THE ULTRAVIOLET ABSORPTION SPECTRA OF THE PYRIMIDINE RIBONUCLEOSIDES AND RIBONUCLEOTIDES*

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It is well known that the high and characteristic absorption of nucleic acids in ultraviolet light is due to the absorption of their purine and pyrimidine components. Several workers have measured the absorption curves of the amino- and oxypurines (1-3) and Jordan has obtained similar data for adenosine and guanosine and for the corresponding nucleotides (3). In the latter instances substitution of the carbohydrate radical in position 9 of the purine ring does not change the absorption appreciably, but, as shown by Gulland and coworkers (4), substitution in the 7 position of either adenine or guanine causes definite changes in the absorption spectra.

Although the absorption curves of the free pyrimidines, uracil, cytosine, and thymine (2, 5, 6), and of thymine desoxyriboside (7) have been determined, similar data have not been published for the corresponding ribonucleosides, cytidine and uridine, or for the nucleotides, cytidylic acid and uridylic acid. With the growing interest in the metabolism of these compounds (8, 9), knowledge of their absorption characteristics would be useful for their determination in biological material. As it has been possible recently to prepare them in appreciable yield and of a high degree of purity (10, 11), we have accordingly measured their absorption in comparison with the free pyrimidines, cytosine and uracil. As all of these compounds may exist in different tautomeric forms, depending on the hydrogen ion concentration of the solvents employed, dilute acid and dilute alkali were used to provide conditions under which one or the other form would be present. For comparison 0.05 M phosphate buffer at pH 7.0 was also used.

EXPERIMENTAL

Materials—Synthetic samples of cytosine and uracil (12) as well as a commercial sample of uracil (Eastman Kodak Company) were used. Both uracil samples were recrystallized several additional times from water and melted with decomposition at 329–333° (when placed in a melt-

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ing point block at 315°). They were dried to constant weight in vacuo over phosphorus pentoxide at 100° before using. The sample of cytosine was recrystallized two additional times from water. It melted with decomposition at 315–318° and was used in the form of the monohydrate.

Cytidine, uridine, cytidylic acid, and diammonium uridylate were prepared by methods developed in this laboratory (10, 11). The first three compounds were dried to constant weight in vacuo over phosphorus pentoxide at 100°. The diammonium uridylate was used as the monohydrate (10).

Solutions—Stock solutions of each compound were prepared containing approximately 0.1 mM in 100 ml. of distilled water. 10 ml. aliquots were diluted to 100 ml. with 0.05 M potassium phosphate buffer at pH 7.0 for measurements at this pH or with sufficient standard hydrochloric acid or sodium hydroxide to give solutions that were 0.01 N with respect to acid or alkali.

Optical Methods—All measurements were made on a model DU Beckman spectrophotometer with the ultraviolet light attachment. Standard calibrated silica cells, 1 cm. wide with a light path of 1 cm., were used throughout. Both the test solution and the appropriate blanks were pipetted into the cells. Extinction readings were usually made with the selector-switch in the 1.0 position, but where the density values were larger than 1.0, greater accuracy was obtained by using the selector-switch in the 0.1 position. The wave-length scale was calibrated by using a mercury vapor lamp, the 253.6 and the 365.0 mμ bands being used. Density measurements on a standard potassium chromate solution (5.00 X 10⁻⁴ M anhydrous potassium chromate in 0.05 M potassium hydroxide) checked the values recorded in the literature (13).

RESULTS AND DISCUSSION

Uracil and Cytosine—The absorption curves of uracil and cytosine in the solvents mentioned above are shown in Figs. 1 and 2. The results with cytosine are in good agreement with the previously published data (6), even though slightly weaker alkali was used in the present experiments. Those with uracil show general agreement as to the wave-lengths at which maxima and minima have been found previously, but differ significantly in the value of the molecular extinction coefficient, \( E_M \). Thus in acid or neutral solution the value for \( E_M \) may be observed to vary from about 8200 found in the present experiments for both 0.01 N hydrochloric acid and phosphate buffer at pH 7.0 to 9500 found at pH 2.6 and 6.5 (5), and to 11,000 found for a solution in a Kolloff buffer at pH 3 (14). Whether the discrepancies are due to differences in the uracil samples, resulting possibly from their preparation under different light conditions (5), or to
the methods of measurement employed is not apparent, but the two preparations used in the present experiments gave identical results within experimental error in three duplicate experiments carried out on each sample. It should be pointed out that no unusual precautions were taken to avoid exposure of the crystalline samples to light other than to avoid direct contact with sunlight.

It is of interest from the standpoint of the tautomeric equilibria concerned that both uracil and cytosine show absorption maxima near 280 m\(\mu\) in alkali. The absorption curves of uracil at pH 7.0 and in 0.01 N alkali are reproduced in Fig. 3 in comparison with the absorption data...
found in 0.001 N alkali and with the previously published data for uracil at a pH estimated to be between 9.0 and 9.5 (5) and with data obtained in a Koltchoff buffer at pH 11 (14). The lack of agreement between our results in 0.001 N alkali at approximately the same pH as that of the Koltchoff buffer is evident. It seems likely that the increased absorption in the buffer solution is due to certain of the buffer constituents, which, however, were not given in the publication cited. It may be seen from a comparison of the other data that the value for the molecular extinction at 260 m\(\mu\) observed in phosphate or in acid decreases as the solution is made alkaline and practically disappears in 0.01 N alkali concomitant with the appearance of the new maximum at 280 m\(\mu\). As the pK value of uracil is 9.45, it is evident that the appearance of the second maximum is correlated with salt formation and probably with the development of a more fully aromatic structure, as the hydrogen atom at position 1 or at position 3 is shifted to 6 or 2, respectively, under these conditions.

