buffers at 60 °C. ⊗ =
pH 3·0, ● = pH 4·35, ■ = 5·80, ○ = pH 6·35, ▲ =
pH 6·90, △ = pH 7·35, □ = pH 8·05.

5-COOH-U predominate, together with a smaller
proportion of an unknown product (R_F 0·51 in
System 2) containing both base ring and sugar moiety.

Steaming experiments

To assess the effect of a free-steaming sterilizing cycle
(102 °C for 35 min) on eyedrop solutions buffered at
pH 6·90 with either the borate or 0·066 M phosphate
buffers, the sterilized solutions were analysed by
h.p.l.c. for F_3TdR content, and F_3TMU content (the
latter by use of a calibration graph prepared by using
authentic F_3TMU under the same conditions). The
results are compared to those obtained for a simple
water solution in Table 4 and highlight the differences
in decomposition route between the buffers.

DISCUSSION

In aqueous solution the decomposition of F_3TdR
proceeds via the hydrolysis of the glycoside bond as
described previously, presumably by the A-1
mechanism of Shapiro & Kang (1969). The steep
temperature dependence of the degradation, while
allowing reasonable shelf lives in terms of F_3TdR
loss, precludes any possibility of steam sterilization
of a simple aqueous eyedrop formulation.

Earlier reports have stated that in pyrimidine
nucleoside hydrolysis no difference between borate
and phosphate buffer systems is found (Nestler &

Table 2. First order rate constants for the hydrolysis of F_3TdR in boric acid/borax buffer systems. Temperature $= 60\,°C$.

pH*	k (days^{-1})	Standard error of k (days^{-1})
5·70	0·1065	0·0033
6·20	0·1013	0·0029
6·70	0·1118	0·0017
7·20	0·1131	0·0029
7·85	0·1306†	0·0049

* Measured on final solution, at room temperature (20 °C) uncorrected.
† Linear portion of regression only.

Garrett 1968; Shapiro & Kang 1969). However, analysis was by changes in u.v. spectrum, and Shapiro & Kang studied only thymidine itself.

In borate buffer systems over the pH range 5·7–7·1 an essentially pH independent first order solvolysis of glycoside occurs (Table 2). The expected hydroxide ion catalysed hydrolysis to 5-COOH-dR is almost entirely suppressed.
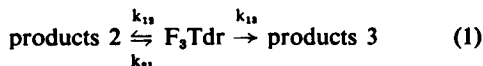
In phosphate buffer systems, however, over a similar pH range, F_3TdR is decomposed with complex kinetics as shown in Fig. 3. At pH 3·0 first order degradation, wholly glycoside hydrolysis, occurs. At higher pH 5-COOH-dR production almost entirely supplants this, with some contribution from a reaction leading to the unknown R_F 0·51 product.

These results are at variance with those of Clough et al (1978) who found first order kinetics in phosphate-buffered saline at 37 °C, but who used the relatively unselective u.v. absorption technique as assay method.

A computer program, which by means of a reiterative least squares procedure was able to fit the observed data to the non-linear regressions corresponding to various model complex reaction schemes was used.

Table 3. Results of free-steaming sterilization of buffered and aqueous solutions of F_3TdR. (Yields of F_3TMU are expressed as % conversion of F_3TdR.) pH = 6·90.

Vehicle	% F_3TdR remaining	F_3TMU (% F_3TdR converted)	Others (Including 5-COOH-dR)
Borate buffer, pH 6·90	47·8	44·3	7·9
Phosphate buffer, pH 6·90	37·5	28·1	34·4
Water	41·9	52·0	6·1

$$\text{products 2} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} F_3Tdr \overset{k_{13}}{\rightarrow} \text{products 3} \qquad (1)$$

Assuming a combination of pH dependence (rate constants linearly related to pH) and pH independence (the glycoside solvolysis is essentially pH independent over the neutral pH ranges, from the
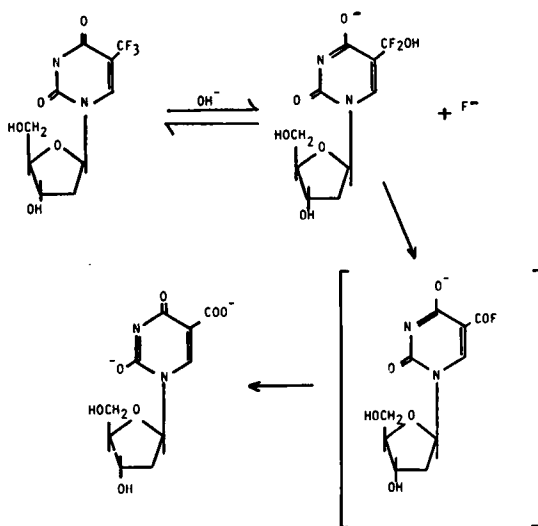
SCHEME 1.

