College of Pharmacy
Babylon University

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Advanced Pharmaceutical Analysis

Lecture No. 2 (Based on Bruice-Organic Chemistry 4th Ed.)

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UV/Vis spectroscopy

UV/Vis spectroscopy provides information about compounds with conjugated double bonds.

Ultraviolet light and visible light have just the right energy to cause an electronic transition—the promotion of an electron from one orbital to another of higher energy.

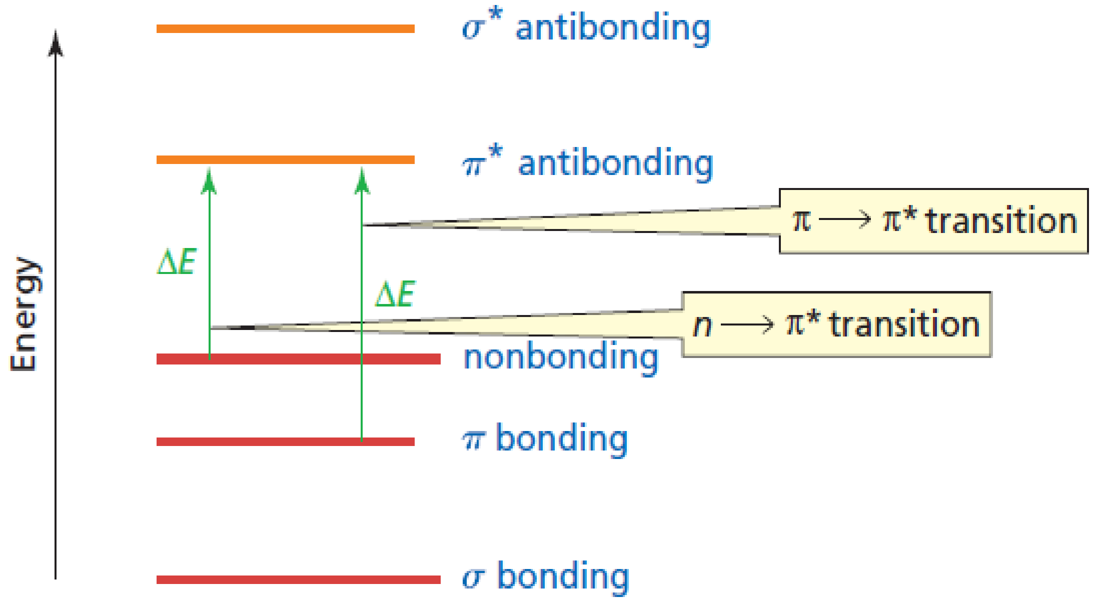
Depending on the energy needed for the electronic transition, a molecule will absorb either ultraviolet or visible light.

Ultraviolet light is electromagnetic radiation with wavelengths ranging from 180 to 400 nm (nanometres); visible light has wavelengths ranging from 400 to 780 nm.

The normal electronic configuration of a molecule is known as its **ground state**—all the electrons are in the lowest-energy molecular orbitals.

When a molecule absorbs light of an appropriate wavelength and an electron is promoted to a higher energy molecular orbital, the molecule is then in an **excited state**.

Thus, an **electronic transition** is the promotion of an electron to a higher energy molecular orbital MO. The relative energies of the bonding, nonbonding, and antibonding molecular orbitals are shown in the below Figure.

