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INTERNATIONAL ATOMIC ENERGY AGENCY
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
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H4.SMR/916 - 7

SEVENTH COLLEGE ON BIOPHYSICS:

Structure and Function of Biopolymers: Experimental and Theoretical Techniques.

4 - 29 March 1996

Applications of Circular Dichroism Spectroscopy to the Study of Chiral Organic Molecules and Biopolymers

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Bologna
ITALY

OPTICAL ACTIVITY

1. Detection of Chirality

2. Characterisation of Chirality
   (Registration, Patent, Identification)

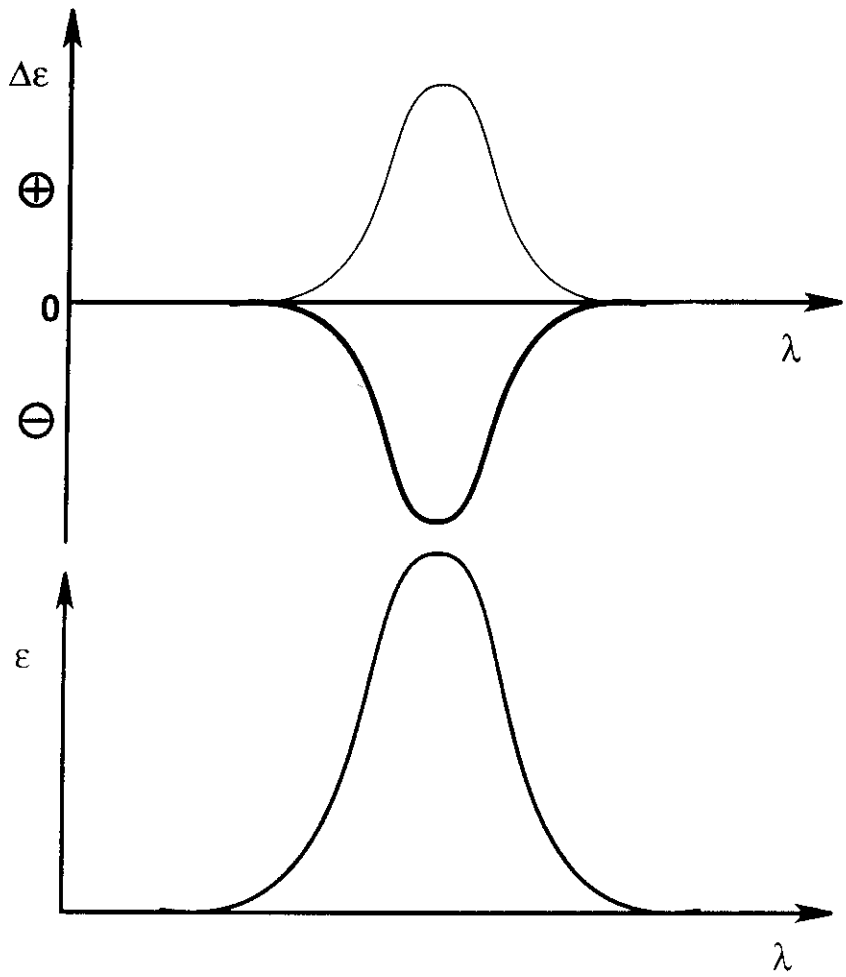
3. Determination of Absolute Stereochemistry
   (Absolute Configuration)

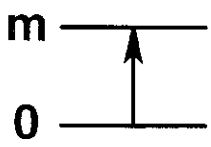
4. Biopolymer Conformation
   (Secondary Structure)

5. Self-assembly Processes

Optical rotation $\alpha = (n_l - n_r)\frac{\pi}{\lambda}$

Circular dichroism $\Delta\varepsilon = (\varepsilon_l - \varepsilon_r)$





experimental band area

$$R_{0m} = \text{cost} \cdot x \int \frac{\Delta\varepsilon}{\nu}\, d\nu$$

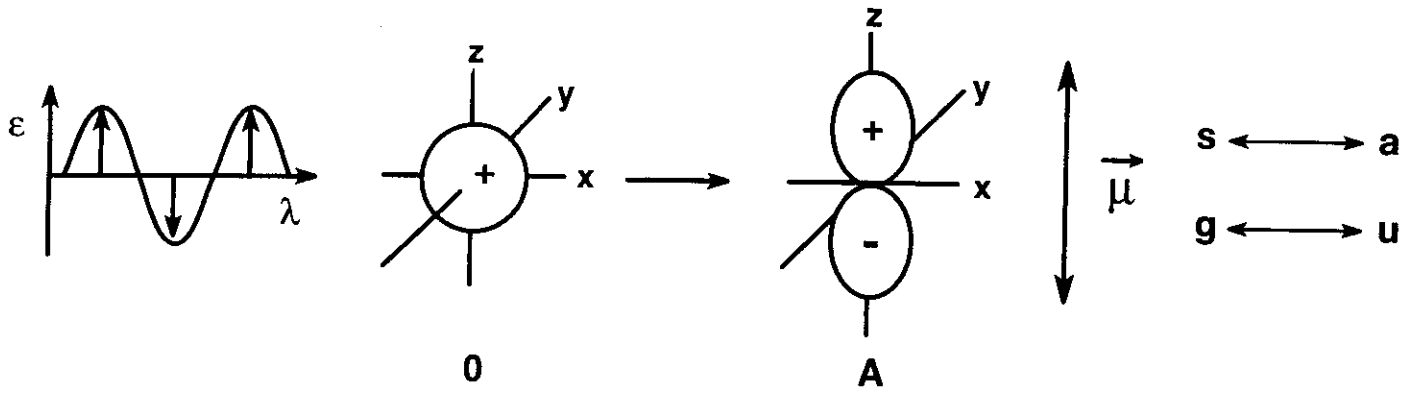
calculated band area

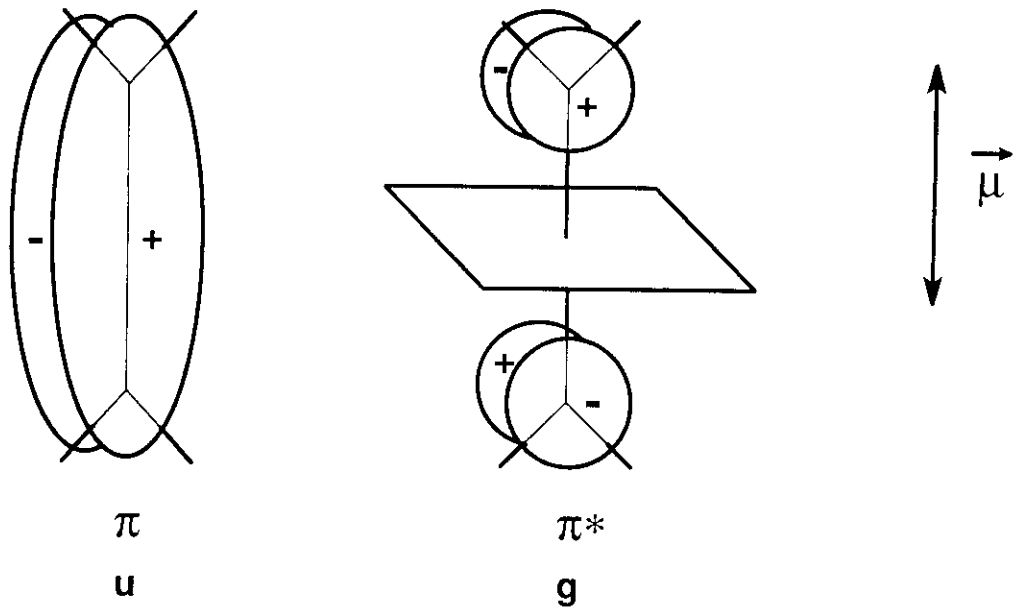
$$R_{0m} = \text{Im}\,\langle \Psi_0 | \hat{\mu} | \Psi_m \rangle \cdot \langle \Psi_m | \hat{\mu} | \Psi_0 \rangle$$

