COMMUNICATIONS TO THE EDITOR

ROTATORY DISPERSION OF NUCLEIC ACIDS IN THE NEAR-ULTRAVIOLET REGION

Sir:

We wish to report the demonstration of several optically active electronic transitions in the near-ultraviolet absorption band, 230–300 mμ, of nucleic acids. The presence of these transitions has been deduced from the observation of anomalous optical rotatory dispersion in the form of multiple Cotton effects. The ultraviolet rotatory dispersion profiles of native deoxyribonucleic acid, DNA, and ribonucleic acid, RNA, share certain features and show a dependence on secondary structure that is consistent with a common element of helical structure in these nucleic acids.[1,2] Moreover, the two dominant Cotton effects exhibit in both RNA and DNA a differential sensitivity to denaturation. This suggests differential response of the associated electronic transitions to hydrogen bonding between bases and the stacking of base pairs in helical polynucleotides. Electronic transitions in purines and pyrimidines have been recognized earlier[3,4]; the significance of the present findings lies in their relation to interactions resulting from helical secondary structure, since the exaltation of optical activity in a helical polymer is dependent upon the formation of exciton bands.[5]

Previously it had been established that the optical rotatory power of polynucleotides and nucleic acids at 589 mμ has a large positive component due to helical secondary structure.[1,6,7,8] More recently, the rotatory dispersion of these substances was shown to be *simple Drude* above 350 mμ with λ_c values suggesting the presence of optically active bands in the ultraviolet region of the spectrum.[9] Thus the current findings provide a spectroscopic basis for the empirical relation between secondary structure and optical rotatory power of polynucleotides.

The measurement of optical rotation of nucleic acids below 300 mμ presents a formidable problem because of their very strong absorbance relative to rotatory strength. To overcome this obstacle a spectropolarimeter was modified to afford increased photosensitivity.[10] With this apparatus it was usually possible to make measurements down to 250 mμ on 0.02% ($A_{260} \cong 4$) solutions of nucleotide material, contained in strain free silica cells of 1 cm. path length, and occasionally to 230 mμ when a very intense lamp was available.

Figures 1 and 2, which contain rotatory dispersion curves of DNA, RNA and a nucleotide mixture provide a realistic picture of the scatter of the data.[11] These dispersion curves display the characteristic complexity resulting from the participation of several optically active transitions. They are not readily resolved, and hence a quantitative description of their magnitudes and shapes is not possible. Nevertheless, qualitative interpretations suggest themselves.

In native or completely helical DNA the pattern is simplest (Fig. 1), containing two distinct peaks at 289 and 257 mμ and a shoulder at 264 mμ. It seems likely that the peak at 289 mμ is attributable to a Cotton effect arising from $n \rightarrow \pi^*$ transitions,[3,12] for it occurs in a region where the absorption of DNA is very small and red-shifts upon disruption of the continuous hydrogen-bonded structure. Treatment leading to the formation of partially helical single-stranded DNA[1] diminishes this peak somewhat (Fig. 1) and upon complete denaturation its magnitude drops to a level comparable to that observed for a mixture of monoribonucleotides (Fig. 2).

The peak at 257 mμ in the dispersion curve of DNA appears to be more sensitive to denaturation. Thus, it is nearly absent in single-stranded DNA which contains a high proportion of residues in *short* imperfect helical regions.[1] Such sensitivity to the degree of ordered stacking is consistent with the transitions' being polarized perpendicular to the helix axis.[5] In this context, this is the behavior expected of $\pi \rightarrow \pi^*$ transitions.

In contrast however, *native* RNA, which is also single-stranded and contains a similar proportion of residues in helical regions[1] shows a $\pi \rightarrow \pi^*$ transition of high rotatory strength (peak at 257 mμ, Fig. 2). This suggests that native RNA possesses a more extensively developed and precise secondary structure, such as would occur if the helical regions were longer and contained fewer defects.[13] Partial denaturation of RNA upon lowering the ionic

(1) P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *Proc. Nat. Acad. Sci.*, **45**, 482 (1959).