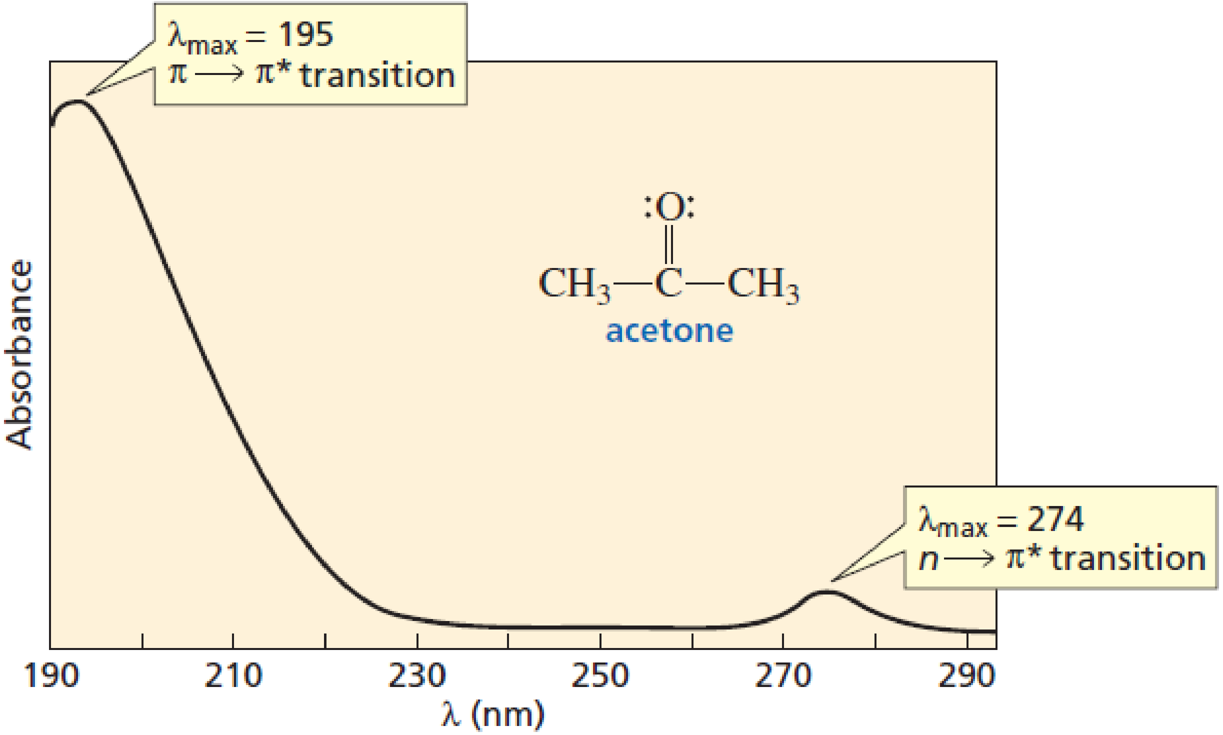


$$E = \frac{hc}{\lambda}$$

h = Planck's constant
c = velocity of light
λ = wavelength

The electronic transition with the lowest energy is the promotion of a nonbonding (lone pair) electron (n) into a π^* antibonding molecular orbital.

The higher energy electronic transition is the promotion of an electron from a π bonding molecular orbital into a π^* antibonding molecular orbital



The UV spectrum of acetone

The λmax (stated as “lambda max”) is the wavelength corresponding to the highest point (maximum absorbance) of the absorption band.

The Beer–Lambert Law

The relationship among absorbance, concentration, and length of the light path is known as the **Beer–Lambert law** and is given by

$$A = cl\varepsilon$$

where

A = absorbance of the sample = $\log \dfrac{I_0}{I}$

I_0 = intensity of the radiation entering the sample

I = intensity of the radiation emerging from the sample

c = concentration of the sample, in moles/liter

l = length of the light path through the sample, in centimeters

ε = molar absorptivity (liter mol^{-1} cm^{-1})

The **molar absorptivity** (formerly called the extinction coefficient) of a compound is a constant that is characteristic of the compound at a particular wavelength.

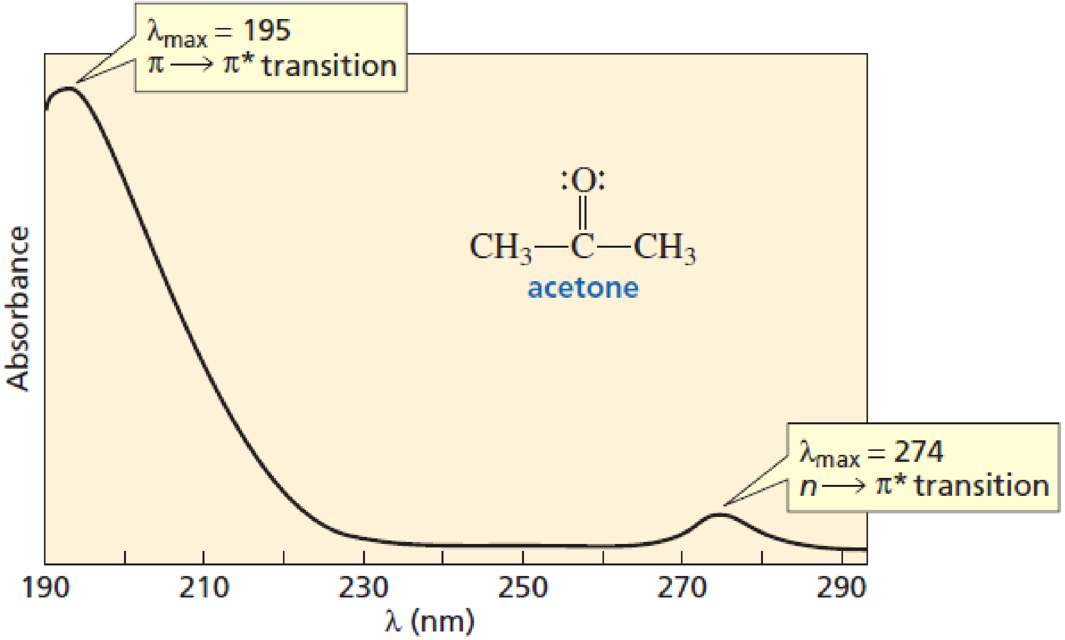
It is the absorbance that would be observed for a 1.00 M solution in a cell with a 1.00 cm path length.

