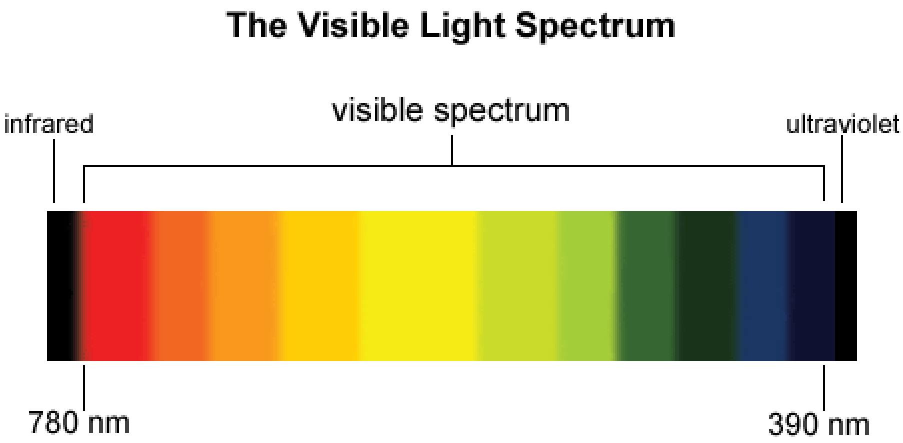
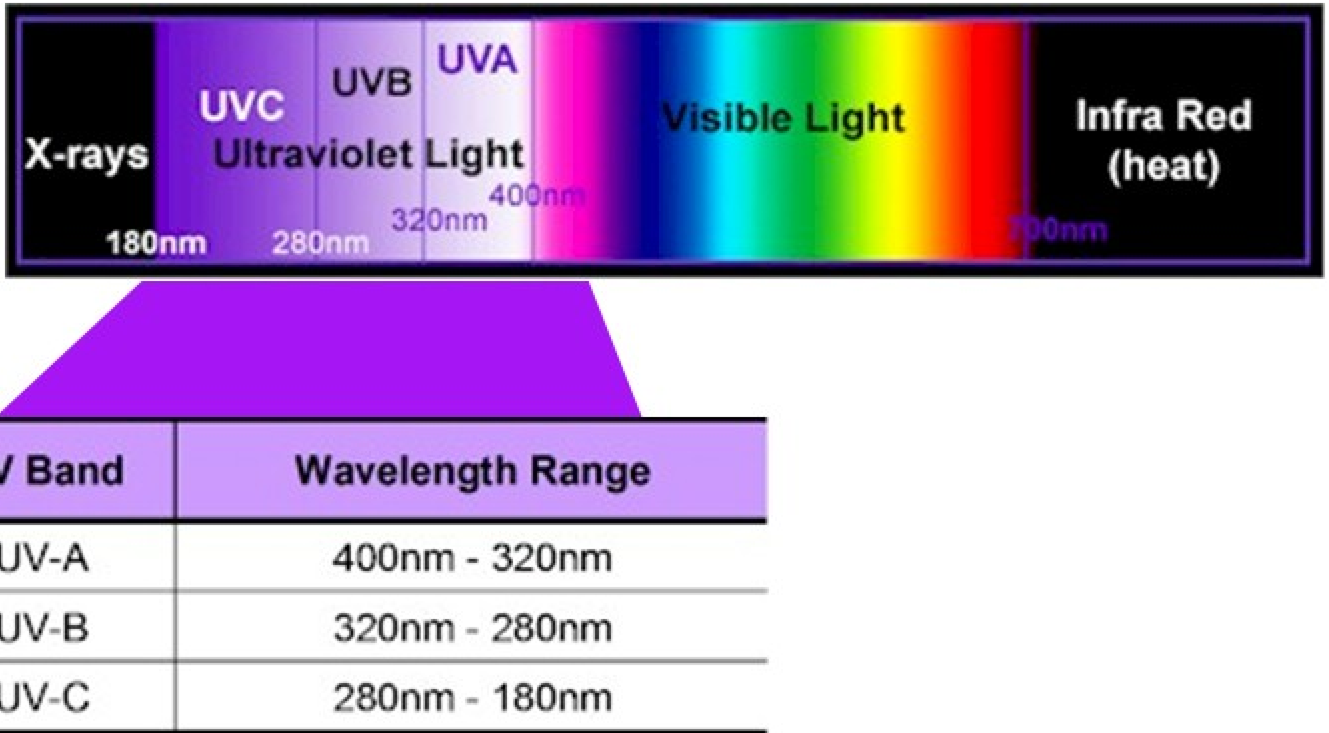
Optical and Ultraviolet Spectroscopy

Visible region:


The Visible Light Spectrum

Ultraviolet region:



UV Band	Wavelength Range
UV-A	400nm - 320nm
UV-B	320nm - 280nm
UV-C	280nm - 180nm

TABLE 10-2

Definitions of Terms and Equations

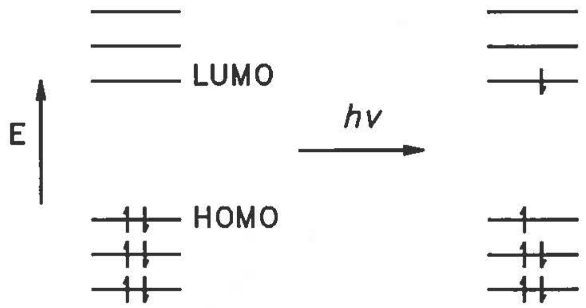
Quantity	*Unit of measure*	*Dimensions*
Wavelength (λ)	Nanometer (nm)	1 nm = 10^{-9} m
	Ångstrom (Å)	1 Å = 10^{-10} m
Frequency (ν)	Hertz, Hz, or s^{-1}	Cycles per second
Energy	Depends on the units of h	6.626×10^{-27} erg s 6.626×10^{-34} J s 9.534×10^{-14} kcal–s/mol 1.583×10^{-34} cal–s/molec

TABLE 10-3

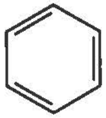
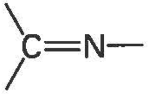
Useful Conversion Factors

cm^{-1}	*Hz*	*kcal mol^{-1}*	*kJ mol^{-1}*
1	3.00×10^{10}	2.86×10^{-3}	1.20×10^{-2}
3.33×10^{-11}	1	9.53×10^{-14}	3.99×10^{-13}
3.50×10^{2}	1.05×10^{13}	1	4.18

Figure 10-2 Idealized representation on a potential energy scale of occupied and unoccupied molecular orbitals in the electronic ground state (left) and electronic configuration of an excited state arising by promotion of an electron from the highest occupied molecular orbital to the lowest unoccupied molecular orbital (right). The electrons and their relative spin ori-



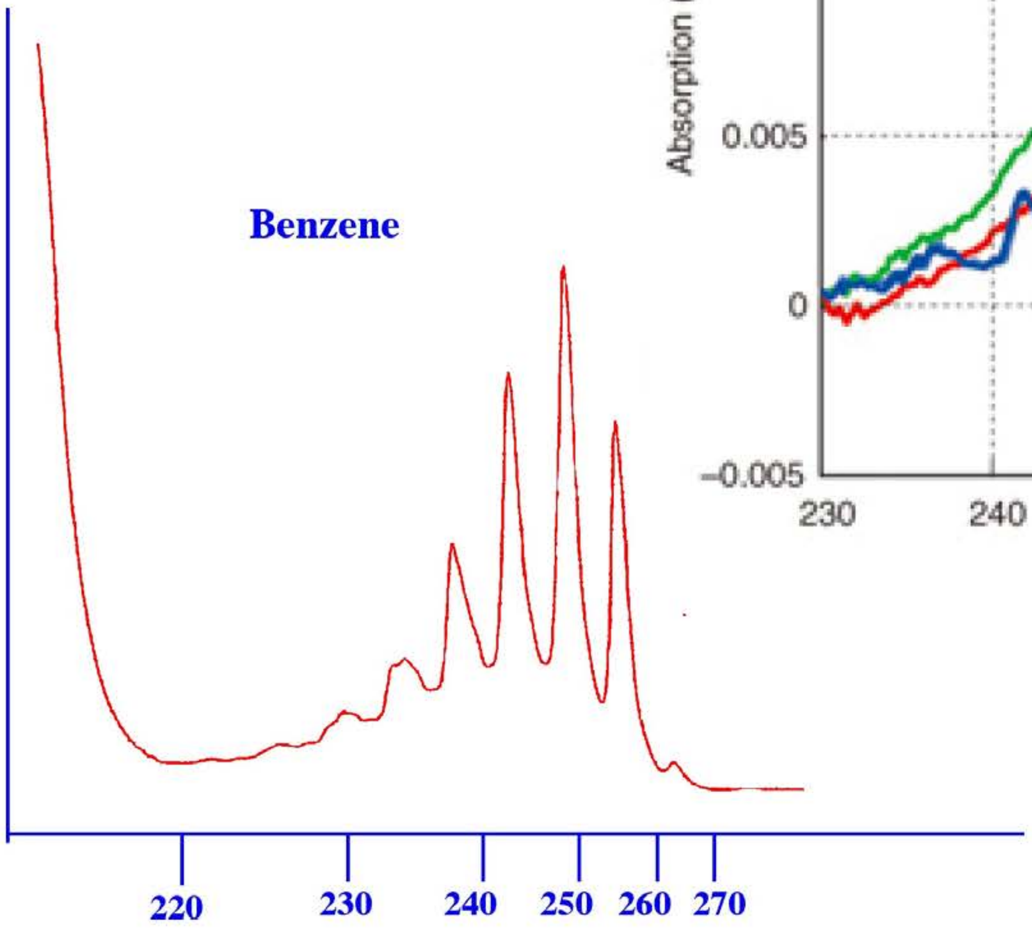
	UV Cutoff (nm)		UV Cutoff (nm)
Acetonitrile UV	190	Ethyl Ether	215
Pentane	190	Iso-Octane	215
Water	190	*n*-Butyl Chloride	220
Hexane UV	195	Glyme	220
Cyclopentane	198	Isobutyl Alcohol	220
Cyclohexane	200	Propylene Carbonate	220
Heptane	200	Ethylene Dichloride	228
Isopropyl Alcohol	205	1,1,2-Trichlorotrifluoroethane	231
Methanol	205	Dichloromethane	233
Ethyl Alcohol	210	Chloroform	245
2-Methoxyethanol	210	*n*-Butyl Acetate	254
Methyl *t*-Butyl Ether	210	Ethyl Acetate	256
n-Propyl Alcohol	210	Dimethyl Acetamide	268
Trifluoroacetic Acid	210	*N*,*N*-Dimethylformamide	268
Tetrahydrofuran UV	212	Dimethyl Sulfoxide	268
n-Butyl Alcohol	215	Toluene	284
1,4-Dioxane	215		

Chromophore	*Example*	*Solvent*	*λ_{max} (nm)*†	*ε (liter mol^{-1} cm^{-1})*
$C{=}C$	1-Hexene	Heptane	180	12,500
$—C{\equiv}C—$	1-Butyne	Vapor	172	4,500
	Benzene	Water	254	205
			203.5	7,400
	Toluene	Water	261	225
			206.5	7,000
$C{=}O$	Acetaldehyde	Vapor	298	12.5
			182	10,000
	Acetone	Cyclohexane	275	22
			190	1,000
	Camphor	Hexane	295	14
$—COOH$	Acetic acid	Ethanol	204	41
$—COCl$	Acetyl chloride	Heptane	240	34
$—COOR$	Ethyl acetate	Water	204	60
$—CONH_2$	Acetamide	Methanol	205	160
$—NO_2$	Nitromethane	Hexane	279	15.8
			202	4,400
$=\overset{+}{N}=\overset{-}{N}$	Diazomethane	Diethyl ether	417	7
$—N{=}N—$	*trans*-Azomethane	Water	343	25
	$C_2H_5CH—NC_4H_9$	Isooctane	238	200

* From J.B. Lambert, H.F. Shurvell, L. Verbit, R.G. Cooks, and G.H. Stout, *Organic Structural Analysis*, Macmillan Publishing, New York, 1976.
† Chromophores often have more than one absorption band.

a = εbc, where ε = extinction coefficient, b = path length, c = concentration and a =log(1/T)

or a = 2-log(%T)


