0.39 g. of silver nitrate) for 15 min. The filtered solution was evaporated to dryness *in vacuo* and the residue distilled (150°, 0.2 mm.) yielding 4-methyl-5-phenyl-1-pentene (0.238 g., 68%); λ_{max} mμ (log ε) in methanol: 242 (2.21), 248 (2.22), 253 (2.22), 259 (2.22), 262 (2.21), 265 (2.11), and 269 (2.11).

Oxidation of the 4-Methyl-5-phenyl-1-pentene.—The pentene derivative (160 mg.) was dissolved in ether (5 ml.), and water (5 ml.) added. After the addition of a small crystal of osmium tetroxide (20 mg.) a solution of sodium metaperiodate (580 mg.) in water (5 ml.) was added during 25 min. to the rapidly stirred solution cooled to 0°. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 3 hr. Sodium iodate was removed by filtration and the filtrate extracted with ether. The aqueous solution was distilled into a saturated aqueous solution of dimedone (125 ml.), which on standing overnight deposited crystals of the formaldehyde dimedone derivative (132 mg., 45%). Evaporation of the dried ether extract afforded 3-methyl-4-phenylbutanal (48.6 mg., 30%).

3-Methyl-4-phenylbutanoic Acid.—3-Methyl-4-phenylbutanal (48.6 mg., 0.30 mmole) was dissolved in acetone (5 ml.) and a solution of chromic acid in sulfuric acid (0.075 ml., 0.20 mmole, made by dissolving 26.7 g. of chromium trioxide in 23 ml. of concentrated sulfuric acid and diluting with water to 100 ml.) added rapidly with stirring. After 3 min., water (20 ml.) was added, the solution saturated with sodium chloride, and then extracted with ether (4 × 20 ml.). The combined ether extract was extracted with aqueous 5% sodium bicarbonate which was then acidified with sulfuric acid and extracted with ether. Evaporation of the dried ether extract afforded 3-methyl-4-phenylbutanoic acid (25.5 mg., 50%). This acid was dissolved in 50% ethanol and titrated with 0.1 *N* sodium hydroxide to a pH of 8. Evaporation of the solution afforded the sodium salt.

Schmidt Reaction on 3-Methyl-4-phenylbutanoic Acid.—The sodium salt of 3-methyl-4-phenylbutanoic acid (15 mg.) was dissolved in concentrated sulfuric acid (0.1 ml.) and cooled to −10°. Sodium azide (20 mg.) was added and the mixture warmed to 40–43° in a stream of carbon dioxide-free nitrogen for 1 hr. The evolved carbon dioxide was absorbed in barium hydroxide solution affording barium carbonate (4.9 mg. 33%). The acidic residue in the flask was diluted with water (5 ml.) and extracted with ether. The ether was washed with 5% sodium bicarbonate and then dried over magnesium sulfate. The residue obtained on evaporation of the ether was sublimed (90°, 10^{-3} mm.) yielding a colorless solid. Recrystallization from petroleum ether (b.p. 60–70°) afforded benzo[*f*]-4-methyl-2,3,4,5-tetrahydroazepin-2-one (IX) as colorless needles, m.p. 116–117° (2.9 mg., 22%). This material was identical (mixture m.p., infrared spectrum) with the product obtained by the Beckmann rearrangement on 3-methyl-α-tetralone oxime.

Benzo[*f*]-4-methyl-2,3,4,5-tetrahydroazepin-2-one.—3-Methyl-α-tetralone oxime[18] (0.267 g.) was stirred with polyphosphoric acid (7.0 g.) at 110° for 10 min. The reaction mixture was poured onto ice and extracted with ether. The dried ether extract was evaporated and the residue crystallized from petroleum ether yielding colorless needles of the lactam (0.147 g., 55%), m.p. 116–117°.

Anal. Calcd. for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.70; H, 7.10; N, 7.99.