$$= \vec{\mu}_{0m} \cdot \vec{m}_{0m} = \mu\, m \cos\theta$$

$\theta = 0$ $R_{0m} = \mu m$ CD⊕

$\theta = 180$ $R_{0m} = -\mu m$ CD⊖





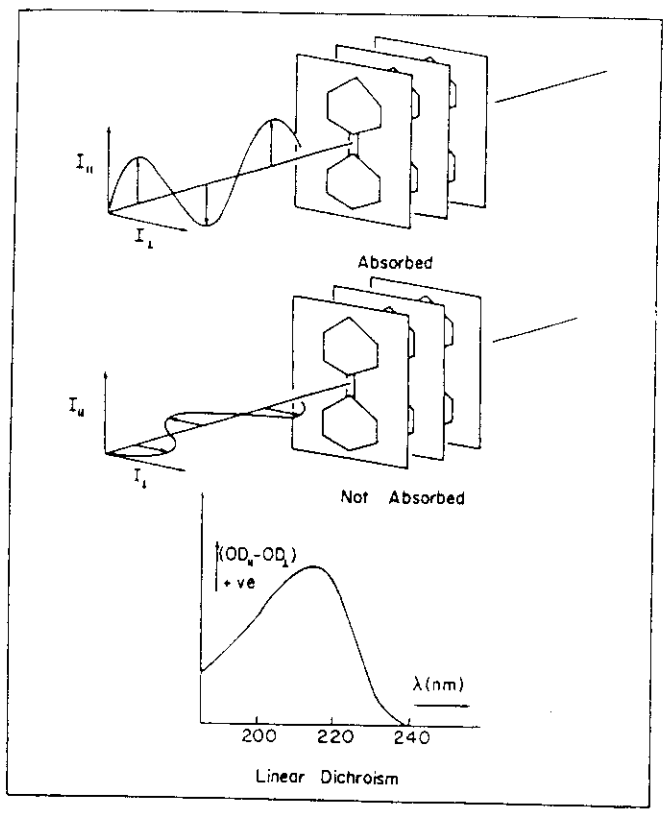
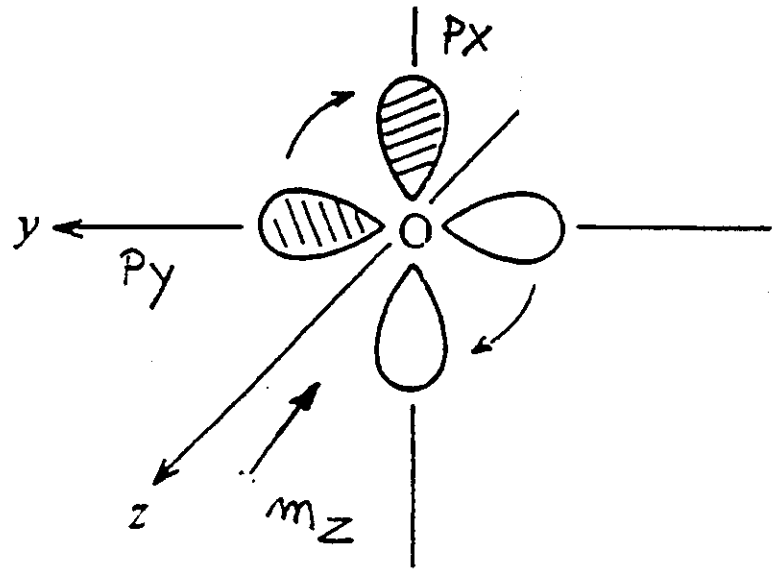
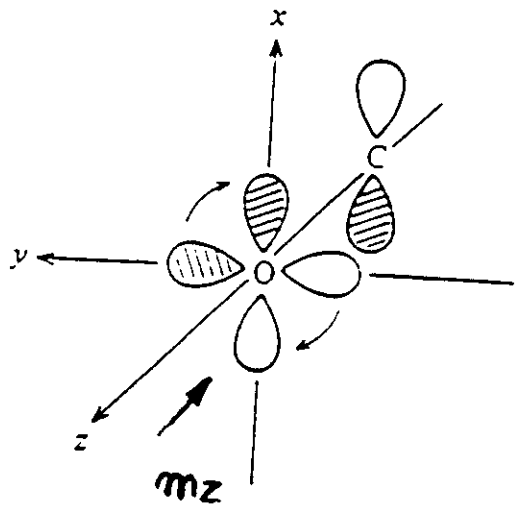


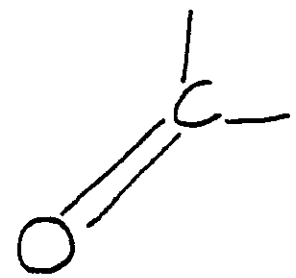
Figure 4. The linear dichroism of oriented Bicyclohexylidene molecules (A. F. Drake and G. Gottarelli)



u ⟷ u

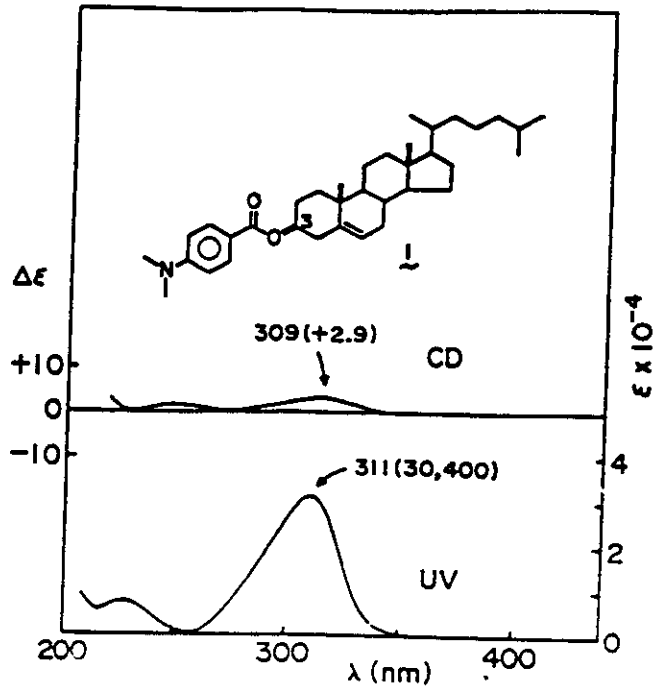
(g ⟷ g)