Benzene
220
230
240
250
260
270

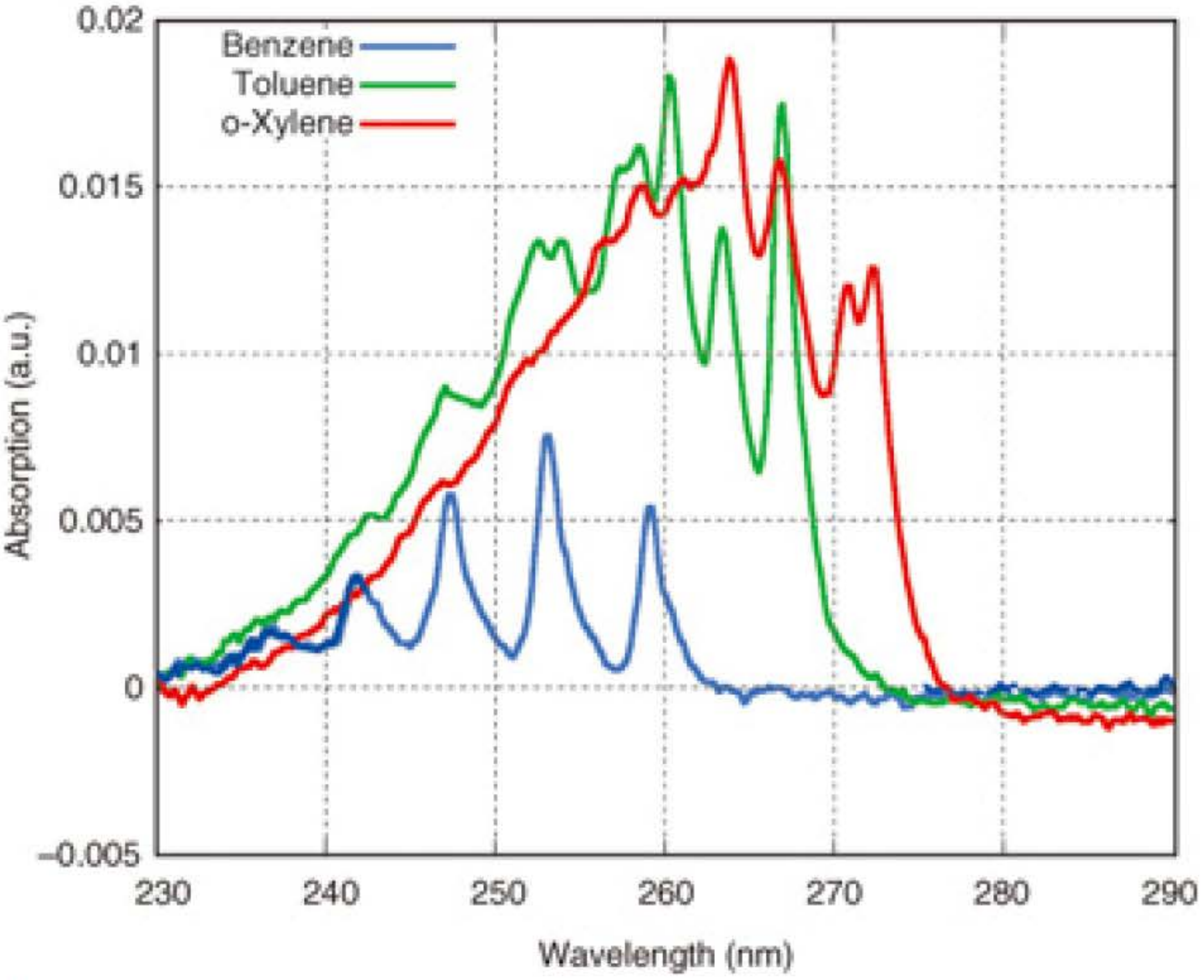

Benzene
Toluene
o-Xylene
Absorption (a.u.)
0.02
0.015
0.01
0.005
0
−0.005
230
240
250
260
270
280
290
Wavelength (nm)

TABLE 11-9
Absorption Data for Benzene and Derivatives*†

Compound	Solvent	λ_{max} (nm)	ε_{max}	λ_{max} (nm)	ε_{max}	λ_{max} (nm)	ε_{max}	λ_{max} (nm)	ε_{max}
Benzene	Hexane	184	68,000	204	8,800	254	250		
	Water	180	55,000	203.5	7,000	254	205		
Toluene	Hexane	189	55,000	208	7,900	262	260		
	Water			206	7,000	261	225		
Ethylbenzene	Ethanol‡			208	7,800	260	220		
tert-Butylbenzene	Ethanol			207.5	7,800	257	170		
o-Xylene	25% methanol			210	8,300	262	300		
m-Xylene	25% methanol			212	7,300	264	300		
p-Xylene	Ethanol			216	7,600	274	620		
1,3,5-Trimethylbenzene	Ethanol			215	7,500	265	220		
Fluorobenzene	Ethanol			204	6,200	254	900		
Chlorobenzene	Ethanol			210	7,500	257	170		
Bromobenzene	Ethanol			210	7,500	257	170		
Iodobenzene	Ethanol			226	13,000	256	800		
	Hexane			207	7,000	258	610	285(sh)	180
Phenol	Water			211	6,200	270	1,450		
Phenoxide ion	aq NaOH			236	9,400	287	2,600		
Aniline	Water			230	8,600	280	1,400		
	Methanol			230	7,000	280	1,300		
Anilinium ion	aq acid			203	7,500	254	160		
N,N-Dimethylaniline	Ethanol			251	14,000	299	2,100		
Thiophenol	Hexane			236	10,000	269	700		
Anisole	Water			217	6,400	269	1,500		
Benzonitrile	Water			224	13,000	271	1,000		
Benzoic acid	Water			230	10,000	270	800		
	Ethanol			226	9,800	272	850		
Nitrobenzene	Hexane			252	10,000	280(sh)	1,000	330(sh)	140
Benzaldehyde	Hexane			242	14,000	280	1,400	328	55
	Ethanol			240	16,000	280	1,700	328	20
Acetophenone	Hexane			238	13,000	276	800	320	40
	Ethanol			243	13,000	279	1,200	315	55
Styrene	Hexane			248	15,000	282	740		
	Ethanol			248	14,000	282	760		
Cinnamic acid									
cis-	Hexane	200	31,000	215	17,000	280	25,000		
trans-	Hexane	204	36,000	215	35,000	283	56,000		
	Ethanol			215	19,000	268	20,000		
Stilbene									
cis-	Ethanol			225	24,000	274	10,000		
trans-	Heptane	202	24,000	228	16,000	294	28,000		
Phenylacetylene	Hexane	202	44,000	248	17,000	hidden			
2,2'-Dimethylbiphenyl	Hexane	198	43,000	228(sh)	6,000	264	800		
Diphenylmethane	Ethanol			220	10,000	262	500		

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* From J.B. Lambert, H.F. Shurvell, L. Verbit, R.G. Cooks, and G.H. Stout, *Organic Structural Analysis,* Macmillan Publishing, New York, 1976.

† If vibrational structure is present, λ_{max} refers to the subband of highest intensity.

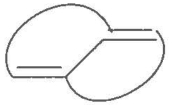
‡ "Ethanol" should be taken to mean 95% ethanol.

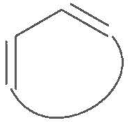
Woodward’s Rules

TABLE 9.4 Calculation of π-π^* λ_{max} (nm) for Conjugated Polyenes

Base Values (nm)


Acyclic 217


Heteroannular 214


homoannular 253

Groups	*Increments (nm)*
Extended conjugation (per C=C)	+30
Each exocyclic C=C	+5
–Alkyl	+5
$-OCOCH_3$	+0
–O–Alkyl	+6
–S–Alkyl	+30
–Cl, –Br	+5
$-N(Alkyl)_2$	+60

Solvent correction: none

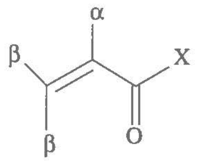
TABLE 9.3 Summary of Empirical Schemes to Identify Substructures by UV λ_{max}

Chromophore	λ_{max} *Base Value (nm)*	*Type of Information*	*Reference*
$H_2C{=}CH{-}(HC{=}CH)_n{-}HC{=}CH_2$	n=0, 214 or 217	Length of chromophore, type and number of substituents	Table 9.4
$H_2C{=}CH{-}(HC{=}CH)_n{-}XC{=}O$	n=0, X=H, 207 n=0, X=R, 215 n=0, X=OR, 200	Length of chromophore, type and number of substituents	Table 9.5
Ar–X	X=H, 254	Location of multiple substituents with *n* or π electrons	Table 9.7

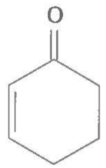
TABLE 9.5 Rules for Calculation of π-π^* λ_{max} (nm) for Conjugated Carbonyls

Base Values

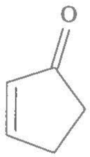
X = H, 207
X = R, 215
X = OH, 193
X = OR, 193



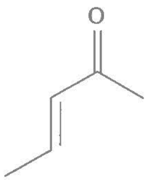
215



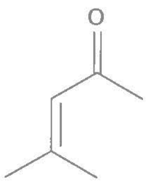
202

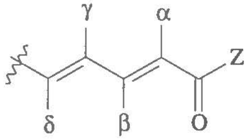


227



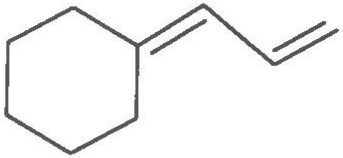
239





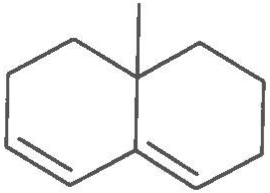
Groups	Increments (nm) α	β	γ	δ and Higher
Extended Conjugation per C=C	+30			
Each exocyclic C=C	+5			
Homoannular diene	+39			
Alkyl	+10	+12	+18	+18
OH	+35	+30		+50
OAc	+6	+6	+6	+6
O–Alkyl	+35	+30	+17	+31
S–Alkyl		+85		
$N(Alkyl)_2$		+95		
Cl	+15	+12		
Br	+25	+30		

Solvent Correction	*nm*
H_2O	+8
EtOH	0
$CHCl_3$	-1
Dioxane	-5
Et_2O	-7
Hydrocarbon	-11



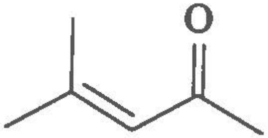
1

base value = 217
2 alkyl substituents = 10
exo double bond = 5
total = 232
experimental = 237



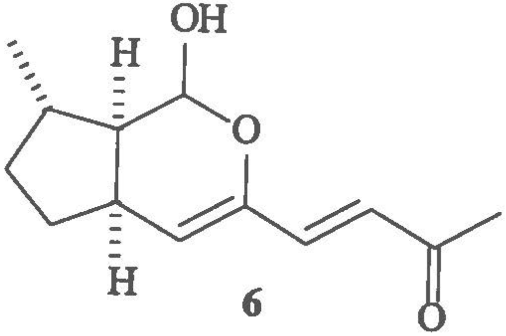
2

base value = 214
3 alkyl substituents = 15
exo double bond = 5
total = 234
experimental = 235



5

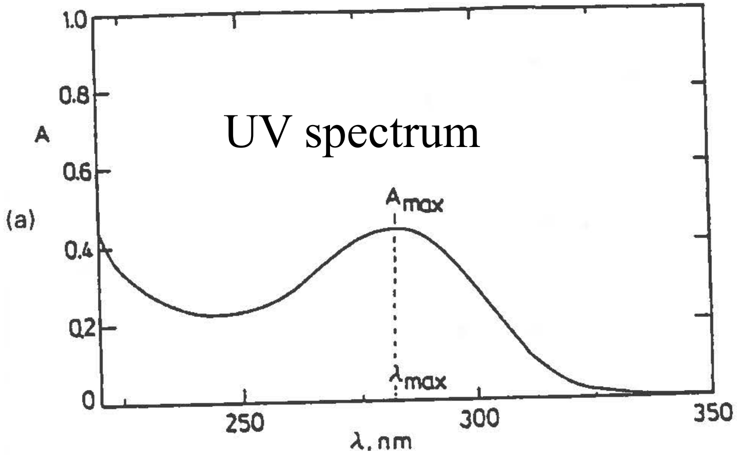
base value = 215
2β alkyl = 24
solvent correction = 0
total = 239
experimental (EtOH solvent) = 237

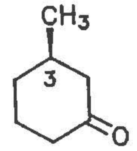


6

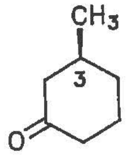
base value = 215
extended conjugation = 30
γ OR = 17
2 δ alkyl = 36
solvent correction = 0
total = 298
experimental (EtOH solvent) = 318, 284

Circular Dichroism and Optical Rotatory Dispersion





3*R*



3*S*

n $\longrightarrow \pi^*$ transition

FIGURE 10-1 (a) UV–vis spectrum of 3(*R*)-methylcyclohexanone, 0.0245 M in methanol, run in a 1.0 cm cuvette. The spectrum is identical for the 3(*S*) isomer. UV–vis curves are typically displayed as absorbance (*A*) on the ordinate and wavelength (λ, in nanometers) on the abscissa. The wavelength at maximum absorbance (A_{max}) is called λ_{max}. UV–vis spectra may also be plotted as ε vs. λ, where ε is the molar absorptivity (molar extinction coefficient). (b) CD curves of 3(*R*)- and 3(*S*)-methylcyclohexanone in methanol. CD curves are typically displayed as Δε versus λ, or molar ellipticity [θ] versus λ. (c) ORD curves of 3(*R*)- and 3(*S*)-methylcyclohexanone in methanol. ORD curves are typically displayed as molecular rotation [ϕ] versus λ. (UV–vis spectrum from Portia Mahal Sabido; ORD–CD curves from Professor J.E. Gurst.)

