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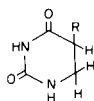
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The spectrophotometric determination of the apparent ionization exponents of 5,6-dihydrouracil (DHU) and 5,6-dihydrothymine (DHT) is described. The values of pK'_a at 37°C and ionic strength of 0.16 are 11.42 for DHU and 11.57 for DHT.

J. Heterocyclic Chem., **19**, 657 (1982).

5,6-Dihydrouracil (DHU, **1**) and 5,6-dihydrothymine (DHT, **2**) are intermediates in the pathways of degradation of the pyrimidine nucleotides. These compounds are capable of ionization, as evidenced by change of ultraviolet absorption of their solutions with change of pH . Absorption is much more intense at high pH than at low. In 0.1 *N* sodium hydroxide there is an absorption maximum at 230 nm (2). Because these compounds are very weak acids and because they are unstable at high pH , it is not feasible to determine their ionization exponents by potentiometric titration, and these values have not hitherto been reported. However, the decay of absorbancy at high pH follows an exponential course and it is possible to extrapolate to zero time to obtain values from which pK'_a can be calculated.



1, DHU (R = H)
2, DHT (R = CH₃)

The spectrophotometric method, which has been applied to the determination of the apparent ionization exponents of many compounds, entails the estimation of the relative concentrations of the two ionization species of the compound in buffers of different pH values. The apparent ionization exponent can be calculated from Equation (1) in which A_1 , A_2 , and A are, respectively, the absorbancies at a single wavelength of solutions of the same

$$pK'_a = pH - \log [(A - A_1)/(A_2 - A)] \quad \text{Equation (1)}$$

total concentration at a value of pH at which only the proton-donor species exists, at a value of pH at which only the proton-acceptor species exists, and at an intermediate value of pH at which both species are present. The present report describes the spectrophotometric determination of the apparent ionization exponents of DHU and DHT.

Results.

The relationships between pH and extinction coefficients at 230 nm as extrapolated to zero time are shown in Figure 1. The calculated values of pK'_a at 37°C and ionic strength of 0.16 are 11.42 for DHU and 11.57 for DHT.

Discussion.

Implicit in the formulation of Equation (1) are the following assumptions: that the two ionic species existing simultaneously absorb independently and additively; that the absorption of each conforms with Beer's Law; and that pH affects only the concentration of a species, not its inherent absorption characteristics. Evidence that these assumptions are valid for the dihydropyrimidines and that spectral changes with pH truly reflect a reversible proton exchange is furnished by the close conformity of measured values to the theoretical relationship of pH to absorbancy as shown in Figure 1.