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The Ultraviolet Spectra of Native and Denatured Deoxyribonucleic Acid[1]

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The ultraviolet absorption spectra of native and denatured calf-thymus deoxyribonucleic acid were studied from 3500 to 1830 Å. The spectral changes which are brought about by heating deoxyribonucleic acid in H_2O or D_2O solution, or by drying in solid films, are similar. The absorption maxima near 1850 and 2600 Å. shift by about 50 and 14 Å., respectively, to longer wave lengths upon denaturation. The integrated intensity of the 2600-Å. band increases upon denaturation by a factor of about 1.43, while the integrated intensity of the 1850-Å. band (of which only the part above 1830 Å. was measured) certainly does not increase by a comparable factor and may possibly decrease slightly.

Introduction

The denaturation of deoxyribonucleic acid (DNA) is accompanied by the well known increase in the absorbance of the ultraviolet band centered at 2600 Å.[2] While many studies of the changes of absorbance of DNA upon denaturation have been reported, none of them extend below about 2100 Å., in spite of considerable theoretical interest in measuring the spectrum as far as possible toward short wave lengths.[3-7] In the present work the spectra of DNA in the native and denatured state were examined from 3500 to 1830 Å., with special care in the short wave-length region. The effect of denaturation on the spectrum was studied both in aqueous solution and in solid films. The study made use of the recent observation that solid DNA undergoes, upon drying, structural changes which are analogous to denaturation.[8]

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Experimental

Two samples of the sodium salt of calf-thymus DNA were used. One was purchased from the Nutritional Biochemicals Corp., Cleveland, Ohio, and the other was kindly donated by Dr. Goeffrey Brown, Biophysics Department, King's College, London. The purity and integrity of these samples were checked by means of their infrared and ultraviolet spectra, orientability and dichroism, melting temperature, and extent of hypochromism at 2600 Å. A negative silver nitrate test showed the absence of chloride ions, and a negative orcinol test showed that the samples contained little or no ribonucleic acid. X-Ray diffraction patterns confirmed the absence of salt, protein, and ribonucleic acid.[9]

The spectra were recorded on a Beckman DK-2 "far ultraviolet" spectrophotometer.[10] The instrument was purged at the rate of about 40 l. per min. with "purified grade" nitrogen (which contains less than 15 parts per million of oxygen[11]), supplied by the Canadian Liquid Air Co. This purging rate was found to be sufficient to eliminate practically all absorption due to oxygen and water vapor. The stray light at short wave lengths[12] was determined by inserting in the sample beam a plate of Vycor 2 mm. in thickness (absorbing 100% below 2200 Å.) or a 1 cm. silica cell containing a 0.01 *M* solution of sodium chloride in water (absorbing 100% below 1940 Å.). The values of stray light of wave length above 1940 Å. and above 2200 Å., determined in the above manner, were the same. The part of each spectrum in

(9) The author wishes to thank Professor M. H. F. Wilkins and Dr. Struther Arnott, King's College, London, for recording and interpreting the X-ray diffraction patterns of DNA samples used in this work.

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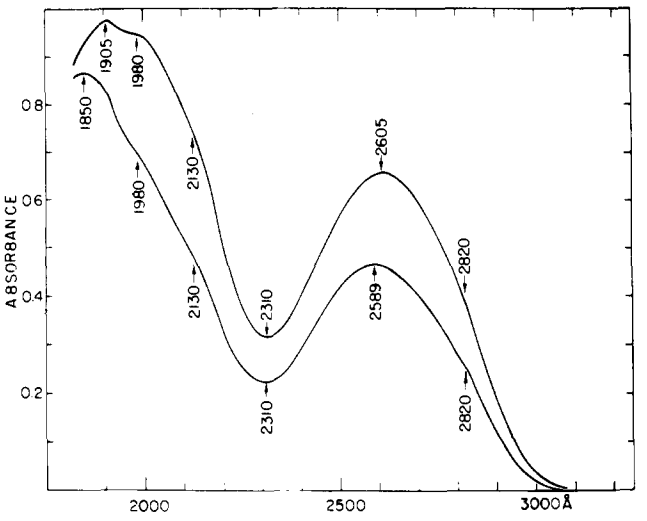

Fig. 1.—Lower curve: spectrum of a solid film of DNA exposed to 93% relative humidity (D_2O); upper curve: spectrum of the same film at 0% relative humidity.