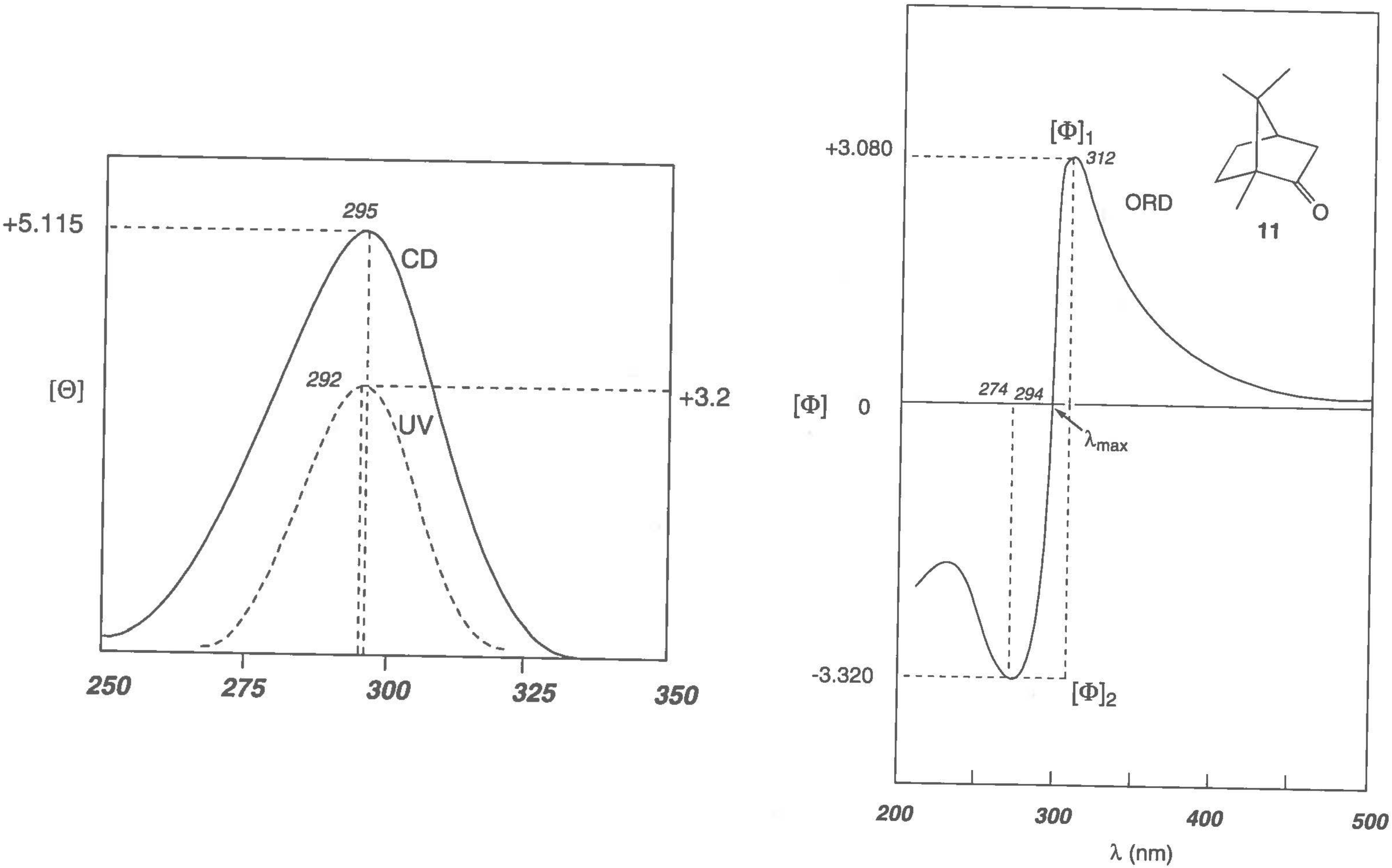
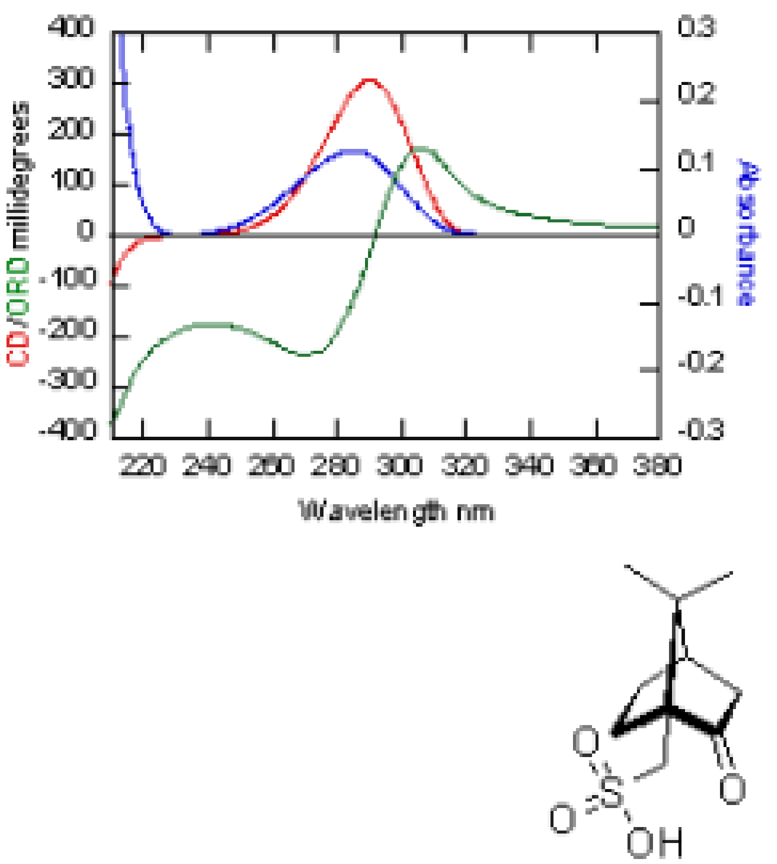
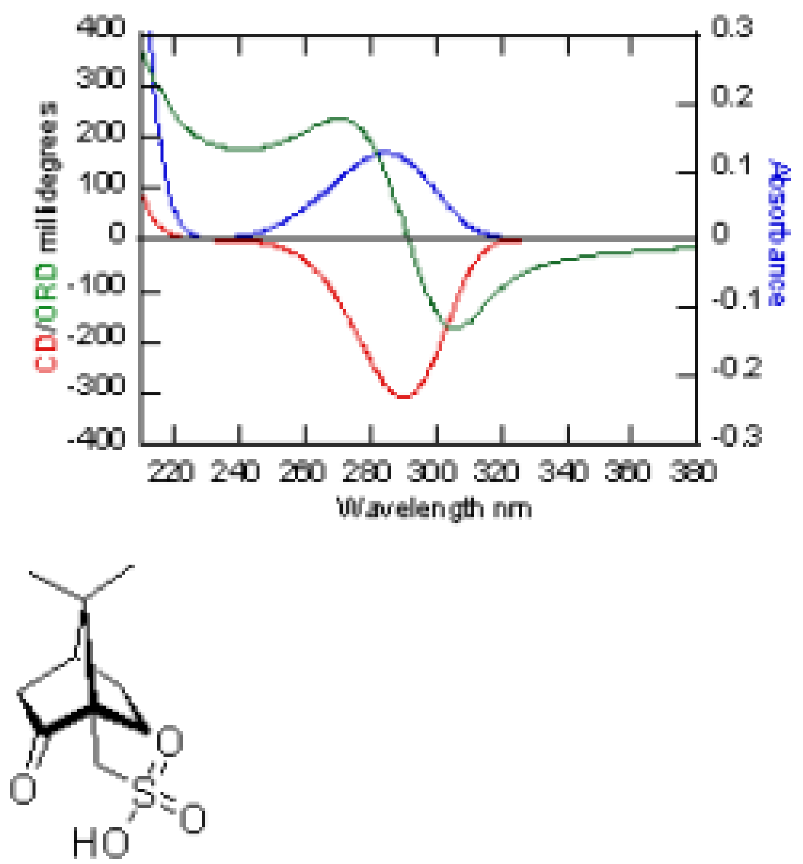


FIGURE 9.6 ORD, CD, and UV of camphor.



(1S)-(+)-Camphor-10-sulphonic acid



(1R)-(-)-Camphor-10-sulphonic acid

How does one identify, and differentiate chiral substances?

For example how do we separate chiral substances?

How does an enzyme differentiate between enantiomers?

Identification and separation requires chirality to identify chirality

How do we identify a chiral substance spectroscopically?

We measure the changes in the plane of polarized light

Is light chiral?

How does a chiral substance bend the plane of polarization?

What does circular dichroism measure?

How does a polarimeter work?

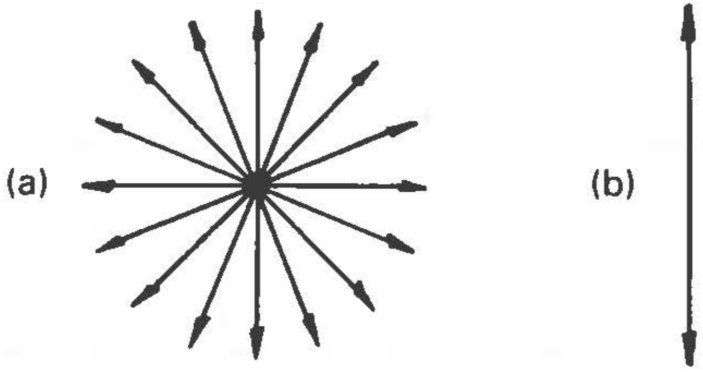


Figure 10-9 Schematic representation of unpolarized and linearly polarized light. (a) Cross section of a narrow beam of ordinary light traveling directly toward the observer. Vibration of the light may be in any direction that is perpendicular to the direction of travel, as indicated by the numerous arrows. (b) A beam of polarized light has vibration in only one direction. This direction is the plane of polarization, in this example the *xz* plane of Figure 10-8.

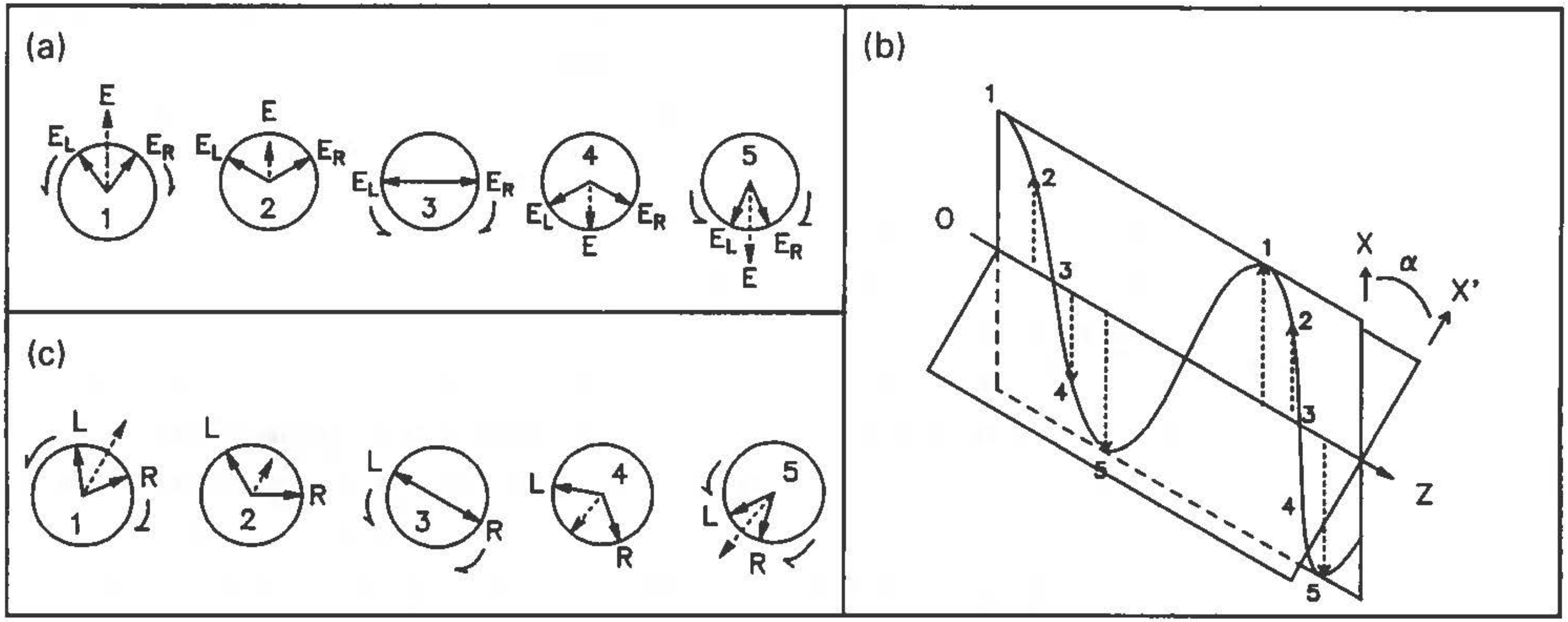


Figure 10-11 (a) and (c) Representations of linearly polarized radiation as the vector sum (E, dotted arrows) of two oppositely rotating beams of circularly polarized radiation E_L and E_R. In (c), the vector for left circularly polarized light (E_L) lags behind that for right circularly polarized light (E_R) relative to the picture in (a). (b) Linearly polarized light waves. The *xz* plane of polarization corresponds to the situation in (a). The *x'z* plane, rotated from the *xz* plane by the angle of rotation α, corresponds to the situation in (c).