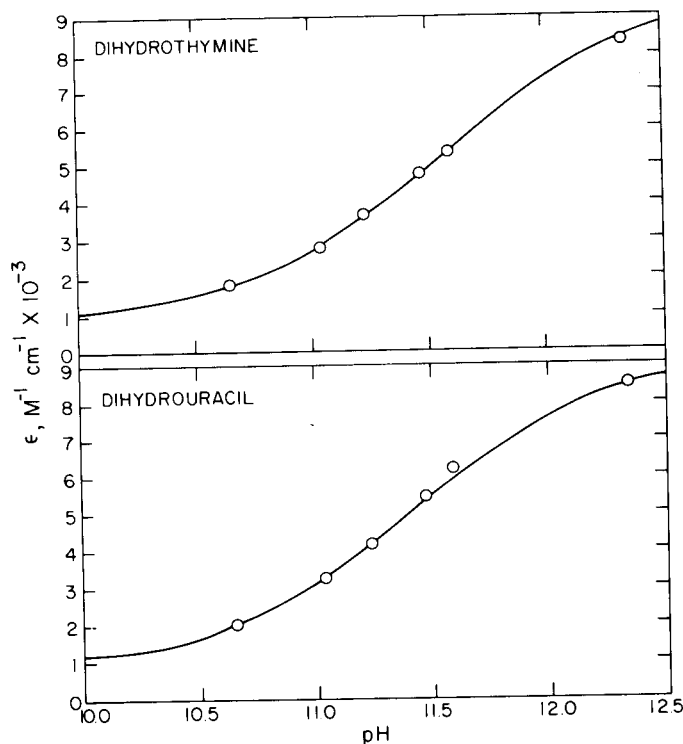


Figure 1. Relation between pH and absorption at 230 nm for solutions of DHU and DHT at 37°C. The theoretical curves are defined by Equation (2) and are drawn with the following parameter. For DHU: $\epsilon_1 = 0.78 \times 10^3$, $\epsilon_2 = 9.34 \times 10^3$, $pK'_a = 11.42$. For DHT: $\epsilon_1 = 0.85 \times 10^3$, $\epsilon_2 = 9.72 \times 10^3$, $pK'_a = 11.57$.

$$\epsilon = (\epsilon_1 10^{pK'_a} + \epsilon_2 10^{pH}) / (10^{pK'_a} + 10^{pH}) \quad \text{Equation (2)}$$

EXPERIMENTAL

Buffer Solutions.

Five phosphate buffers of high pH were prepared by addition of sodium hydroxide to solutions of disodium hydrogen phosphate as described by Bates and Bowers (3). The final concentrations with respect to phosphate were 0.025 M . Potassium chloride was added in such quantities that the final ionic strengths were 0.16. The buffers were prepared at 1.11 times the desired final concentrations to allow for dilution with stock solutions of the dihydropyrimidines. The pH values of these phosphate buffers were 10.65, 11.04, 11.24, 11.47, and 11.59. In addition, absorption measurements were made in 0.1 N sodium hydroxide (pH 12.34) and in a 0.124 M borate buffer of pH 9.45. Absorptions in these two latter solutions were used in estimation of the absorptions of the pure proton-acceptor and proton-donor species. Stock solutions of DHU and DHT (from Sigma Chemical Co.) were prepared at concentrations of 10^{-3} M and diluted 1:10 with the buffers to give final concentrations of 10^{-4} M .

Measurement of pH .

These measurements were made at $37^{\circ}C$ with a Metrohm E 300 B pH meter with Metrohm EA 121 UX combined electrode assembly for low alkali error. No corrections were necessary. For the buffers of high pH , the primary standard of pH was a 0.05 M solution of trisodium phosphate prepared from disodium hydrogen phosphate and a carbonate-free solution of sodium hydroxide. It was assigned a pH value of 11.74 at $37^{\circ}C$ (4). For measurement of the pH of the borate buffer, the primary standard was a solution containing 0.025 moles of potassium dihydrogen phosphate + 0.025 moles of disodium hydrogen phosphate per liter. It was assigned a pH value of 6.84 at $37^{\circ}C$ (5).

Ultraviolet Absorption Measurements.

A Cary Model 15 recording spectrophotometer with sample chamber thermostatted at $37^{\circ}C$ was used. As soon as possible after dilution of a

stock solution with buffer, the solution was transferred to a cuvette, which was stoppered and placed in the sample chamber. The reference cuvette contained a solution identical except for omission of the dihydropyrimidine. Absorbancy was recorded continuously at 230 nm. Except in the buffer of lowest pH , absorbancy decreased with time. Decay of absorbancy was exponential, and semi-logarithmic plots permitted extrapolation to zero time to obtain the values shown in Figure 1 and used in the calculations.

Calculation of Ionization Exponents.

A simple method for calculating pK'_a is by successive approximations. The inflection point of the absorbancy vs. pH curve as estimated visually was taken as a first approximation of pK'_a . This was substituted in Equation (1) with the values of A for the solution of highest and lowest pH to give two simultaneous equations from which A_1 and A_2 were calculated as first approximations. These values were used in Equation (1) with the measurements on the buffers of intermediate pH to calculate values of pK'_a . The mean of these values was used as a second approximation of pK'_a . This was used as before to obtain second approximations of A_1 and A_2 . The process was repeated until no significant change was seen.

REFERENCES AND NOTES

- (1) This investigation was supported by Public Health Service Research Grants GM 13606 and 18715 from the National Institute of General Medical Sciences. Thomas C. Butler was recipient of Public Health Service Research Career Program Award 5 KO6 GM 19429 from the National Institute of General Medical Sciences.
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