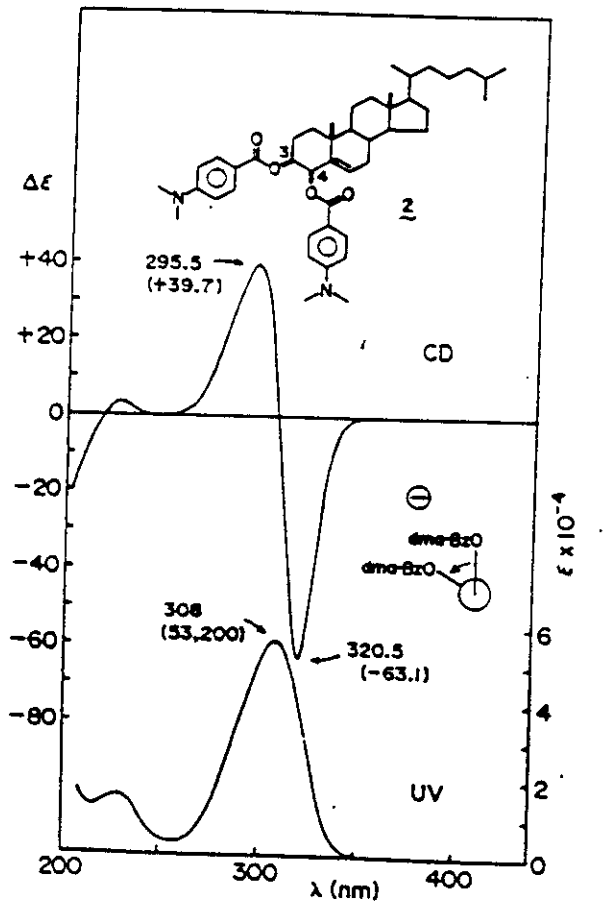




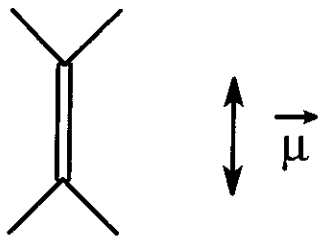
n → π*

ca. 300 nm


Δε
+10
0
−10
309(+2.9)
CD
311(30,400)
UV
200
300
400
λ (nm)
ε x 10⁻⁴
4
2
0
1

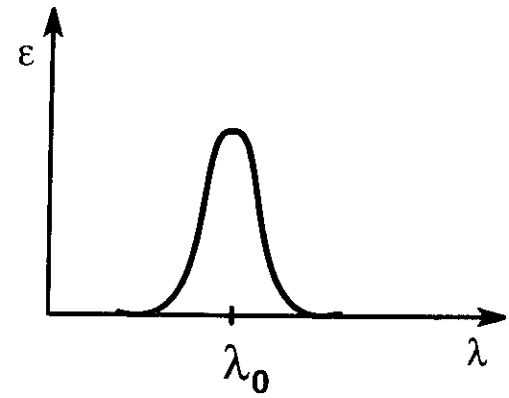

Δε
+40
+20
0
−20
−40
−60
−80
295.5
(+39.7)
CD
308
(53,200)
320.5
(−63.1)
UV
200
300
400
λ (nm)
ε x 10⁻⁴
6
4
2
0
2