(2) J. R. Fresco, B. M. Alberts and P. Doty, *Nature*, **188**, 98 (1960).

(3) M. Kasha, in "Light and Life," W. D. McElroy and B. Glass, editors, The Johns Hopkins Press, Baltimore, Md., 1961, p. 31.

(4) S. F. Mason, *J. Chem. Soc.*, 1240 (1959).

(5) W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Nat. Acad. Sci.*, **43**, 723 (1957).

(6) J. R. Fresco, *Federation Proc.*, **18**, 229 (1959); *Trans. N. Y. Acad. Sci.*, Ser. *II*, **21**, 653 (1959).

(7) J. R. Fresco and E. Klemperer, *Ann. N. Y. Acad. Sci.*, **81**, 730 (1959).

(8) G. K. Helmkamp and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **83**, 138 (1961).

(9) Summarized in J. R. Fresco, *Tetrahedron*, **13**, 185 (1961).

(10) The voltage input across the 1P28 photomultiplier of a Rudolph Spectropolarimeter, model 800 S, was increased by up to 360 volts d.c., thereby increasing its sensitivity, and at the same time the photomultiplier was cooled to about −50° in order to suppress the consequent increase in dark current and noise level. Satisfactory cooling of the photomultiplier was achieved by insulating it from the analyzer prism and surrounding it with copper coils through which cold nitrogen gas from a liquid nitrogen reservoir was driven under slight pressure. A General Electric A-H6 mercury arc was employed to provide an intense and relatively stable energy source. The modified polarimeter enabled photoelectric detection of as little as 1/1000 the energy limit of the ordinary instrument.

(11) Dispersion data were derived from multiple readings (4-6) every 2 mμ, and each dispersion was repeated at least twice. Consecutive readings at a given wave length generally were reproducible to within 5%, and the contour of the dispersion likewise was reproducible. However, absolute rotation values could not be reproduced to better than ±10%, even though blank values were taken in sequence at each wave length, and the symmetrical rocking angle was fixed at 5°.

(12) A. Rich and M. Kasha, *J. Am. Chem. Soc.*, **82**, 6197 (1960).

(13) R. A. Cox and P. Doty, unpublished observations.

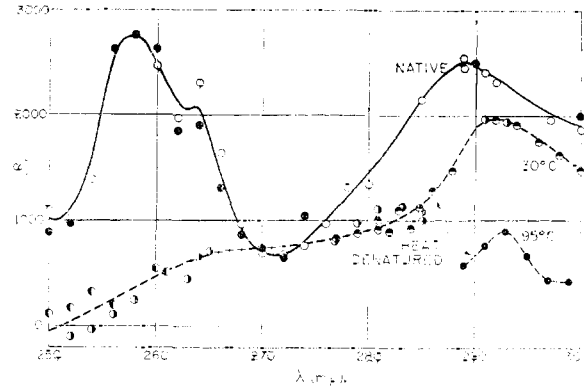

Fig. 1.—Ultraviolet rotatory dispersion of DNA (calf thymus) in 0.15 *M* NaCl + 0.015 *M* Na Citrate, *p*H 7; DNA was denatured at 95°, then cooled to 30°.

