Anomalous Optical Rotatory Dispersion of Flavine Nucleotides

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FLUORESCENCE[1] and absorption spectroscopy[2] indicate that in aqueous solvents flavine-adenine dinucleotide (FAD) takes up a folded conformation in which the flavine and adenine rings are in close

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[1] G. Weber, *Biochem. J.*, 1950, **47**, 114.
[2] L. G. Whitby, *Biochem. J.*, 1953, **54**, 437.

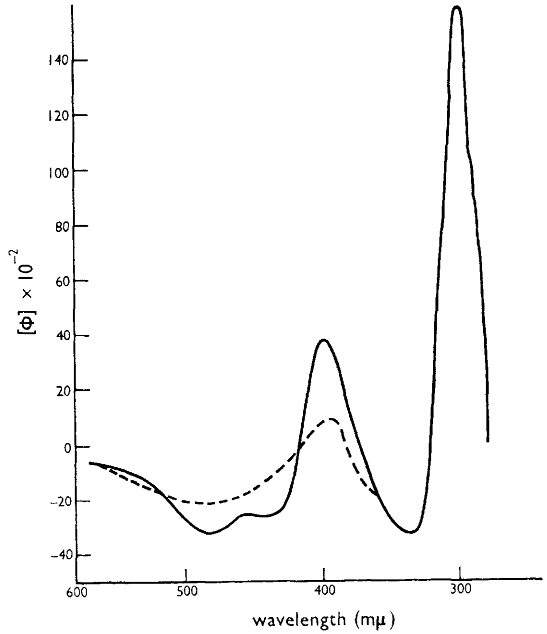
proximity. Since the side-chain of the flavine chromophore is optically active it is to be expected that the optical rotatory dispersion (o.r.d.) of FAD should depend on this intramolecular interaction.

TABLE

Cotton effect and fluorescence of flavine nucleotides

Compound	Solvent (temp. = 25°c)	Amplitude of Cotton effect $a = ([\Phi]_{peak}-[\Phi]_{trough})/100$	Relative fluorescence intensities
FAD	water (pH = 7·0)	70°	25
FAD	6M-urea (pH = 7·0)	31	50
FAD	DMSO/H_2O (50:50 v/v)	35	50
FMN	water (pH = 7·0)	~27	100
FMN	6M-urea (pH = 7·0)	~26	100
FMN	DMSO/H_2O (50:50 v/v)	small	80
Riboflavine	water (pH = 7·0)	small anomaly, amplitude not measurable	

We have studied the o.r.d.[3] of riboflavine, flavine mononucleotide (FMN), and FAD between 240 and 550 mμ and interpreted the data in the light of fluorescence studies. FAD exhibits multiple Cotton effects with peaks at 360 and 260 mμ and troughs at 430 and 280 mμ, and a negative shoulder at 400 mμ showing that each of the three absorption bands at 447, 370, and 260 mμ is optically active, although the Cotton effects associated with the two long-wavelength bands are not clearly resolved. FMN and riboflavine show similar anomalies in the 300 to 500 mμ region but the amplitudes of the Cotton effects are considerably smaller than for FAD (see the Table), and difficult to measure accurately. The shape of the o.r.d. curves and the amplitudes of the Cotton effects in the visible and near-ultraviolet region are the same for an equimolar mixture of FMN and adenosine-5′-phosphate as for FMN. This shows that the observed Cotton effects in FAD are not due to instrumental artefacts caused by high light absorption by the flavine chromophore. This is further supported by the lack of dispersion anomalies in an equimolar mixture of lumiflavine (6,7,9-trimethylisoalloxazine) and D-ribose, and by the fact that the amplitudes of the Cotton effects are linearly related to the concentration of FAD and FMN over a concentration range of $0·2 \times 10^{-4}$—$1·1 \times 10^{-4}$. (The optical densities corresponding to these concentrations at 447 mμ in a 1 cm. cell are between 0·25 and 1·25).



O.r.d. of flavine-adenine dinucleotide (———): *FAD in water;* (– – – –): *FAD in* 6M-*urea*

The relatively large anomalies in FAD compared to FMN appear to be associated with the intramolecular complex. This is supported by the following observations. The Cotton effects decrease in 6M-urea or in dimethyl sulphoxide (DMSO) as the solvent. Under these conditions the fluorescence intensity of FAD approaches that of FMN or riboflavine indicating that intramolecular quenching through complexing is less significant

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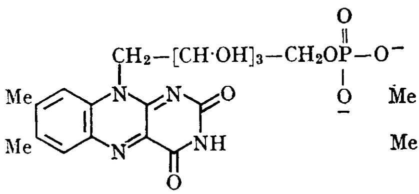
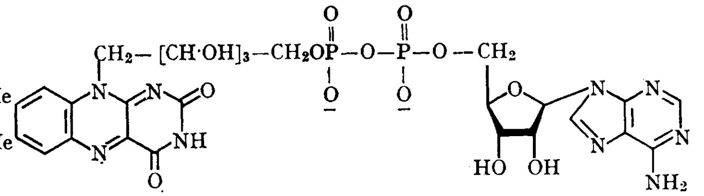
[3] O.r.d. measurements were made on a Bendix–Ericsson recording spectropolarimeter.

[4] S. F. Velick in "Light and Life," W. D. McElroy and B. Glass, ed., The Johns Hopkins Press, Baltimore, 1961, p. 108.

(*cf.* also ref. 4). Similarly the Cotton effects in the visible region disappear at pH = 12.



FMN



FAD

The small anomalies observed for FMN and riboflavine are probably also a result of some restriction on the rotation of the side-chain by the flavine ring, as suggested by e.s.r. data,[5] particularly since the optically-active side-chains are otherwise electronically insulated from the ring by the intervening methylene group.

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[5] A. Ehrenberg in "Electronic Aspects of Biochemistry," B. Pullman, ed., Academic Press, New York, 1964, p. 379.