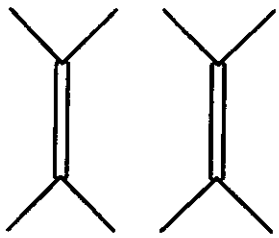
Coupled Oscillator



$\vec{\mu}$

$\pi \rightarrow \pi^*$





1 2

1 2

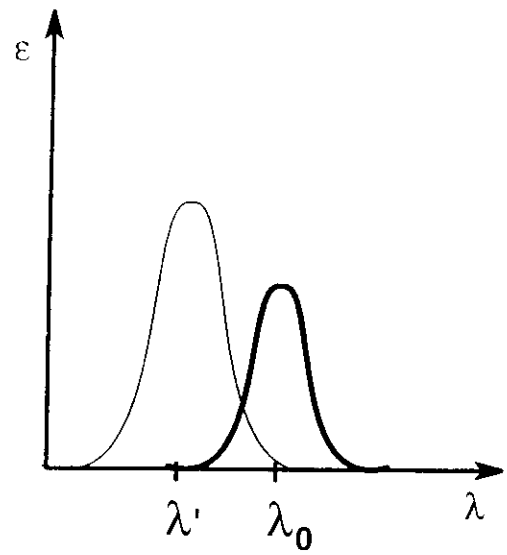
high-energy

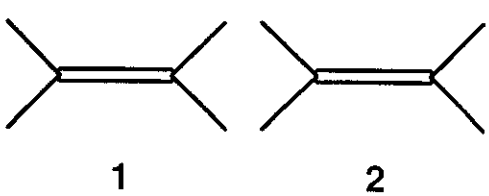
$\vec{\mu}_{ris}$

1 2

low-energy

$\vec{\mu}_{ris} = 0$





1 2

$\vec{\mu}_{ris}$

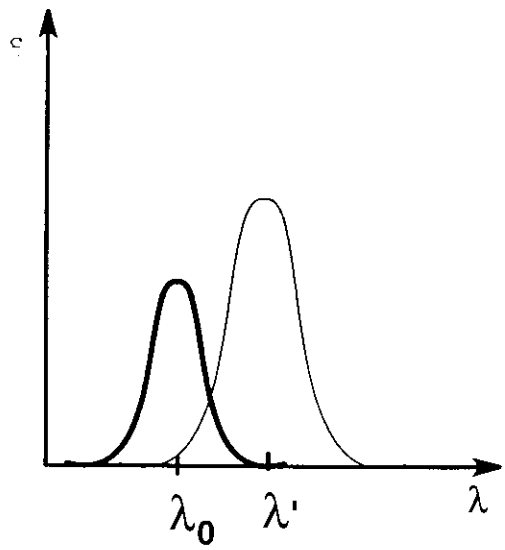
1 2

low-energy

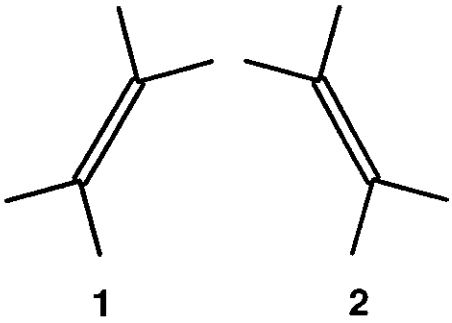
$\vec{\mu}_{ris} = 0$

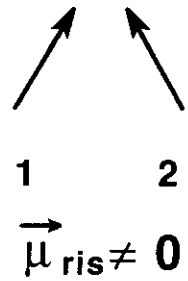
1 2

high-energy

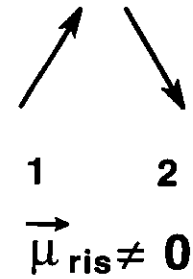


Coplanar

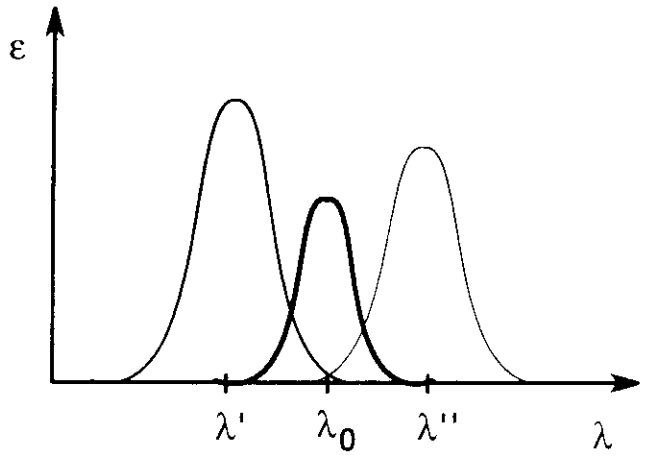




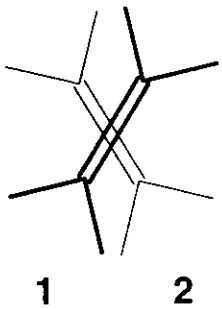
high-energy

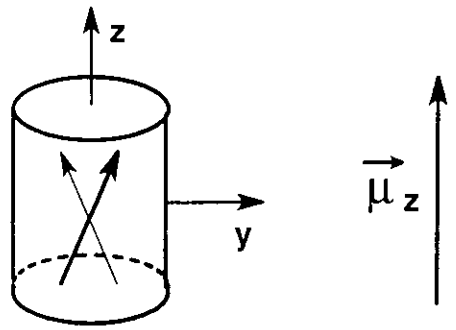


low-energy

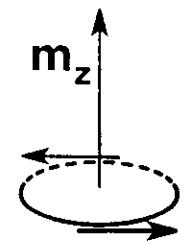


Non coplanar and chiral

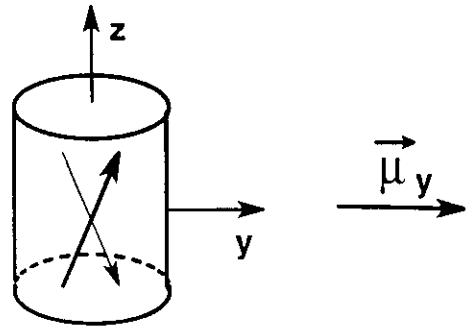




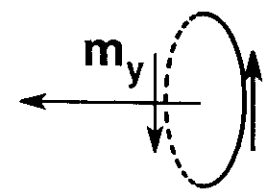
high-energy



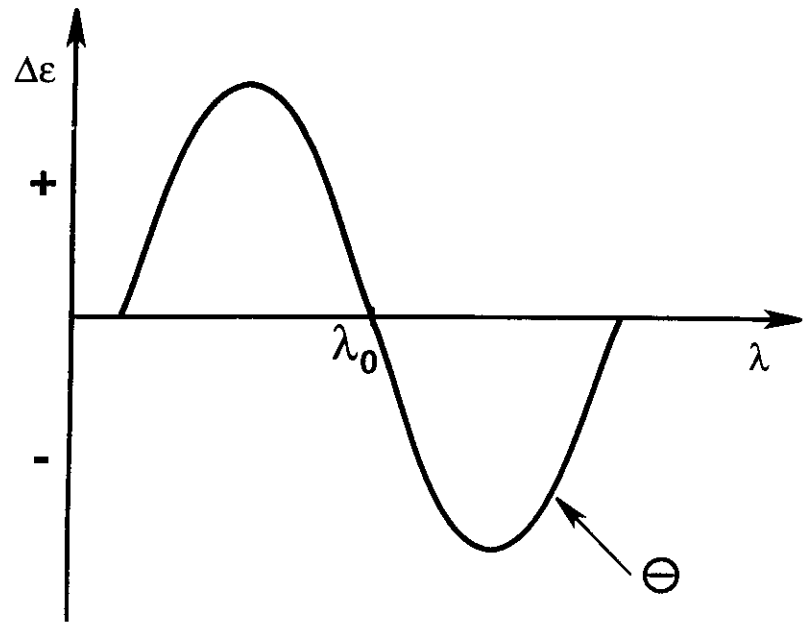
R positive (cos θ = 1)



low-energy

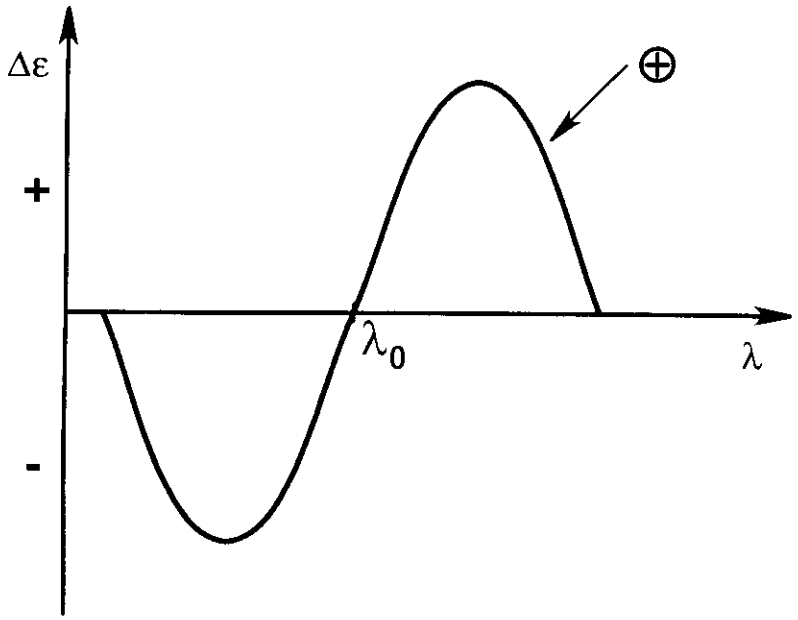


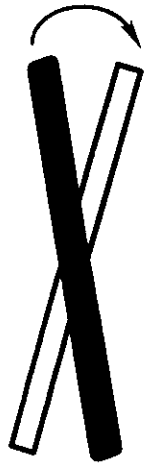
R negative (cos θ = -1)



⊖

negative chirality



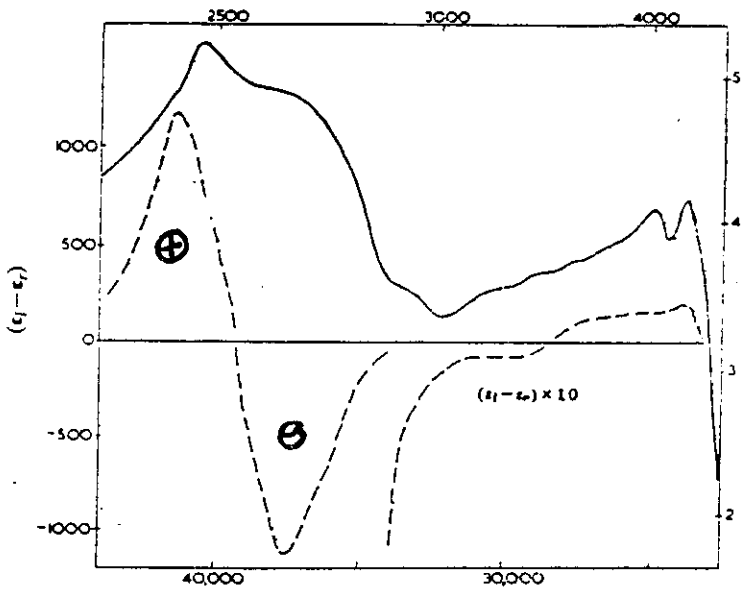


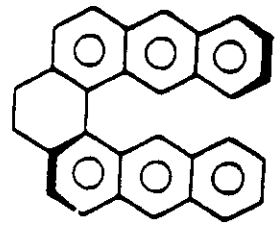
⊕

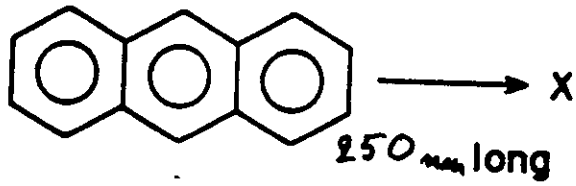
positive chirality

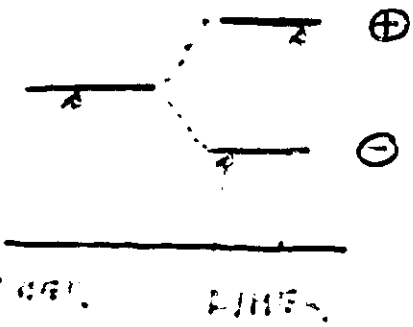
Kuhn 1930

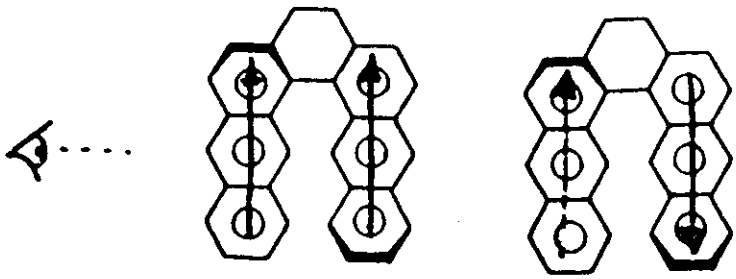
$$R = \vec{\mu} \cdot \vec{m}$$

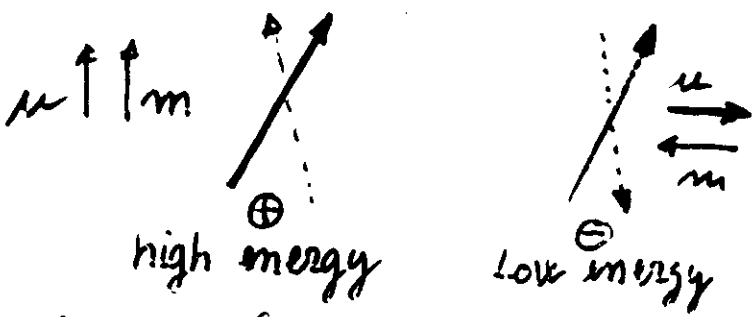












Mason, Grinter, Trans. Faraday Soc., 1964, 274.