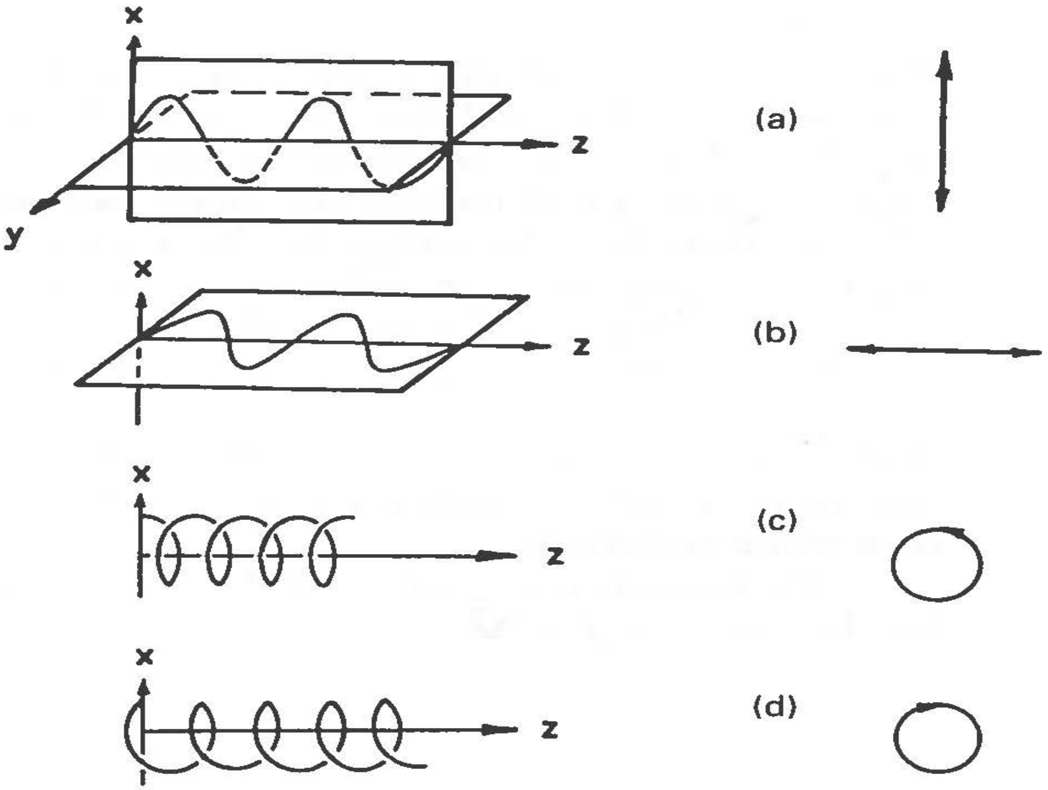
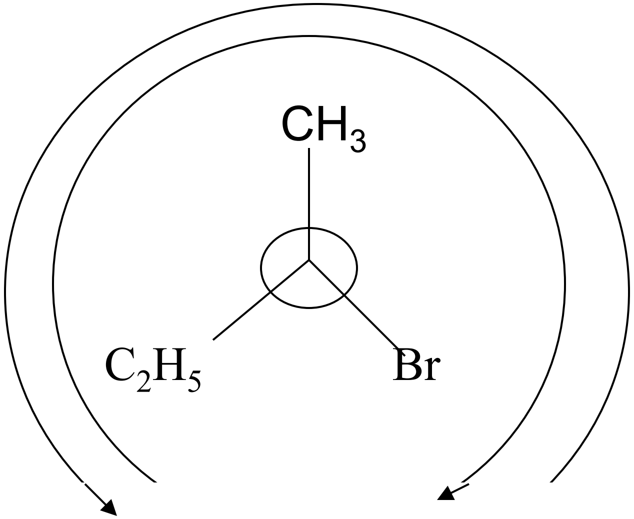


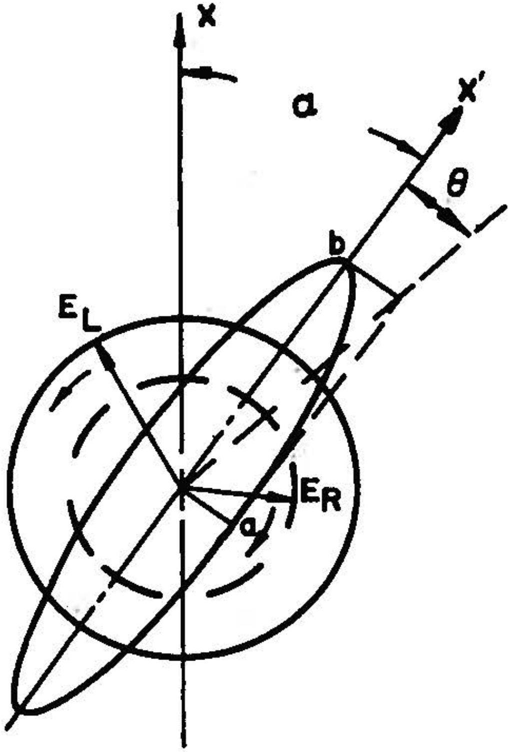
Figure 10-10 Linearly and circularly polarized radiation. (Left) The light wave as a function of time. (Right) Cross-section of the light wave. Light polarized (a) linearly and vertically, (b) linearly and horizontally, (c) left circularly, (d) right circularly. Light having a right-handed helical pattern is termed right circularly polarized. The cross-sectional clockwise rotation of the electric field vector is obtained as the helix is moved forward without rotation through a perpendicular plane.

When light enter a new medium, its speed changes slightly.



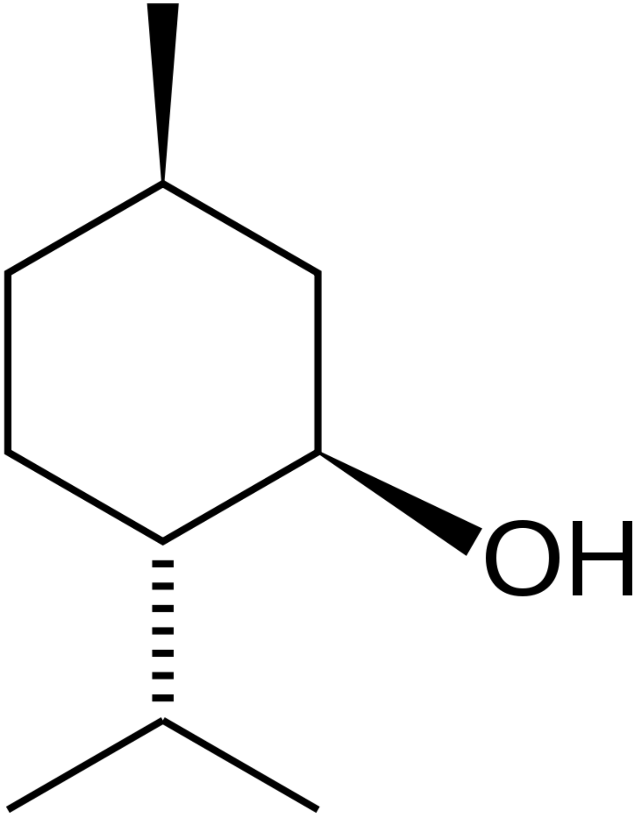
The two components are affected differently as they travel through the medium. The two components slow down differently, causing the plane of polarization of the light to bend (Polarimeter).They are also absorbed slightly differently causing circular dichroism

Figure 10-13 Elliptically polarized light caused by the unequal speed and unequal absorption of left and right circularly polarized light by a chiral medium. The tangent of the ratio of the minor axis *a* to the major axis *b* is θ, the angle of ellipticity. The major axis of the ellipse lies along the *x*′ axis and forms the angle of rotation, α, to the original plane of polarization, the *xz* plane of Figures 10-11 and 10-12.



$[\alpha]_{\lambda}^{T}$ = observed rotation/[(conc g/100ml)*length of tube in decimeters]

$[\alpha]_{D}^{298.15\ K} = \alpha_{obs}/l$ (dm) c (g/mL) for neat liquids



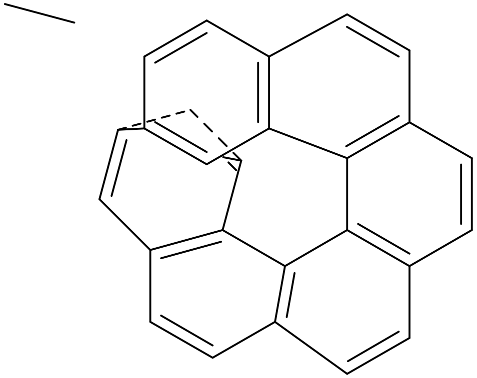
L- (-)-Menthol

enantiomer	$[\alpha]_D$
(+)-menthol	+50.1°
(−)-menthol	-50.2°

Measurement of rotation $[\alpha]_\lambda^T$ = specific rotation

$[\alpha]_D^{298.15\ K}$ uses the sodium D line at 298.15 K (yellow line 589 nm)

λ = variable ORD



Hexahelicene $[\alpha]_D^{298.15\ K} = 3750 \pm 200$ ° (c $5.4*10^{-3}$, chloroform)

How do you measure a rotation > 360 °

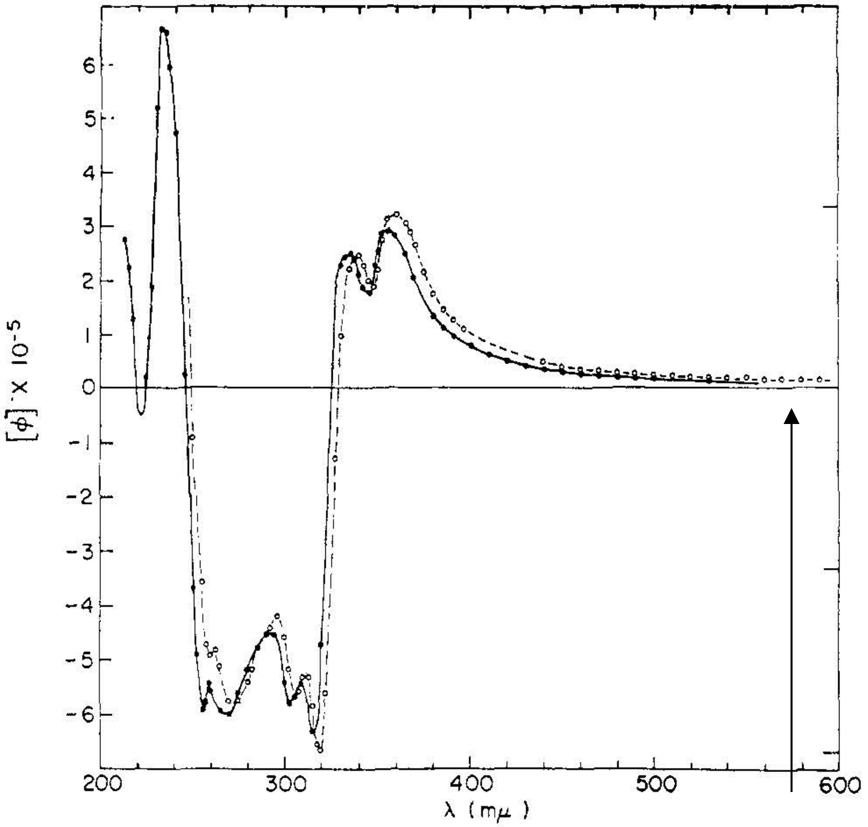


Figure 1. ORD of (+)-hexahelicene in chloroform, ○, and in methanol, ●.