Table 4. Calculated first order rate constants (days^{-1}) for the overall hydrolysis of trifluorothymidine in phosphate buffered solutions.* (For Assignments of k_{12}, k_{21}, k_{13}, See text.)

	Case 1				Case 2			
pH†	k_{12}	k_{21}	k_{13}	Correlation Coefficient	k_{12}	k_{21}	k_{13}	Correlation Coefficient
4·35	0·1018	3·734	0·1883	0·997	0·0975	3·46	0·416	0·991
5·80	0·1389	,,	0·5905	0·993	0·1363	2·614	,,	0·995
6·35	0·1530	,,	0·743	0·999	0·1510	2·293	,,	0·999
6·90	0·1671	,,	0·8955	0·999	0·1656	1·972	,,	0·998
7·35	0·1787	,,	1·02	0·997	0·1777	1·71	,,	0·997
8·05	0·1966	,,	1·214	0·997	0·1964	1·301	,,	0·995

* At pH 3·0 k for the single first order glycoside hydrolysis $= 0·137$ days^{-1}.
† Measured on final solution at room temperature, uncorrected.

borate buffer results) good fit of the experimental data was obtained for two schemes. The calculated rate constants for each case are shown in Table 4.

Case 1. k_{12}, k_{13} pH dependent, k_{21} pH independent.

We may speculate that $k_{13} = k_{solvolysis} + k_{5-COOH-dR\ prodn}$ where the pH independent solvolysis has $k_{solvolysis} = 0.11$ days^{-1}. The equilibrium reaction presumably involves the production of the unknown product R_F 0·51 (t.l.c. system 2).

Case 2. k_{12}, k_{21} pH dependent, k_{13} pH independent.

k_{13} refers to the pH independent solvolysis and also includes the now pH independent unknown product production. This implies in turn that $k_{12} = k_{5-COOH-dR\ prodn}$. Thus in Scheme 1 the production of the monodefluorinated intermediate is an equilibrium with overall base catalysis.

Conclusions

At present there is insufficient data to allow a proper choice between the alternative models in phosphate buffers, or to explain the suppression of the base-catalysed reaction in borate buffers which is not reported by previous workers presumably because of their use of analysis by u.v. spectral change.

It appears that the high temperature coefficient of trifluorothymidine hydrolysis in aqueous solutions constitutes a major barrier to steam sterilization of any eyedrop formulation containing it.

REFERENCES

Britton, H. T. S. (1955) Hydrogen Ions. Vol. I, Chapman & Hall, London, p 361

Clough, D. W., Wigdahl, B. L., Parkhurst, J. R. (1978) Antimicrob. Agents Chemother. 14: 126–131

Garrett, E. R., Seydel, J. K., Sharpen, A. J. (1966) J. Org. Chem. 312: 219–2227

Garrett, E. R., Suzuki, T., Weber, D. J. (1964) J. Am. Chem. Soc. 86: 4460–4468

Heidelberger, C., Parsons, D. G., Remy, D. C. (1964) J. Med. Chem. 7: 1–5

Lee, M. G. (1979) Int. J. Pharmaceutics 5: 19–24

Maloney, E. D., Kaufman, H. E. (1963) Invest. Ophthalmol. 2: 55–57

Nestler, H. J., Garrett, E. R. (1968) J. Pharm. Sci. 57: 1117–1124

Shapiro, R., Kang, S. (1969) Biochemistry 8: 1806–1810

U.S. Pharmacopeia (1975) XIX, p 703.

Wellings, P. C., Awdry, P. N., Bors, F. H., Jones, N. C., Brown, D. C., Kaufman, H. E. (1972) Am. J. Ophthalmol. 73: 932–942