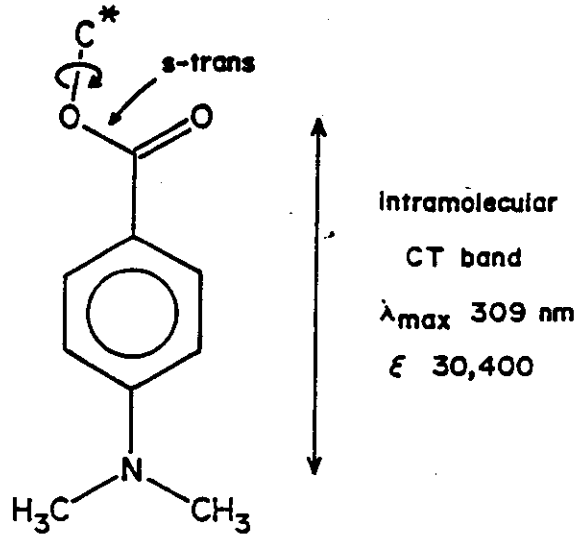


Figure 1-7. Direction of the transition dipole moment in p-dimethylaminobenzoate chromophore.

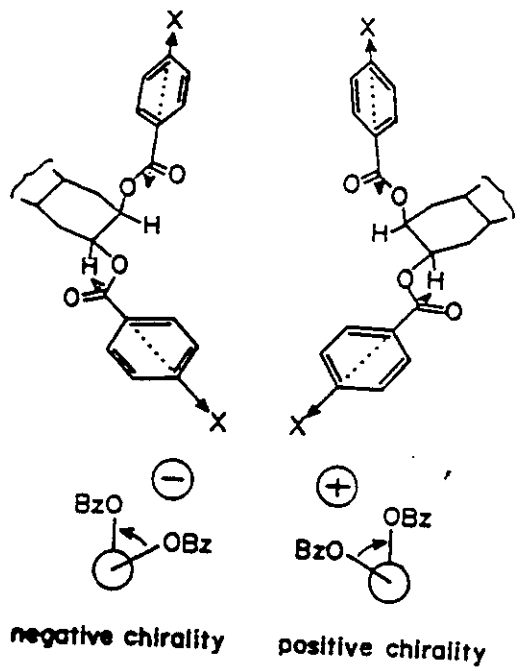
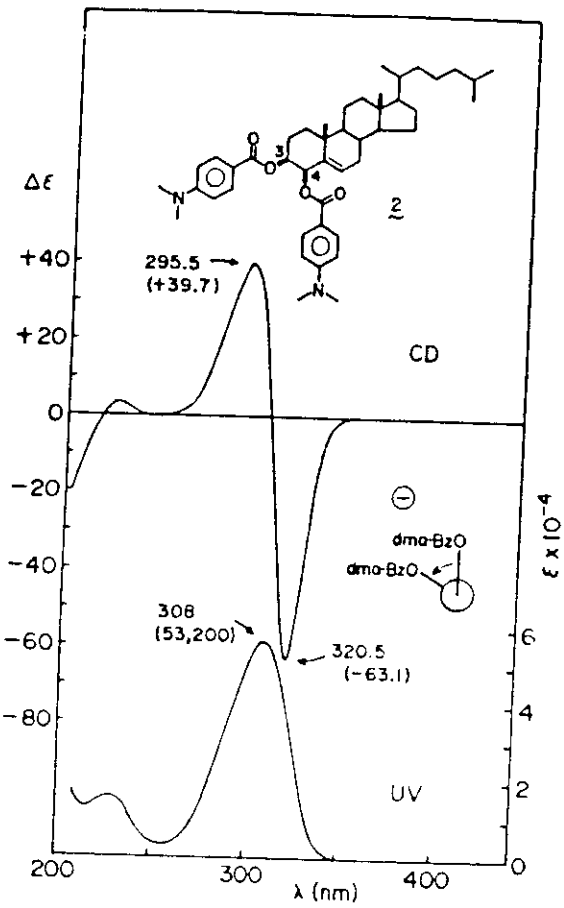
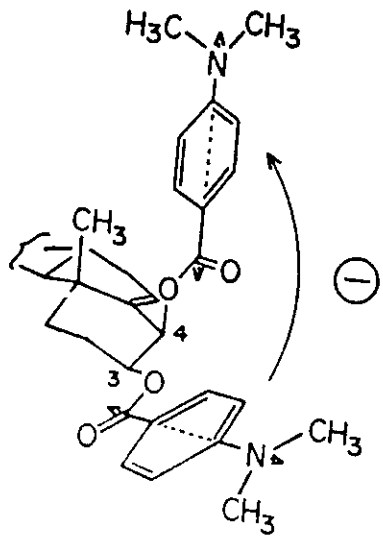
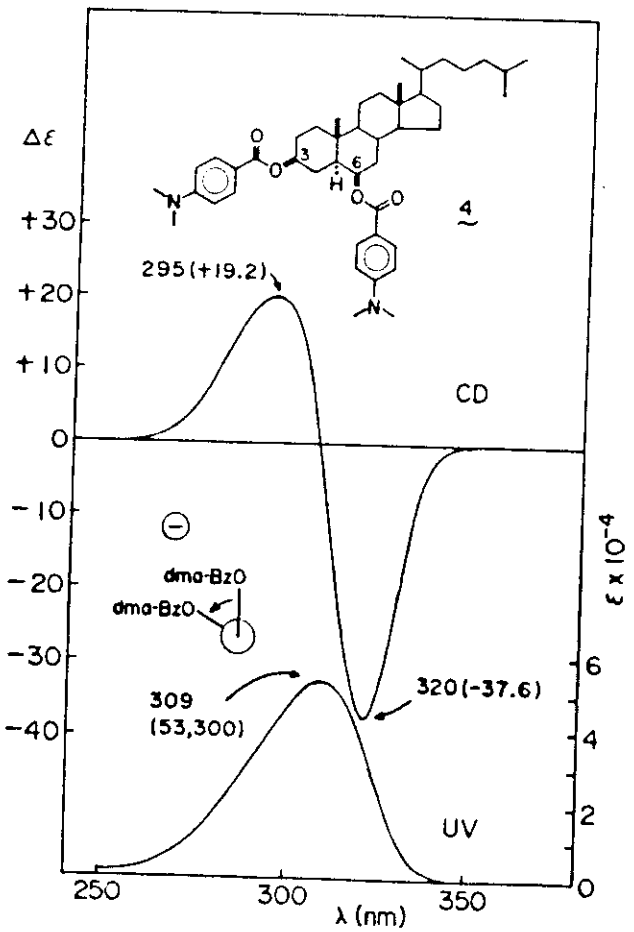
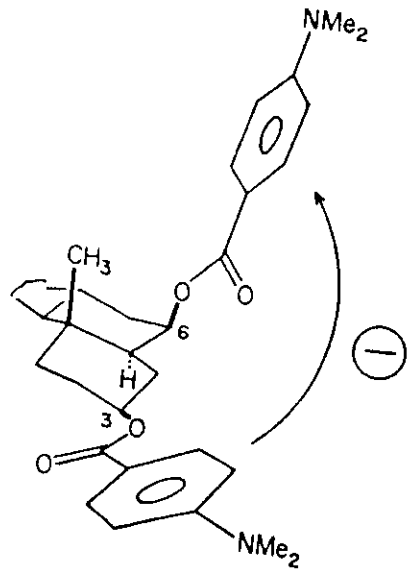


Figure 1-2. Chiralities of α-glycol dibenzoates. [Reprinted from reference 18.]