The CD spectrum in chloroform between 370 and 450 mμ (Figure 3) is interesting in that the bands at 413 and 397 mμ are opposite in sign. This suggests that these two bands are most likely part of two distinct electronic transitions. A

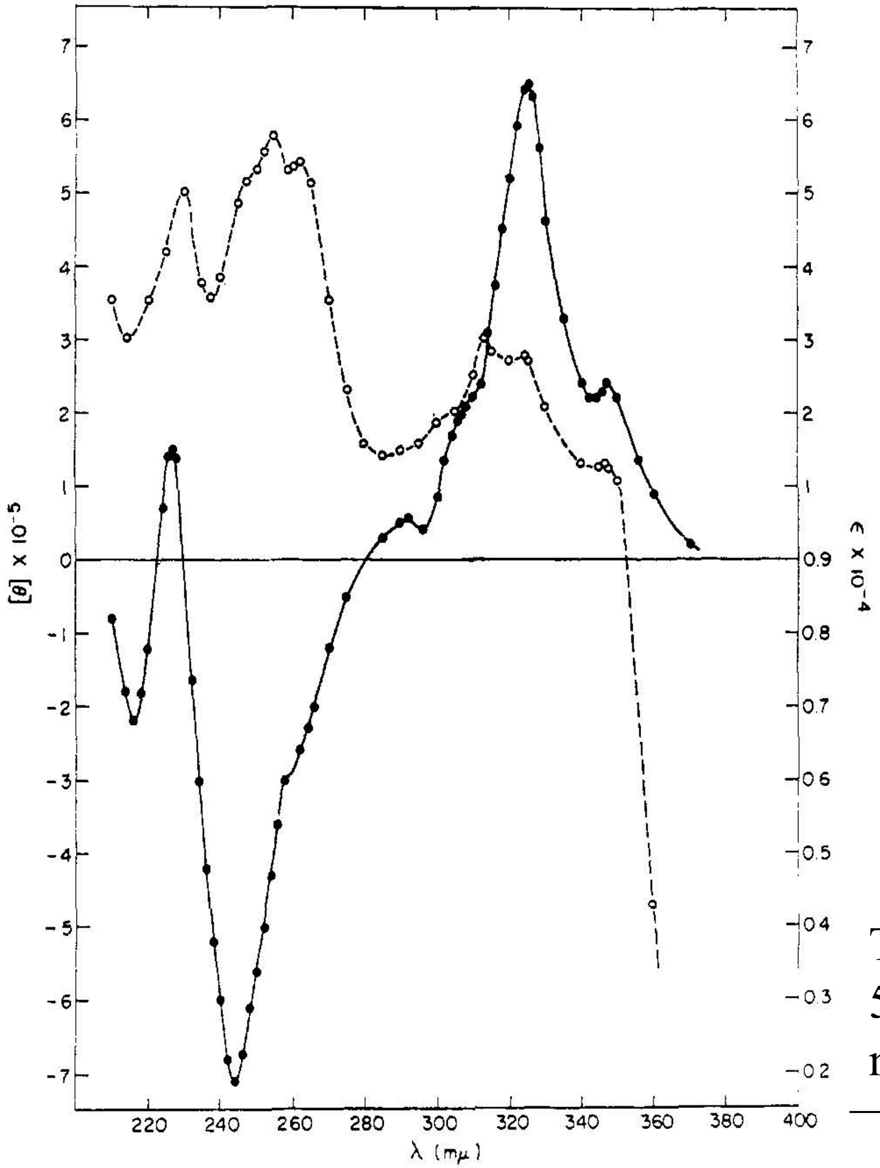


Figure 2. CD, ●, and ultraviolet, ○, spectra of (+)-hexahelicene in methanol.

To 589 nm →

Dissymmetric chromophore: example the chiral carbon in 3 methylcyclohexanone

Inherently dissymmetric chromophore: when the entire molecule is responsible for the chirality (helix)

Say the actual rotation of a molecule is +380; What would you measure?

+20°

You dilute it in half, what would you expect?

+10°

What would you get: +190°

How do you know a rotation is +200° and not -160°?

Dilute it in half +100° vs – 80 °

TABLE 11-10

Important Chromophores of Proteins*

Residues	*Chromophore*	*Location (nm)*	*log ε_{max}*	*Assignment*
Peptide bond	CONH	162	3.8	$\pi^+ \longrightarrow \pi^-$
		188	3.9	$\pi^0 \longrightarrow \pi^-$
		225	2.6	$n \longrightarrow \pi^-$
Aspartic, glutamic	COOH	175	3.4	$n \longrightarrow \pi^*$
		205	1.6	$n \longrightarrow \pi^*$(?)
Aspartate, glutamate	COO^-	200	2	$n \longrightarrow \pi^*$
Lysine, arginine	N—H	173	3.4	$\sigma \longrightarrow \sigma^*$
		213	2.8	$n \longrightarrow \sigma^*$
Phenylalanine	Phenyl	188	4.8	
		206	3.9	$\pi \longrightarrow \pi^*$
		261	2.35	
Tyrosine	Phenolic	193	4.7	
		222	3.9	$\pi \longrightarrow \pi^*$
		270	3.16	
Tyrosine (ionized)	Phenolate ion	200?	5	
		235	3.97	$\pi \longrightarrow \pi^*$
		287	3.41	
Tryptophan	Indole	195	4.3	
		220	4.53	$\pi \longrightarrow \pi^*$
		280	3.7	
		286	3.3	
Histidine	Imidazole	211	3.78	$\pi \longrightarrow \pi^*$(?)
CysSH	S—H	195	3.3	$n \longrightarrow \sigma^*$
CysS—	S—	235	3.5	$n \longrightarrow \sigma^*$
Cystine	—S—S—	210	3	$n \longrightarrow \sigma^*$
		250	2.5	

*Modified from J. Donovan, *Physical Principles and Techniques of Protein Chemistry,* Part A, S. Leach, ed., Academic Press, New York, 1960.

TABLE 11-11

Circular Dichroism Properties of Aromatic Amino Acids and of Cystine

Amino Acid (as the Hydrochloride)	*Chromophore*	*λ (nm)*	*[θ] (deg · cm² dmole⁻¹)*	*References**
α-Phenylglycine	Benzenoid	267, 260, 254	−1,000	a
		218	34,000	
Phenylalanine	Benzenoid	266, 263, 257	50-75	a, b
		217	14,000	
Tyrosine	Phenolic	274	1,200	b, c
		225	8,000	
Tyrosine	Phenolate ion	293	1,000	b, d
		230	−2,000	
		210	7,000	
Tryptophan	Indole	286, 276, 269	1,500–2,500	c
		225	20,000	
		209	−15,000	
Histidine	Imidazole	216	8,000	b, c
Cystine	Disulfide	250	−2,000	b, e, f
		220	20,000	
		196	−44,000	

*KEY: a = L. Verbit and P.J. Heffron, *Tetrahedron,* **24,** 1231 (1968); b = M. Legrand and R. Viennet, *Bull. Soc. Chim. France,* 479 (1965); c = L. Verbit and P.J. Heffron, *Tetrahedron,* **23,** 3865 (1967); d = T.M. Hooker, Jr., and J.A. Schellman, *Biopolymers,* **9,** 1319 (1970); e = P.C. Kahn and S. Beychok, *J. Am. Chem. Soc.,* **90,** 4168 (1968); f = J.P. Casey and R.B. Martin, *J. Am. Chem. Soc.,* **94,** 6141 (1972).

Identifying tertiary structure of proteins

anti-parallel β sheet - - -

β turn - · -

α helix ———

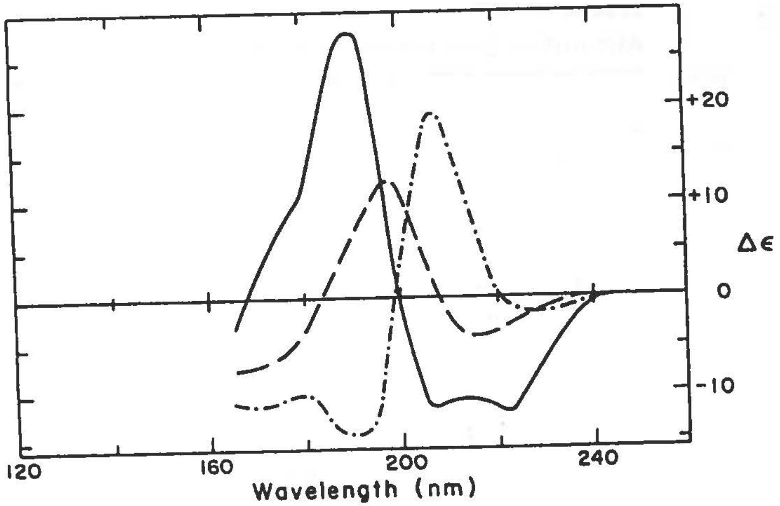


Figure 11-16 The CD in aqueous solution of poly-L-glutamic acid at pH 4.5 representing the α-helix (———), poly(Lys-Leu) in 0.1 M NaF at pH 7 representing the antiparallel β sheet (– – –), and poly(Ala$_2$-Gly$_2$) representing the β turn (— · —). (Reproduced with permission from the *Annual Review of Physical Chemistry* in W.C. Johnson, ed., *Ann. Rev. Phys. Chem.*, **29**, 93 [1978].)

Random coil

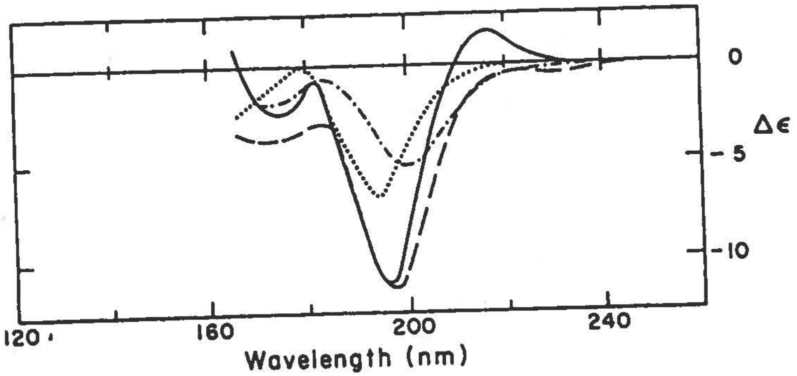
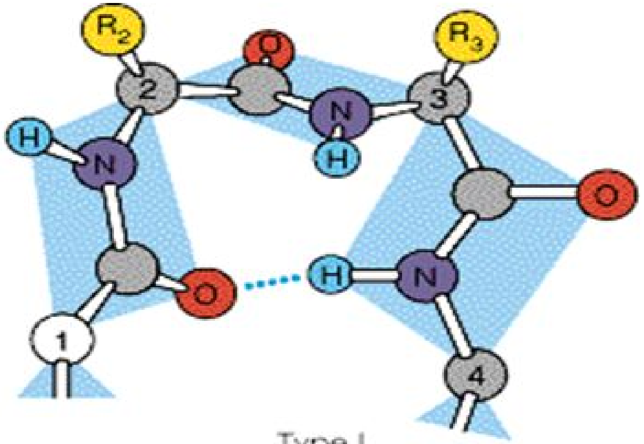
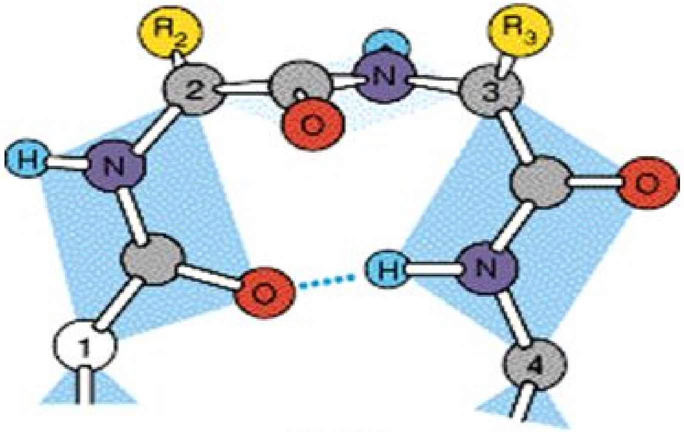


Figure 11-17 The CD of a number of peptides in aqueous solution thought to represent the unordered amide chromophore. Poly-L-glutamic acid at pH 8 (———), poly(Pro-Lys-Leu-Lys-Leu) in a salt-free solution (– – –), collagen at 45° C in a 0.01 M sodium phosphate buffer pH 3.5 (— · —), and *N*-acetyl-L-alanine-*N*'-methylamide (·······). (Reproduced with permission from the *Annual Review of Physical Chemistry.* W.C. Johnson, ed., *Ann. Rev. Phys. Chem.*, **29**, 93 [1978].)