The molar absorptivity of acetone, for example, is 9000 at 195 nm and 13.6 at 274 nm. The solvent in which the sample is dissolved when the spectrum is taken is reported because molar absorptivity is not exactly the same in all solvents.

So the UV spectrum of acetone in hexane would be reported as λmax 195 nm (ε_{max} = 9000, hexane), λmax 274 nm (ε_{max} = 13.6, hexane).

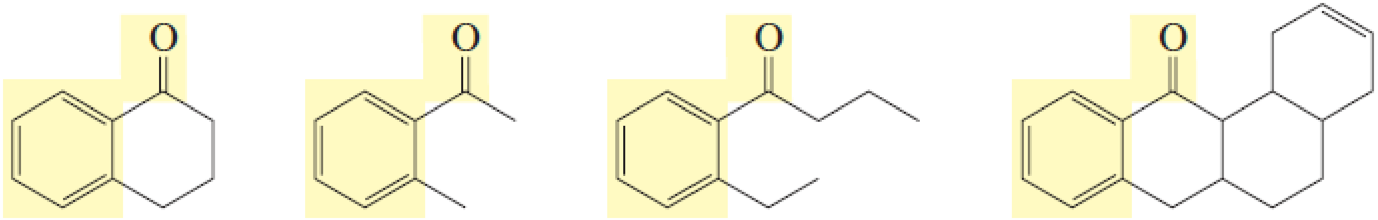
Because absorbance is proportional to concentration, the concentration of a solution can be determined if the absorbance and molar absorptivity at a particular wavelength are known.

The two absorption bands of acetone are very different in size because of the difference in molar absorptivity at the two wavelengths. Small molar absorptivities are characteristic of transitions, so these n ⟶ π* transitions can be difficult to detect. Consequently, π ⟶ π* transitions are usually more useful in UV/Vis spectroscopy.



In order to obtain a UV or visible spectrum, the solution is placed in a cell. Most cells have 1-cm path lengths. Either glass or quartz cells can be used for visible spectra, but quartz cells must be used for UV spectra because glass absorbs UV light.

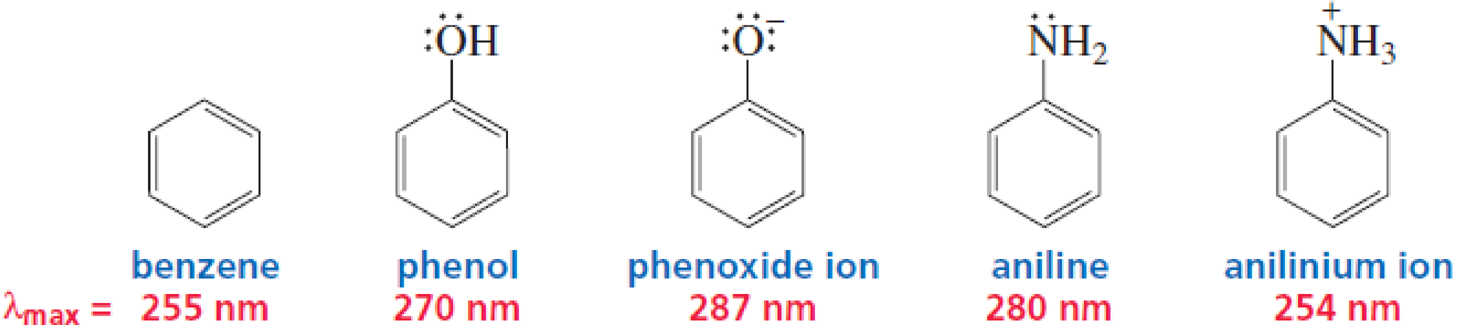
A **chromophore** is that part of a molecule that absorbs UV or visible light. The carbonyl group is the chromophore of acetone. The following four compounds all have the same chromophore, so they all have approximately the same λmax .



An **auxochrome** is a substituent that when attached to a chromophore, alters the λmax and the intensity of the absorption, usually increasing both; OH and NH_2groups are auxochromes.

The lone-pair electrons on oxygen and nitrogen are available for interaction with the π electron cloud of the benzene ring, and such an interaction increases λmax

Because the anilinium ion does not have an auxochrome, its λmax is similar to that of benzene.