Δε
+40
+20
0
-20
-40
-60
-80
295.5
(+39.7)
CD
dma-BzO
dma-BzO
308
(53,200)
320.5
(-63.1)
UV
ε x 10⁻⁴
6
4
2
0
200
300
400
λ (nm)


H3C
CH3
N
CH3
O
O
4
3
O
O
N
CH3
CH3


Δε
+30
+20
+10
0
-10
-20
-30
-40
295(+19.2)
CD
dma-BzO
dma-BzO
309
(53,300)
320(-37.6)
UV
ε x 10⁻⁴
6
4
2
0
250
300
350
λ (nm)


NMe2
CH3
O
O
6
H
3
O
O
NMe2

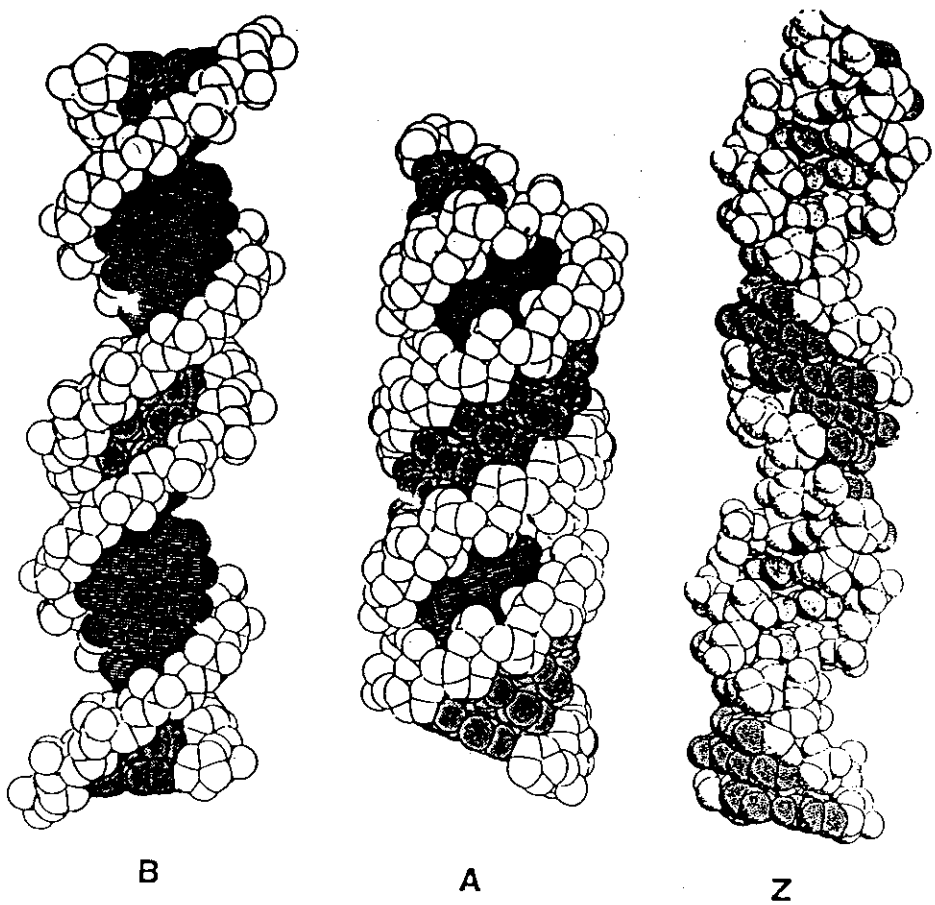


Figure 2.7 Three well-known (but highly idealized) forms of DNA: "B" and "A" are right-handed with 10 and 11 phosphates per helical turn, respectively, while "Z" is left-handed with 12 phosphates per turn. Real right-handed DNA in solution averages about 10.5 phosphates per turn, or halfway between "B" and "A". Pictures of "A," "B" from C.J. Alden and S-H. Kim (1979) *Journal of Molecular Biology* 132, 411–34. Picture of "Z" from H.R. Drew and R.E. Dickerson (1981) *Journal of Molecular Biology* 152, 723–36 (with atoms shown somewhat smaller).

Worked Example 10.4 CD Spectra of DNA

Nucleic acid bases are intrinsically optically inactive, but they are induced to have activity in polynucleotides. Such spectra normally show both a positive band around 270 nm and a negative band around 240 nm because of stacking interactions among the bases, which cause a splitting of the $n \rightarrow \pi^*$ transitions. Figure 10.9 shows the CD spectra of the A and B forms of DNA, which are obtained by changing the humidity of the sample from 75% to 92%. Why do these changes occur?

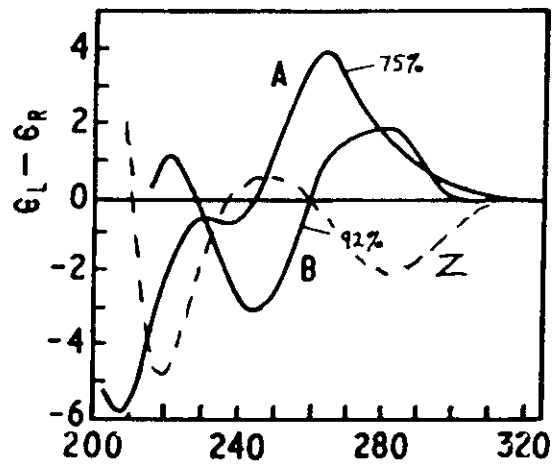
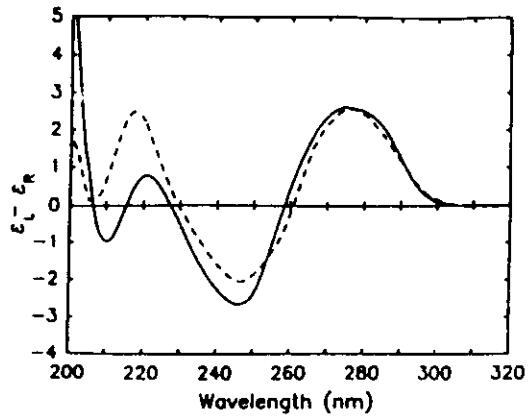


Figure 10.9

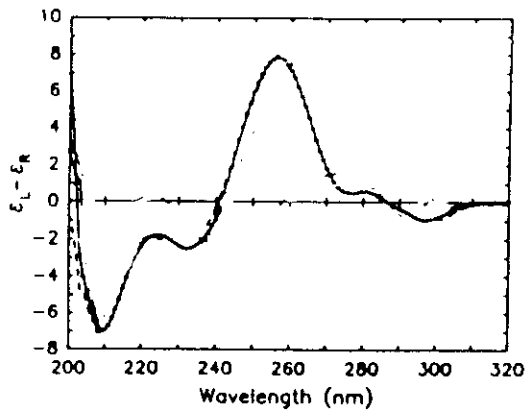
Solution

The CD spectra of DNA arise from the helical structure, which provides an asymmetric environment for the bases, resulting in optical activity. The rotational strength of a transition depends on the interaction of transition dipoles between adjacent groups and therefore on the geometrical relationship between them (see Appendix V). Any changes in the angle between adjacent chromophores alters the transition probability, the position of the absorption bands, and also the rotational strengths. The CD spectrum is therefore very sensitive to local environment. The changes in the spectrum with hydration imply that the structure of the DNA has been altered. (In the A form, there are about 11 residues per turn in a double helix, with the base pairs tilted 20° away from the perpendicular to the helix axis. In the B form, the double helix has about 10 residues per turn, with the bases perpendicular to the helix axis).