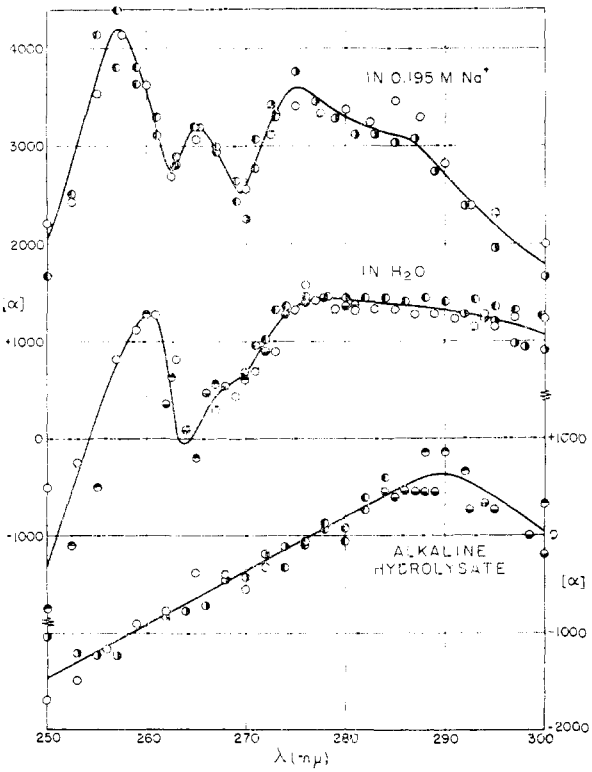

Fig. 2.—Ultraviolet rotatory dispersion of microsomal RNA (calf liver) and its alkaline hydrolysate in 0.15 *M* NaCl + 0.015 *M* Na Citrate, *p*H 7, *T* 22°.

strength results in a loss of detail and diminished rotatory power (Fig. 2). Completely denatured RNA can be expected to show a profile approaching that of its constituent mononucleotides.

These first observations contain considerable detail not evident in the corresponding absorption spectra, and demonstrate that ultraviolet rotatory dispersion is a promising approach to the investigation of the microstructure in nucleic acids. It is in studies with homopolynucleotides and pairs that we can expect to sharpen detail no doubt obscured in natural nucleic acids containing a multiplicity of bases and base sequences.

We are grateful to Mr. Peter Urnes for assistance in the instrumental work, and to Professor Michael Kasha for helpful discussions. This work was supported by grants from the United States Public Health Service, C-2170, the National Science Foundation and the American Heart Association.

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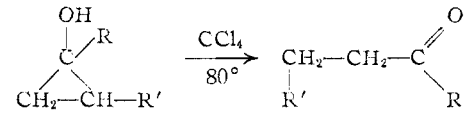
RECEIVED MAY 29, 1961

A FREE-RADICAL CATALYZED REARRANGEMENT OF CYCLOPROPANOLS

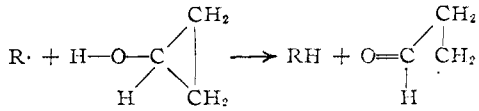
Sir:

We wish to report that in carbon tetrachloride or chloroform solution at 80° cyclopropanols undergo a rapid ring-opening rearrangement reaction, apparently proceeding by a free radical mechanism. For cyclopropanol itself the product, in very high yield, is propionaldehyde, and analogous products, methyl ethyl ketone and γ-phenylpropionaldehyde, are formed from 1-methyl- and *trans*-2-phenylcyclopropanol,[1] respectively.



In a typical experiment 0.5 ml. samples of 0.5 *M* cyclopropanol in carbon tetrachloride were sealed in 2-ml. tubes, under air or oxygen, and heated at 80°. The progress of the reaction was followed by gas chromatography and infrared and nuclear magnetic resonance spectroscopy. Cyclopropanol disappeared with a half-life of approximately thirty minutes and the only detectable product was propionaldehyde. If the samples were sealed with the exclusion of oxygen, or if di-*t*-butyl-*p*-cresol were added to samples sealed in the presence of air, little reaction occurred even after long periods of heating. Cyclopropanol samples sealed in the absence of oxygen but containing added peroxides again were converted rapidly to propionaldehyde. The rate was not slowed by the addition of triethylamine, nor appreciably enhanced by the addition of acid. 1-Methylcyclopropanol and *trans*-2-phenylcyclopropanol, in preliminary studies, appear to react in a similar manner.

These rearrangements appear remarkable to us for several reasons. In the first place cyclopropyl acetate and methyl cyclopropyl ketone are completely inert under the conditions of the reaction. This observation together with the fact that radical abstractions from cyclopropanes are known to be difficult,[2] suggests that the reaction may involve an attack on the O–H bond of the alcohol, probably with simultaneous ring opening. If the reaction



(1) C. H. DePuy, L. R. Mahoney and K. L. Eilers, *J. Org. Chem.*, in press.

(2) J. R. McNesby and A. S. Gordon, *ibid.*, **79**, 825 (1957).