The Beta-turn

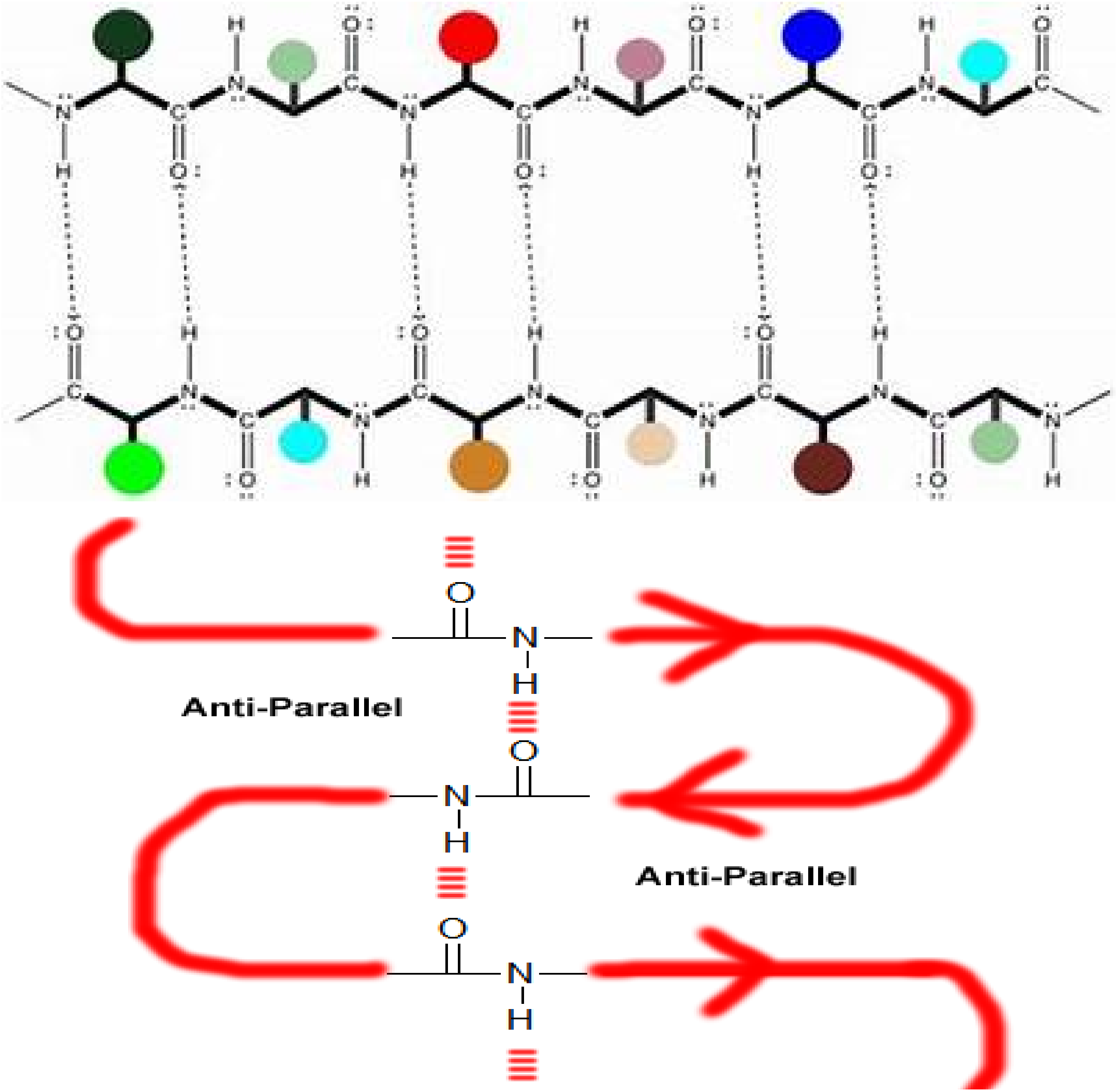


Type I



Type II

Since proteins are globular structures, it is evident that the chains have to be able to turn and reorient themselves.The simple beta-bend is one in which the peptide chain forms a tight loop with the C=O oxygen of one residue H-bonded with the amide proton of the residue three positions down the chain.



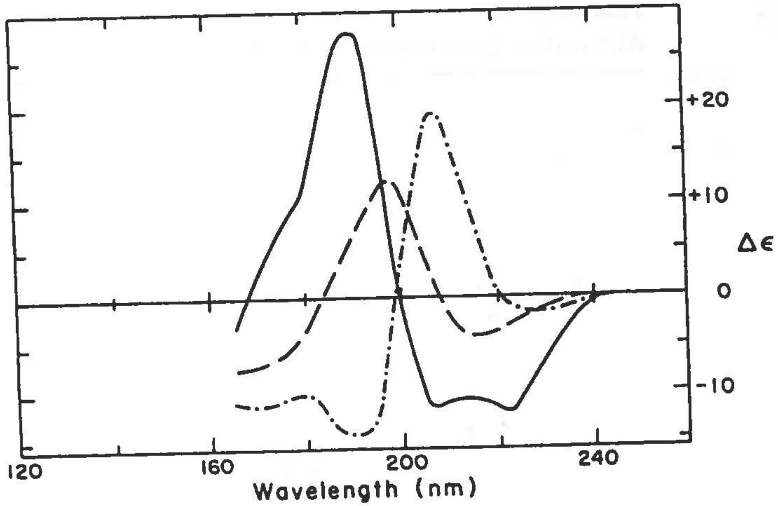
Anti-parallel β sheet

Identifying tertiary structure of proteins

anti-parallel β sheet - - -

β turn - · -

α helix ———



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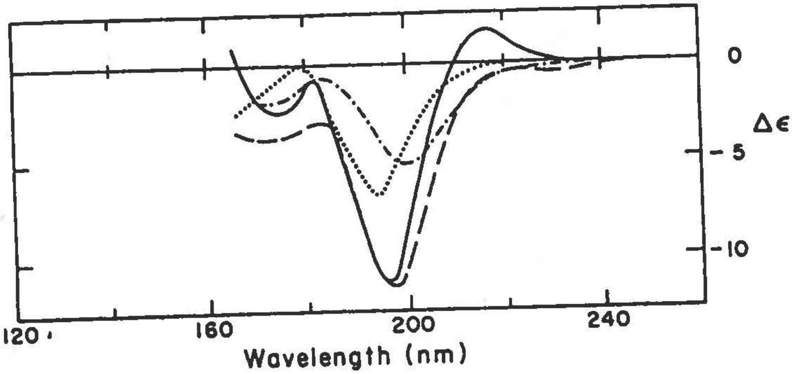
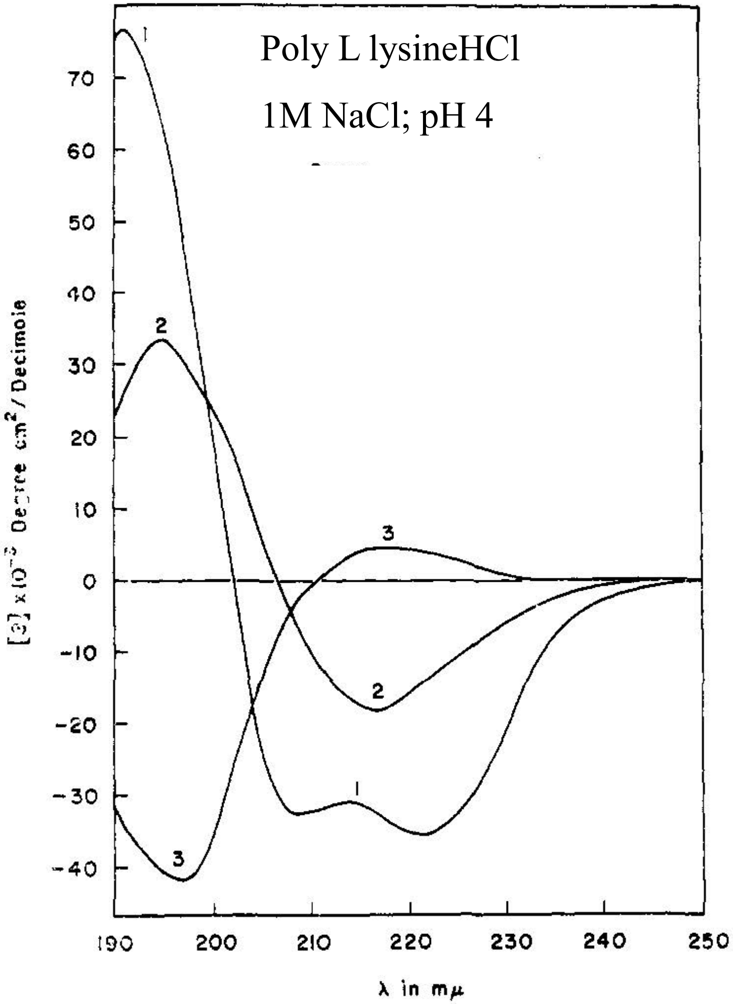


Figure 11-17 The CD of a number of peptides in aqueous solution thought to represent the unordered amide chromophore. Poly-L-glutamic acid at pH 8 (———), poly(Pro-Lys-Leu-Lys-Leu) in a salt-free solution (– – –), collagen at 45° C in a 0.01 M sodium phosphate buffer pH 3.5 (— · —), and *N*-acetyl-L-alanine-*N*′-methylamide (·······). (Reproduced with permission from the *Annual Review of Physical Chemistry*. W.C. Johnson, ed., *Ann. Rev. Phys. Chem.*, **29**, 93 [1978].)

Applications of ORD/CD

CD



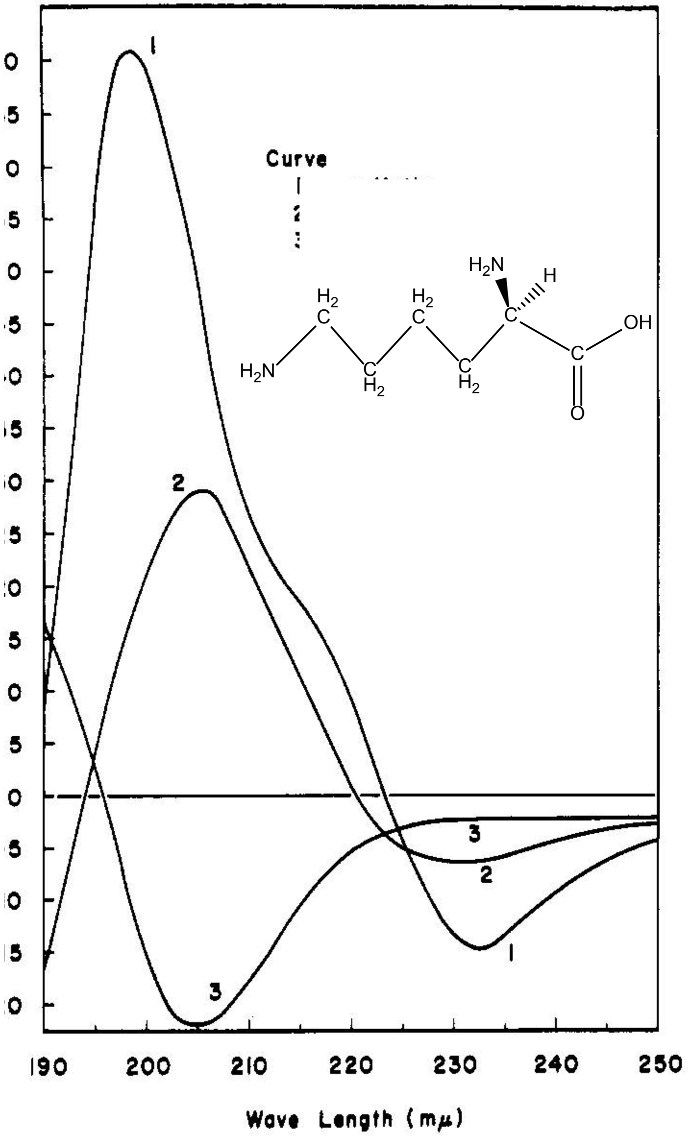
Poly L lysineHCl

1M NaCl; pH 4

FIGURE 1: Circular dichroism spectra of poly-L-lysine in the α-helical, β, and random conformation.