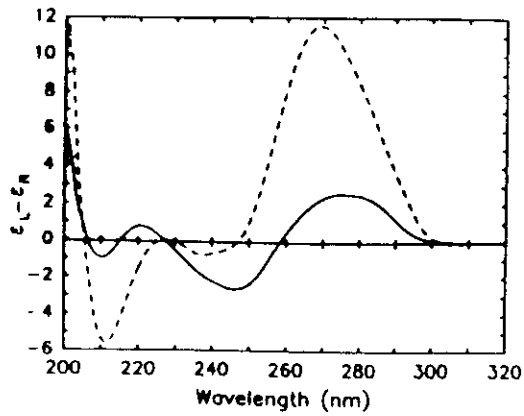
DNA B

FIG. 1. CD spectra of T7 phage DNA before (—) and after (- - -) heat denaturation. The DNA was in 2 m*M* Na^+ (phosphate buffer), pH 7. The extinction coefficient at 260 nm was 6570 M^{-1} cm^{-1} for the native DNA. In all figures, data are plotted as $\varepsilon_L - \varepsilon_R$ in units of M^{-1} cm^{-1}, per molar concentration of nucleotide. Unless otherwise noted, spectra in this and subsequent figures were taken at 20°.



RNA

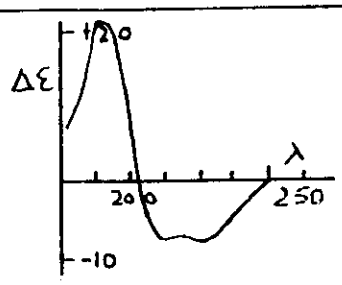
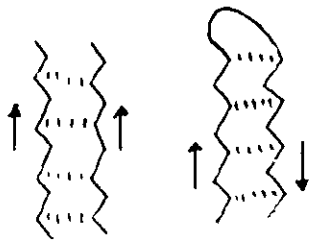
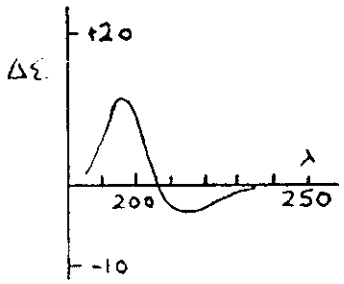
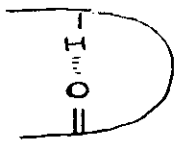
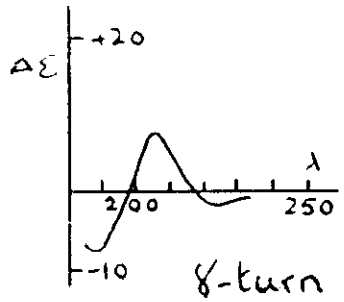
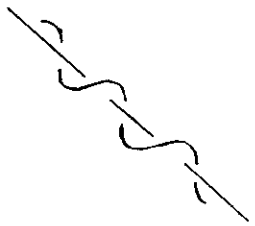
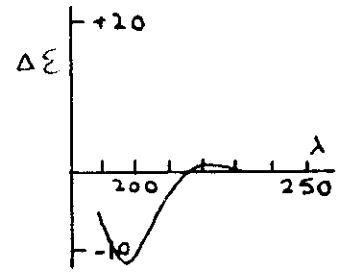
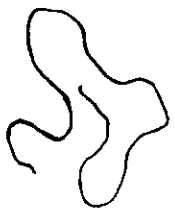
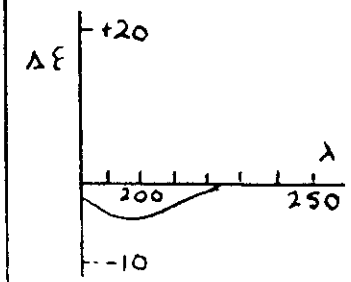
poly [r(AC) r(G·U)]



B → A

FIG. 4. CD spectra of T7 phage DNA at 0% ethanol (—) and at 80% (w/w) ethanol (- - -).

PROTEIN CONFORMATION AND CD SPECTRA

CONFORMATION	SHAPE	CD
α-helix (H-bonded) H		
β-sheet (parallel and anti-parallel) (H-bonded) P, A		
β-turn (Type I, II, III, IV...) (Some turns in proteins not H-bonded) γ-turn (H-bonded) T		
Left-handed helix (Polyproline II) (3_1-helix) (Not H-bonded)		
Irregular structure (Not H-bonded)		

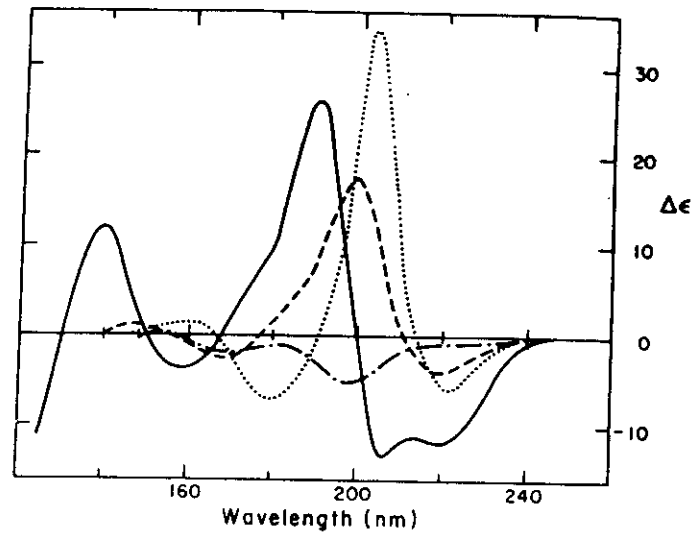


Figure 36. CD spectra of repeating polypeptides with a definite secondary structure in solution. The α-helix (————) is an average of poly(γ-methyl-L-glutamate) in F_6iPrOH (from Johnson and Tinoco, 1972) and poly(L-alanine) as a film (from Young and Pysh, 1973). The antiparallel β-sheet (- - -) is BOC (L-alanine)$_7$OMe as a film (redrawn from Balcerski et al., 1976). The parallel β-sheet (· · · ·) is BOC(L-valine)$_7$OMe as a film (redrawn from Balcerski et al., 1976). The unordered collagen in F_3iPROH (· - · -) is redrawn from Jenness et al. (1976).

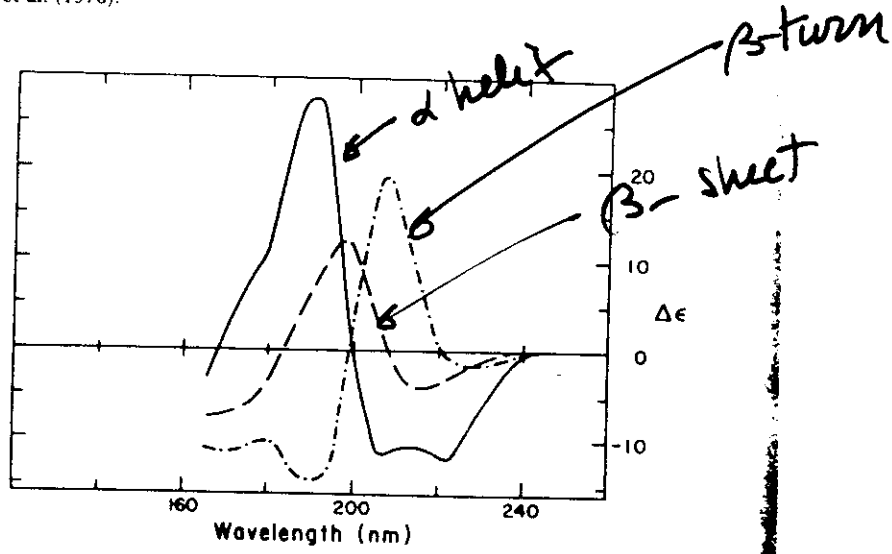
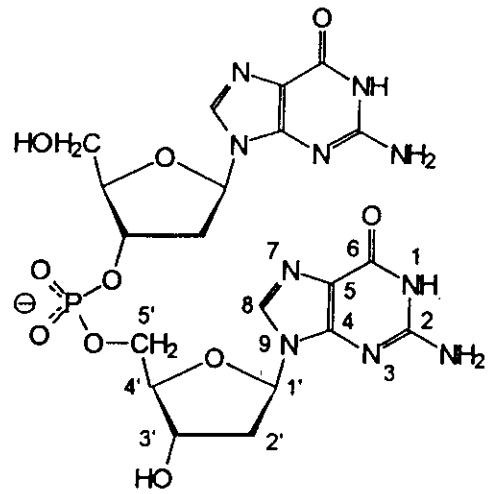
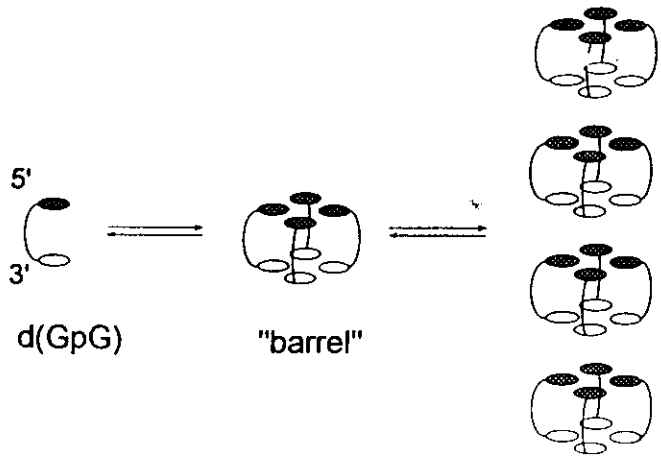