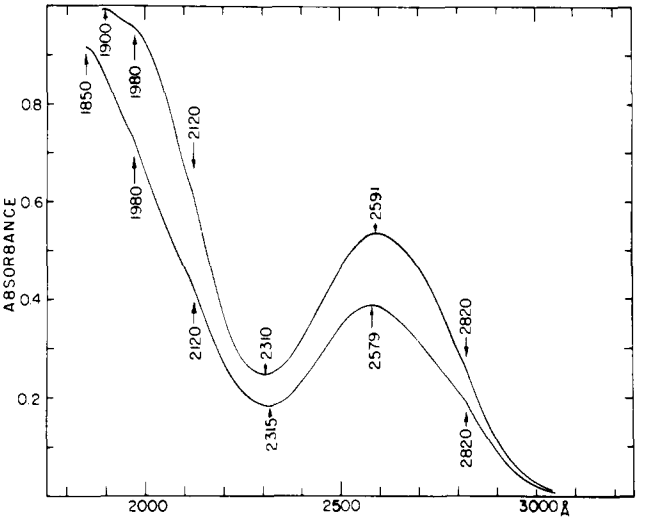

Fig. 2.—Lower curve: spectrum of a solution of 0.002 DNA in D_2O in a 1 cm. cell at 20°; upper curve: spectrum of the same solution at 90°.

which the proportion of stray light was 0.5% or greater was disregarded, which resulted in a short wave-length cut-off at 1830 to 1920 Å., depending on the transmission of the sample and of the reference. This procedure kept the error in absorbance, caused by stray light, below 1% for samples having an absorbance of about 0.6. The spectra were recorded at slow rates of scanning and at narrowest practicable slits and were reproducible to about ±0.002 absorbance unit. Wave-length reproducibility of the instrument was ±1 Å. and wave-length accuracy about ±5 Å.

Since the short wave-length limit of the spectrum of DNA in solution is determined by the onset of the ultraviolet absorption of liquid water, and since addition of NaCl shifts this onset to longer wave lengths, the spectra of DNA were examined in NaCl solutions of concentration varying from 0.1 to 0.001 *M*, as well as in pure H_2O and in D_2O for which the onset of the ultraviolet absorption occurs about 30 Å. farther than for H_2O. No changes in the spectrum of native or heat-denatured DNA due to the differences in the ionic strength of the solutions were detected, although the temperature of denaturation and the degree of renaturation upon cooling varied markedly with ionic strength, as has been discussed by Marmur and co-workers.[13]

All the solutions were prepared and kept at 1°, which prevented spontaneous denaturation of DNA at low salt concentration. The pH of the solutions was about 6.0. Matched fused silica cells of 10 mm. or 1 mm. path length were used with appropriate solvent in the reference cell. Dissolved oxygen was removed from the sample and reference solutions by purging with nitrogen. The samples were heated and cooled in the Beckman temperature-regulated cell holder No. 92527 with the cells sealed to prevent evaporation.

Solid films of DNA were prepared by evaporation of a drop of concentrated solution spread on the inside surface of a 1 cm. fused silica cell. A matching empty cell was used in the reference beam. The specimen was converted to the denatured form by drying over anhydrous magesium perchlorate, and to the native form by being exposed to an atmosphere of 86 or 93% relative humidity.[8] After overnight equilibration the cell was sealed and the spectrum was recorded. In some experiments an atmosphere of D_2O was used instead of H_2O.

The contribution of increased scattering at short wave lengths to the absorption spectrum of solid samples was neglected. The contribution of scattering at long wave lengths appeared to be small, since the films were optically clear and showed no changes in transmittance with wave length above 3150 Å., *i.e.*, outside of the DNA absorption region. The agreement between the spectra of solid films of varying thicknesses and between the spectra of solid films and those of solutions makes a large contribution of scattering unlikely also at short wave lengths. Moreover, the spectrum of dry solid DNA was also found to agree with the spectrum of a dry solid film of DNA reported by Preiss and Setlow,[14] in the region from 2600 to 1830 Å., where the two spectra overlap.