Uridine and Uridylic Acid—The absorption curves of uridine and uridylic acid are presented in Figs. 4 and 5. A comparison of the graphs shows that the two compounds gave almost identical results over the wave-lengths studied. Substitution of the phosphate group in the 3' position of the ribose side chain has no appreciable effect on the pyrimidine ring structure present in uridine itself. The absorption curves for uridine and uracil, however, are significantly different. In acid or in neutral solution the curves are similar in appearance, but the absorption maximum of uridine has a value of about 9800, as compared to about 8200 for uracil. In alkali, although the maximum with uridine is decreased to 7400, there is no corresponding shift towards the longer wave-lengths. A possible explana-
tion for the difference in the two cases is that substitution of the labile hydrogen atom in position 3 of uracil with the stable ribosidic linkage has eliminated the possibility of the fully aromatic pyrimidine ring which may be formed with either uracil or cytosine in the presence of alkali.

A comparison of the absorption curves of uridine and of thymine desoxyriboside (6) shows a striking similarity between the two under the same conditions. In acid or in alkaline solution nearly identical values for the molecular extinction coefficient are found for both compounds. The presence of the methyl group in the 5 position of the pyrimidine ring in thymine desoxyriboside, however, results in a slight shift of the maximum and minimum towards the longer wave-lengths. While no similar proof of the location of desoxyribose at the 3 position of the pyrimidine ring in thymine desoxyriboside has been offered, as in the case of uridine, the similar absorption characteristics of the two compounds in acid and in alkali provide evidence that this is actually the case. Thymine in which a hydrogen atom is present in the 3 position shows a similar shift in absorption maximum to 290 m\(\mu\) in alkali as that found for uracil (7).

Cytidine and Cytidylic Acid—The absorption curves of cytidine\(^1\) and cytidylic acid are shown in Figs. 6 and 7 and their absorption character-

\(^{1}\)In recent publications (15) Hotchkiss and Reichard independently present absorption data for cytidine at various wave-lengths. The values given are similar but not identical to those calculated from the data found for the highly purified samples used in the present publication.
istics in comparison with uracil, cytosine, uridine, and uridylic acid are summarized in Table I. It may be seen that the curves for cytidine and cytidylic acid are identical within experimental error. In acid the same maximum was found at 280 μm and the same minimum at 240 μm, and the value for the molar extinction coefficient was approximately 12,750 in each case. Curves of similar shapes that were characteristically different from those in acid were found in both phosphate and in alkali. Under these latter conditions, as with cytosine, the absorption maxima for both compounds were also shifted to 270 μm. Values of the molecular extinction coefficient at this wave-length were reduced to about 9000, but the differences between the results in phosphate and in alkali were not as pronounced as in the case of cytosine. At the same time the amount of absorption at 230 μm was greatly increased, the curves showing a definite shoulder or plateau at this point in contrast to those for cytosine under the same conditions. The curves for cytidine and for cytidylic acid in alkali and in phosphate could scarcely be distinguished from each other. Because of the high absorption of cytidine and cytidylic acid at 270 to 280 μm, it is evident that either compound may be confused with the aromatic amino acids, tyrosine and tryptophan, which also absorb strongly at these wave-lengths (13) p. 185).

Of the three solvents employed, the largest differences in absorption between the amino and oxy compounds occurred when phosphate buffer at pH 7 was used. As the use of such a buffer provides more reproducible conditions than the use of distilled water, it would appear to be more suitable for characterization of the individual compounds. It is also apparent, however, that with uridine and uridylic acid the position of absorption at 230 μm was greatly increased, the curves showing a definite shoulder or plateau at this point in contrast to those for cytosine under the same conditions. The curves for cytidine and for cytidylic acid in alkali and in phosphate could scarcely be distinguished from each other. Because of the high absorption of cytidine and cytidylic acid at 270 to 280 μm, it is evident that either compound may be confused with the aromatic amino acids, tyrosine and tryptophan, which also absorb strongly at these wave-lengths (13) p. 185).

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the absorption maximum remains the same, regardless of the solvent used, while in all the other cases significant shifts occur as either acid or alkali is used, and that absorption measurements in both of these solvents can be of value in the identification of the individual pyrimidine derivative.

SUMMARY

The absorption spectra of uridine and cytidine in comparison with the corresponding free pyrimidine bases, uracil and cytosine, and with the corresponding nucleotides, uridylic acid and cytidylic acid, have been determined in phosphate buffer at pH 7.0, in 0.01 N hydrochloric acid, and in 0.01 N sodium hydroxide.

The absorption curves of uridine closely resemble those for uridylic acid and are different in several respects from those for uracil or for cytidine or cytidylic acid under similar conditions. The last two compounds mentioned show very nearly the same absorption characteristics, which are also different from the free base, cytosine. Phosphate buffer at pH 7.0 is a more suitable solvent for the characterization of either the free pyrimidines or the nucleosides or nucleotides.

The close similarities between the absorption curves of uridine and thymine desoxyriboside in acid and in alkali are offered as evidence that the desoxyribose group in thymine desoxyriboside is substituted in the 3 position of thymine.

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