Figure 37. CD spectra for definite secondary structures is aqueous solution. The α-helix (————) is poly(L-glutamic acid) at pH 4.5 (redrawn from Johnson and Tinoco, 1972). The antiparallel β-sheet (— — —) is poly(L-lysine-L-leucine) in 0.1 *M* NaF at pH 7 (redrawn from Brahms et al., 1977). The β-turn (· - · -) is poly(L-alanine$_2$-glycine$_2$) in 0.1 *M* NaF at pH 7 (redrawn from Brahms et al., 1977).



d(GpG)

Chart



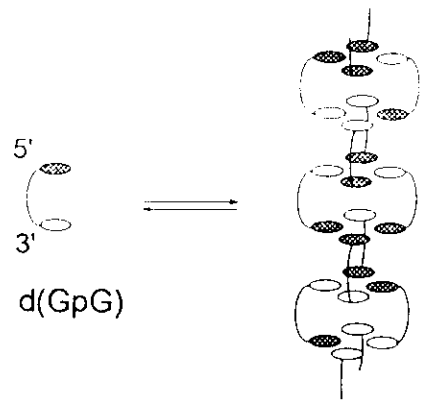


Figure 6

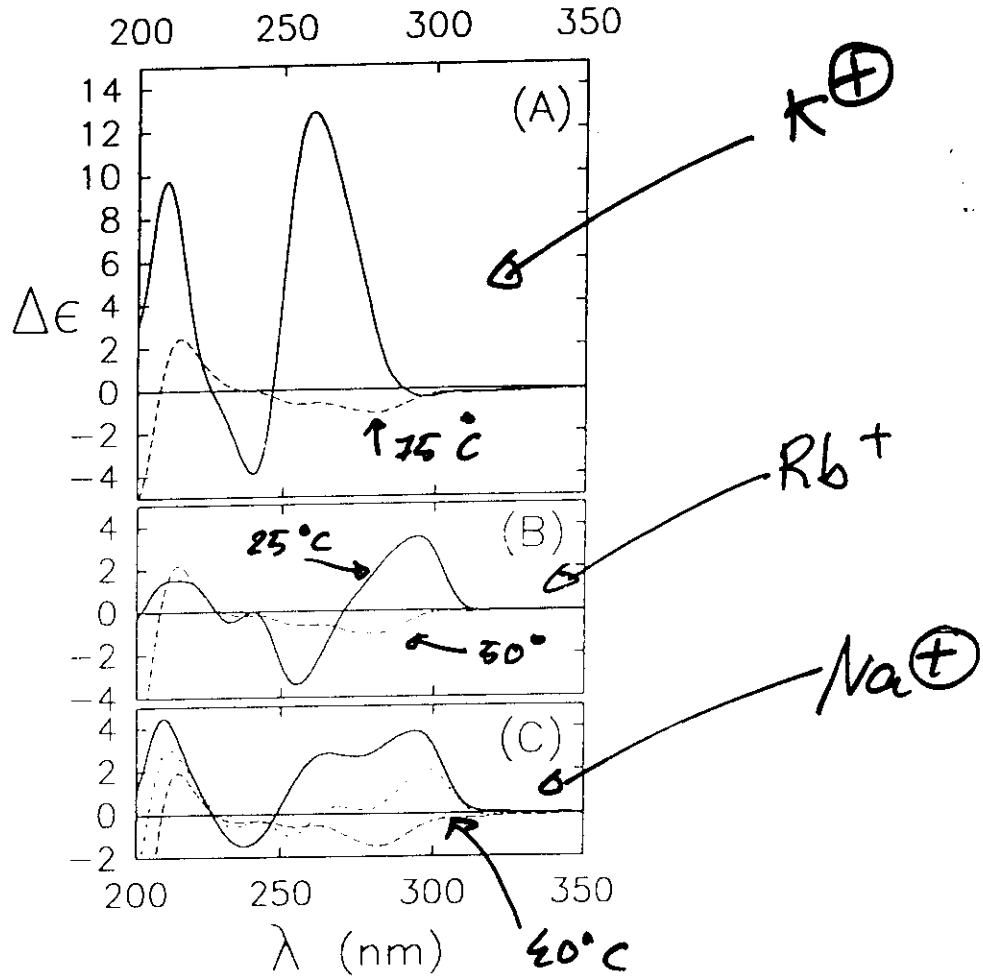


Figure 8

d(GpG)

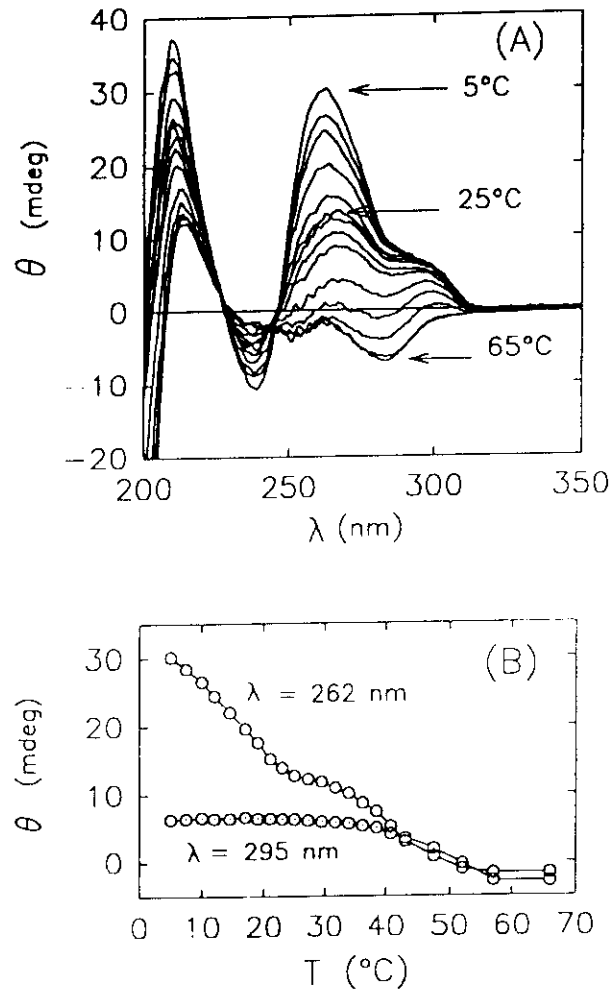

(A)
5°C
25°C
65°C
θ (mdeg)
λ (nm)
40
30
20
10
0
−10
−20
200
250
300
350
(B)
λ = 262 nm
λ = 295 nm
θ (mdeg)
T (°C)
30
20
10
0
0 10 20 30 40 50 60 70

Figure 4