Results and Discussion

Spectrum of Native DNA.—The spectra of DNA in H_2O and D_2O solutions were found to be identical. The spectra in D_2O will be presented here, since they extend down to 1850 Å., about 30 Å. farther than the spectra in H_2O. A typical spectrum of a solid film of DNA at high relative humidity is shown as the lower curve in Fig. 1 and that of a solution of approximately 0.002% DNA in D_2O is shown as the lower curve in Fig. 2. It would be expected that the absorption spectra of native DNA in aqueous solution and in the highly hydrated solid films should be identical, since the DNA molecules in the solid film at 86 or 93% relative humidity are already highly surrounded by water[15] and have the same helical structure as in aqueous solution.[8,16] It is in fact observed that the absorption spectra of aqueous solutions and of solid films are very similar in every respect, except for a small difference in the intensity of absorbance in the 1900-Å. region which will be discussed below.

In the wave-length region studied, the spectrum of native DNA consists of the well-known band with a maximum near 2600 Å. and of a second, more intense band with a maximum near 1850 Å. For solid films the peak absorbance of the 1850-Å. band is about 1.8 times as high as that of the 2600-Å. band, while for D_2O solutions the factor is somewhat greater, and is greater still for the H_2O solutions. The higher absorbance of solutions in this region is most probably caused by the influence of dissolved DNA on the absorption of H_2O or D_2O in this region, which is appreciable even at 1-mm. path length. The effect of dissolved substances on the ultraviolet spectrum of water is often very marked even at very low concentrations.[17] The spectra of the solid DNA films would be free from this effect. At 0.5-μ path length, which is the approximate thickness of the solid films, the absorbed H_2O does not measurably absorb down to 1850 Å., and D_2O at least down to 1830 Å. It may be noted that any contribution of light scattering to the spectrum of the solid films would lead to a *higher*, and not *lower* absorbance at short wave lengths.

Beside the main band maxima, several weak but distinct shoulders occur. The wave lengths of the shoulders, maxima, and minima are indicated in Fig. 1 and 2 and are averaged from about twenty recorded spectra of each kind. The average deviation was ±2 Å. for the maximum near 2600 Å., ±10 to ±30 Å.

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for the shoulders, and ±5 Å. for the minimum near 2310 Å. The exact position of the maximum near 1850 Å. is somewhat uncertain in the spectra of solutions, which only extend to 1850 Å., but was reproducible to ±5 Å. in the spectra of solid DNA, which extend 20 Å. farther.

Effect of Denaturation on Spectrum above 2100 Å.—The spectra of DNA denatured by heating in solution and by drying in a solid film are shown in the upper curves of Fig. 1 and 2. The band near 2600 Å. shifts by 12 to 16 Å. to higher wave lengths upon denaturation; a shift from 2580 to 2590 Å. was reported by Fresco.[18] The shape of the band is little affected by denaturation, except that the relative intensity of the shoulder at 2820 Å. decreases. The absorption of denatured DNA is higher than that of native DNA throughout the 2600-Å. band. This was ascertained with special care, since a cross over near 2960 Å. had previously been reported.[18] Above 2100 Å. the average value of the ratio of absorbance of denatured DNA to that of native DNA is 1.42 for thermal denaturation in solution (after allowing for the 3.4% increase in volume of the solution between 20 and 90°) and 1.44 for denaturation in solid by drying. The integrated intensity of the 2600-Å. band increases by about the same factor. The similarity of the spectral changes which occur in the solid and in solution shows that these processes involve loss of base-stacking to about the same extent. An exposure of the dried film to an atmosphere of high relative humidity results in a very rapid return to a lower absorbance identical with that of the original native DNA. This complete reversibility occurs even if the dried film has been previously maintained for an hour at 90°. Apparently the two DNA strands cannot unwind and separate in the solid state either at room temperature or at 90°. The complementary bases remain "in register" and may rapidly rematch upon rehydration.