Greenfeld & Fasman, *Biochem*. **1969** *8* , 4108

ORD



1: The ORD of poly-L-lysine in the α-helical, β, and random conformations.

Biochem. **1967**, *6*, 1630

- Vibrational Circular Dichroism

How does circular dichroism relate to infrared spectroscopy

From Jas.co

Vibration circular dichroism.
VCD is a method to measure the difference of absorbance intensity between left-hand and right-hand circularly polarized light as shown in Figure 1.

VCD can be applied to almost all organic compounds in the same way as infrared spectroscopy. In addition, by comparing the measurement results with calculated results by ab-initio molecular orbital calculations, the absolute configuration of the sample can be determined. The FDA accepts the absolute configurations.

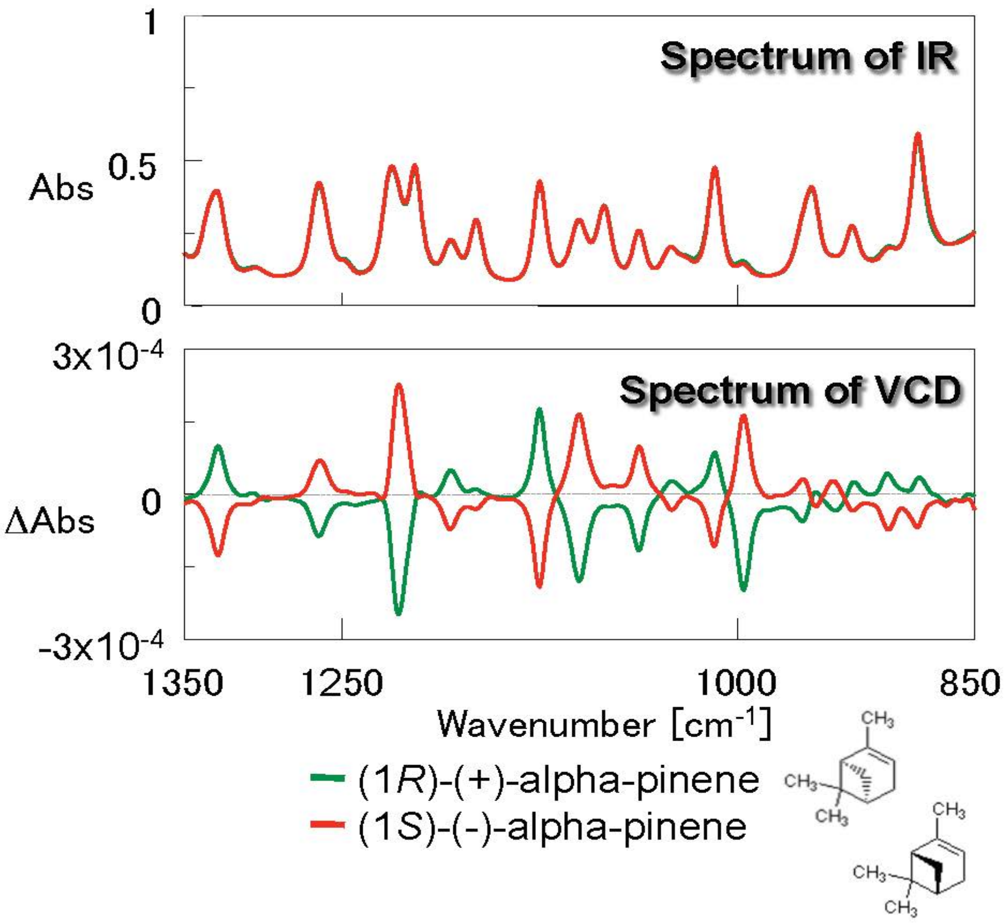
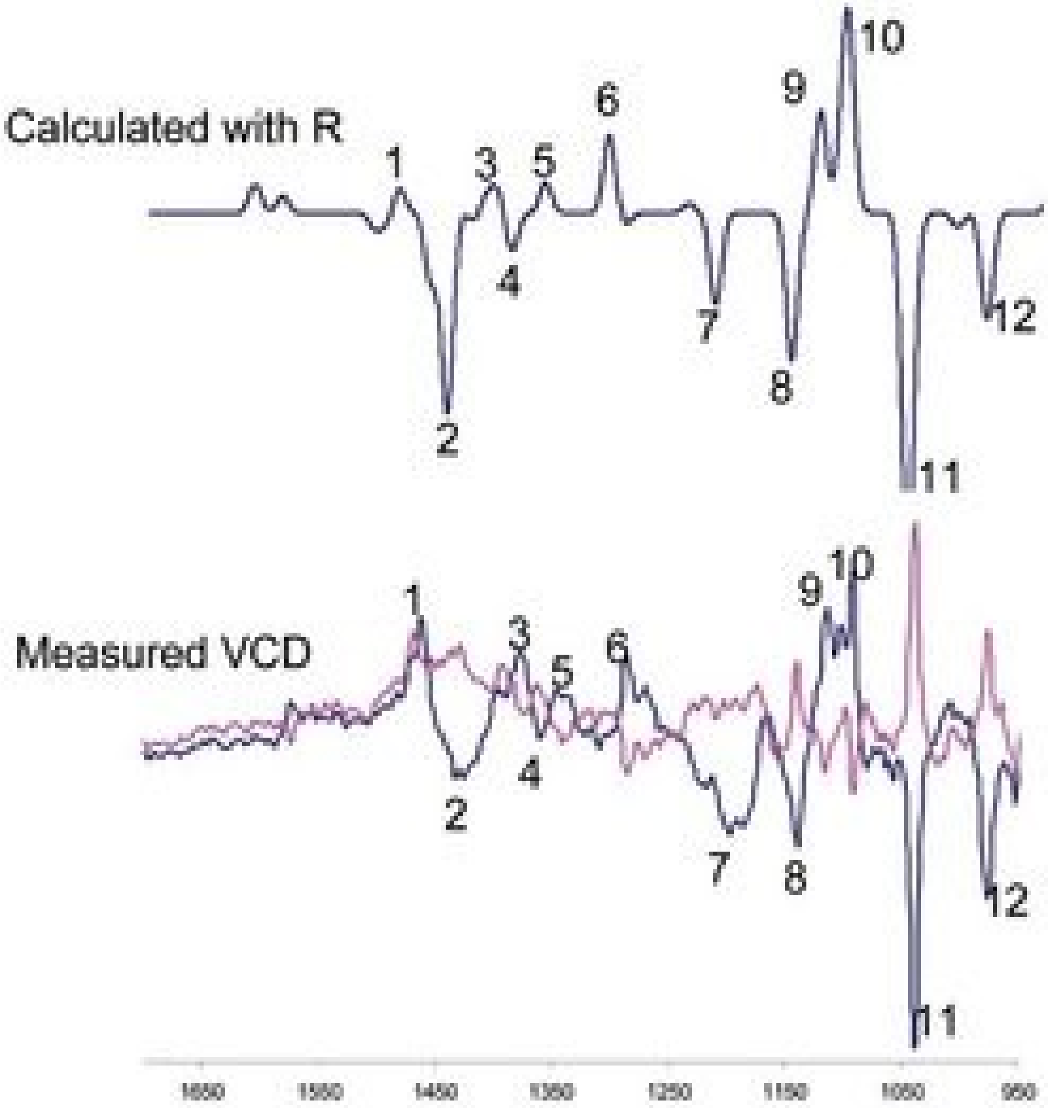
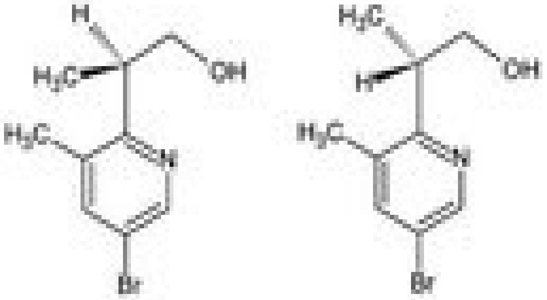


Figure 3: Spectra of alpha-pinene (neat, 50 µm pathlength BaF_2 liquid cell)

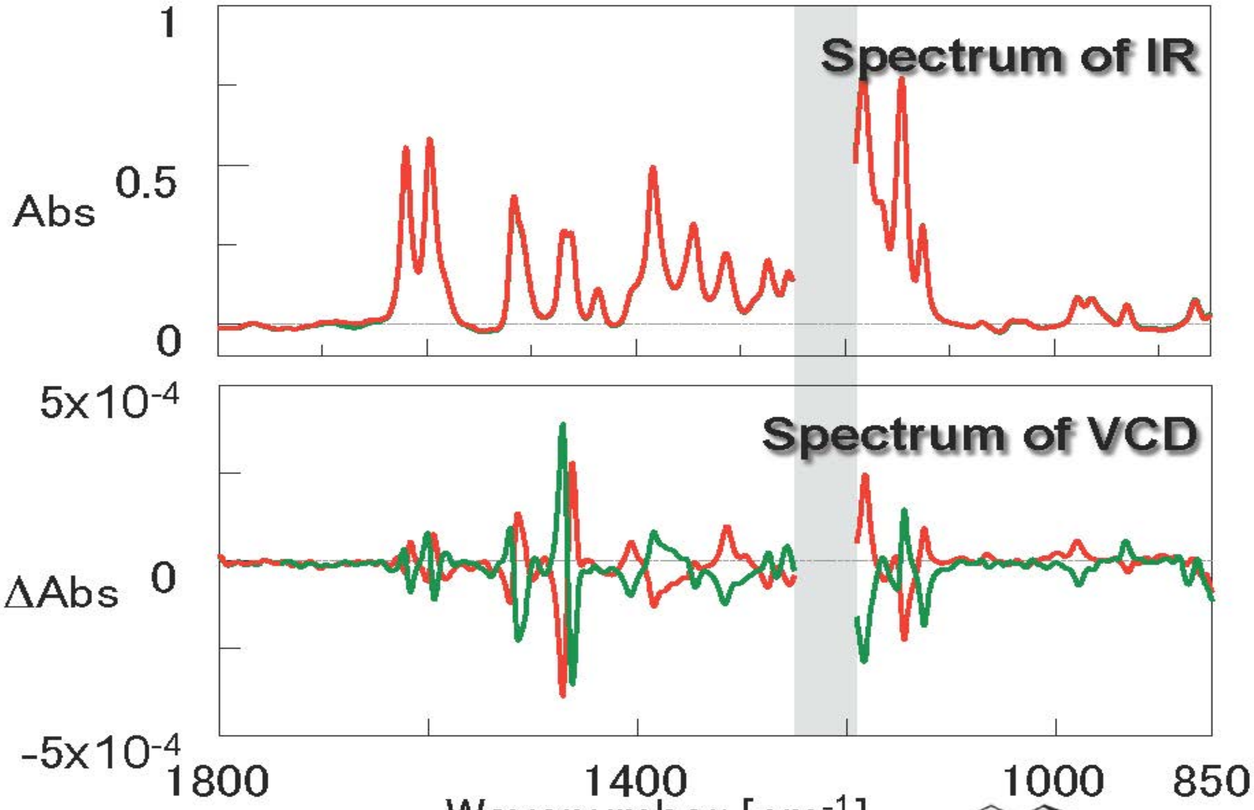


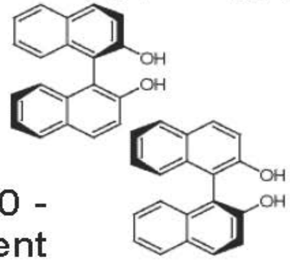
2-(5-bromo-3-methylpyridin-2-yl) propan-1-ol