Weiss and collaborators[19,20] suggested the possibility that the hypochromism near 2600 Å. in nucleic acids may be due to the differences in the environment of the nucleotide bases in the native and denatured configurations. Upon denaturation in solution, the bases are removed from a highly shielded position in the interior of the helical polynucleotide and become surrounded by the polar water molecules. However, the effect of environment cannot explain why dry solid DNA in complete absence of water has the high absorbance at 2600 Å. characteristic of denatured DNA. On the other hand, the loss of hypochromism in dry DNA can be explained by the disappearance of regular parallel stacking of base-pairs upon drying, and is in line with the more generally accepted explanations of hypochromism.[3-7,21]

Effect of Denaturation on Spectrum below 2100 Å.—The band near 1850 Å. shifts to higher wave lengths by about 50 Å. (1400 cm.$^{-1}$) upon denaturation, also becoming somewhat sharper, while the shoulder at 1980 Å. becomes more prominent. The wave-length shift is in the direction and of the order of magnitude expected as a result of exciton splitting of energy levels for a parallel arrangement of transition dipoles when the bases are stacked in the DNA helix.[21] The magnitude of such a shift would be expected to be highly dependent on band intensity, and thus larger for the 1850-Å. band than for the less intense band near 2600 Å., the integrated intensity of which is lower by a factor of about 3.

The ratio of the absorbance of denatured DNA to that of native DNA decreases rapidly below 2100 Å. and the two absorbance curves almost certainly cross just below 1830 Å. One would expect from theory,[6,7,21] that the sum of the integrated intensities of all the electronic transitions in a molecule should be independent of simple changes in conformation such as the helix-coil transition. Since the intensity of the absorption band at 2600 Å. *increases* upon denaturation, the intensity of some other electronic transitions lying farther in the ultraviolet must *decrease*. The peak absorbance of the 1850-Å. band increases upon denaturation, but by a factor of only about 1.11, much smaller than the factor of 1.44 for the band at 2600 Å. The integrated intensity can only be computed for the part of the 1850-Å. band accessible to the present measurements. The area under the curve of absorbance against frequency, computed from the minimum at 2300 Å. to the band maximum (1850 Å. for native DNA and 1905 Å. for denatured DNA), increases upon denaturation by a factor of only about 1.07. Considering the uncertainty in extending this result to the whole band, it can be concluded that the integrated intensity of the 1850-Å. band certainly does not increase upon denaturation by a factor comparable with that for the 2600-Å. band, and could actually decrease slightly. To test this point, the spectra of native and denatured DNA will have to be recorded below 1830 Å. and as far as possible into the vacuum ultraviolet region. Such an experiment could not be done with DNA in aqueous solution, but would be feasible using hydrated and dry solid films, supported on CaF_2 or BaF_2 plates. It would be necessary, below about 1850 Å. for H_2O, and below about 1830 Å. for D_2O, to subtract the contribution of the water of hydration to the absorption spectrum, which may prove somewhat difficult.

The Shoulders at 2820, 2120, and 1980 Å.—The weak shoulders observed in the spectrum of DNA appear to be real. They were observed repeatedly in the spectra of different DNA preparations, and it is unlikely that they could be due to an impurity. A solution of adenosine, thymidine, guanosine, and cytidine in D_2O, in the approximate proportion in which the corresponding four bases occur in DNA, was found to exhibit shoulders at similar wave lengths. The shoulder at 2820 Å. was observed earlier by Fresco,[18] who assigned it to an $n \rightarrow \pi^*$ transition. This assignment has support from the optical rotatory dispersion spectrum of DNA[22] and from the dichroic study of oriented DNA films.[23] A possible assignment of the shoulders near 2120 and 1980 Å. to further $n \rightarrow \pi^*$ transitions must await the extension of optical rotation and dichroic studies of DNA to shorter wave lengths.

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