R-enantiomer

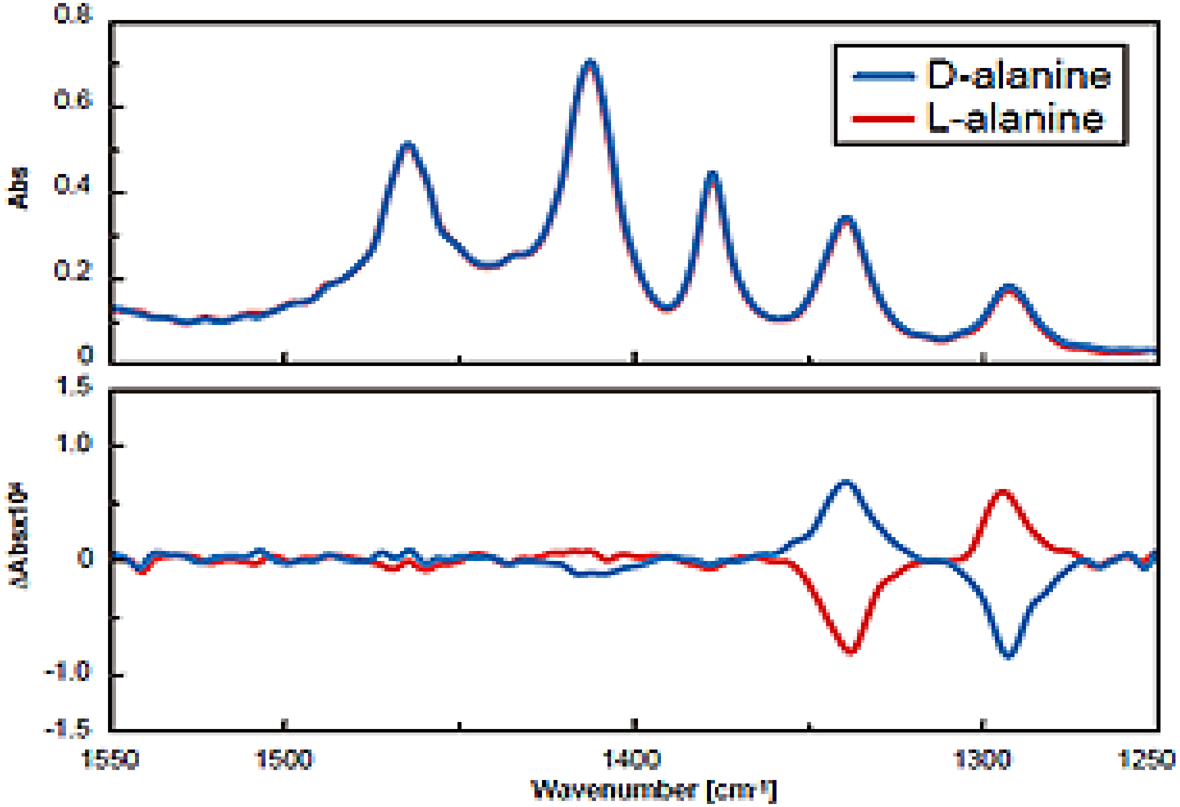
S-enantiomer



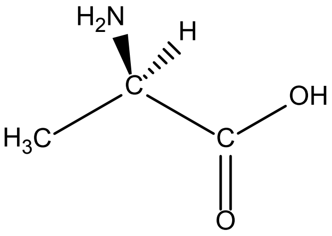


* Since the noise in the range of 1250 - 1190 cm^{-1} is excessive due to solvent absorption, the data in this range are cut from the spectral figures.

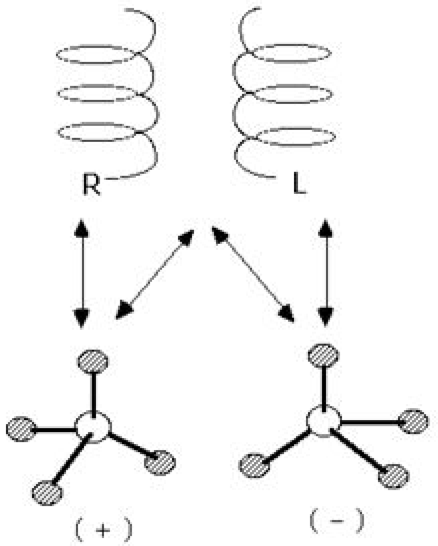
Figure 4: Spectra of 1,1'-Bi-2-naphthol (solvent: $CHCl_3$, concentration: 0.162 M, 50 μm pathlength BaF_2 liquid cell)

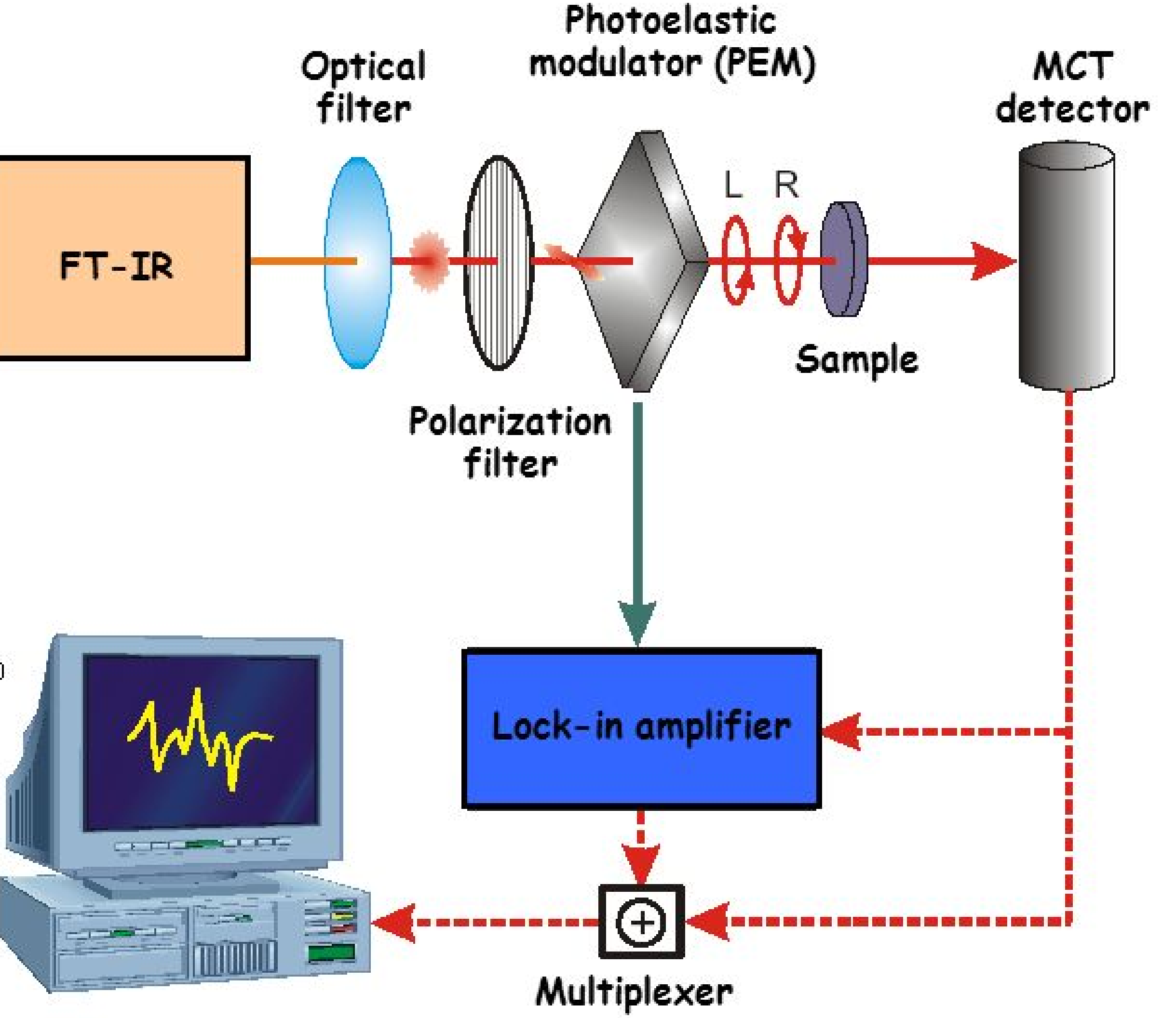


VCD and IR spectra of amino acid



Vibrational circular dichroism (VCD) spectroscopy





$$\Delta\varepsilon_{01}^{a} \sim R_{01}^{a} = \frac{\hbar}{2}\left(\frac{\partial\vec{\mu}}{\partial Q_a}\right)_0 \cdot \left(\frac{\partial\vec{m}}{\partial P_a}\right)_0$$

R : rotatory strength

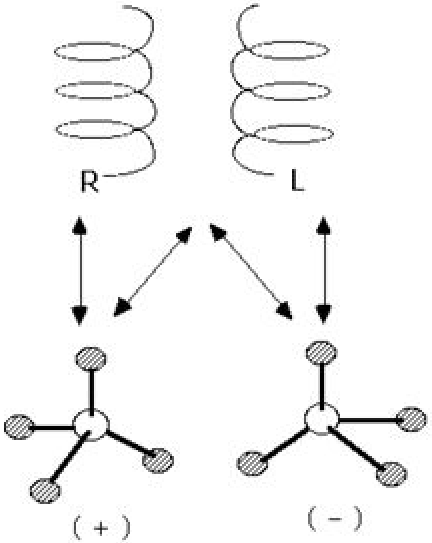
$\vec{\mu}$: electric dipole moment

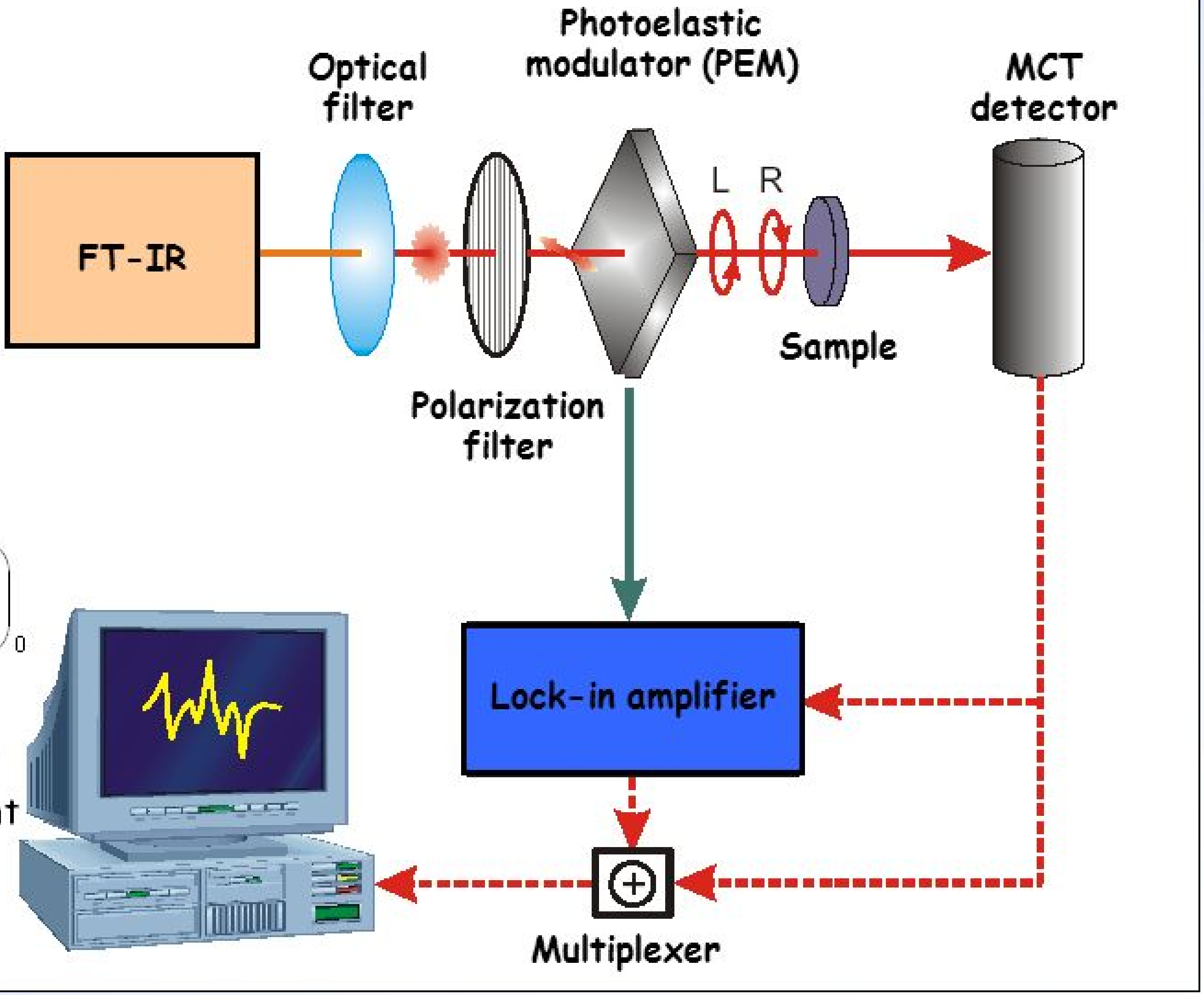
$\vec{m}$: magnetic dipole moment

Q : displ. in normal coord.

P: conjugate momentum

Vibrational circular dichroism (VCD) spectroscopy





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