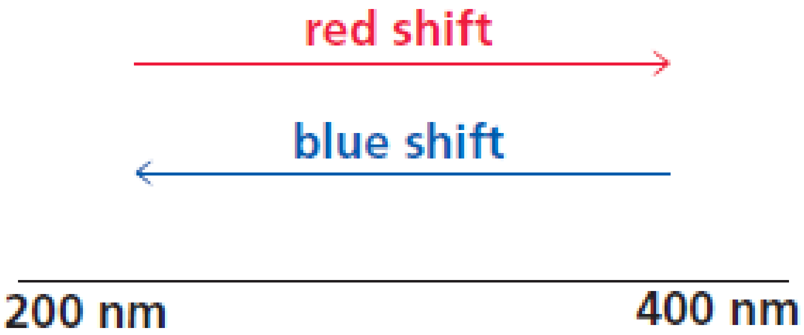


Removing a proton from phenol and thereby forming phenoxide ion (also called phenolate ion) increases the λmax because the resulting ion has an additional lone pair.

Protonating aniline (and thereby forming the anilinium ion) decreases the λmax because the lone pair is no longer available to interact with the π cloud of the benzene ring.

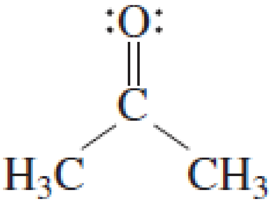
Because wavelengths of red light are longer than those of blue light, a shift to a longer wavelength is called a **red shift**.

A shift to a shorter wavelength is called a **blue shift**. Deprotonation of phenol results in a red shift, whereas protonation of aniline produces a blue shift.

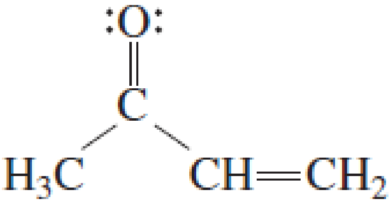


Effect of Conjugation on λ_{max}

The $n \rightarrow \pi^*$ transition for methyl vinyl ketone is at 324 nm, and the $\pi \rightarrow \pi^*$ transition is at 219 nm. Both λ_{max} values are at longer wavelengths than the corresponding λ_{max} values of acetone because methyl vinyl ketone has two conjugated double bonds.



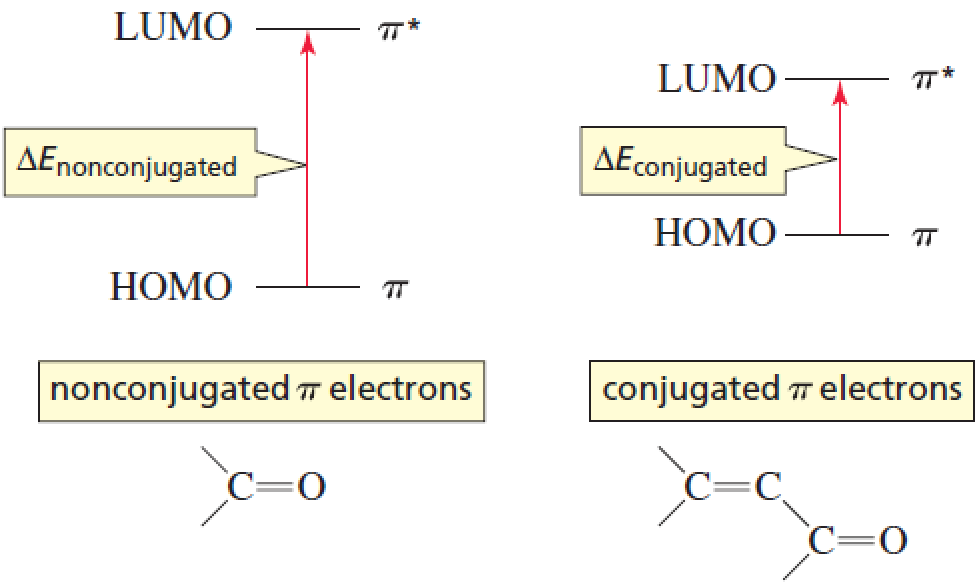
acetone



methyl vinyl ketone

$n \longrightarrow \pi^*$	λ_{max} = 274 nm (ε_{max} = 13.6)	λ_{max} = 331 nm (ε_{max} = 25)
$\pi \longrightarrow \pi^*$	λ_{max} = 195 nm (ε_{max} = 9000)	λ_{max} = 203 nm (ε_{max} = 9600)

The more conjugated double bonds there are in a compound, the less energy is required for the electronic transition, and therefore the longer is the wavelength at which the electronic transition occurs.



LUMO — π^*

$\Delta E_{\text{nonconjugated}}$

HOMO — π

nonconjugated π electrons

LUMO — π^*

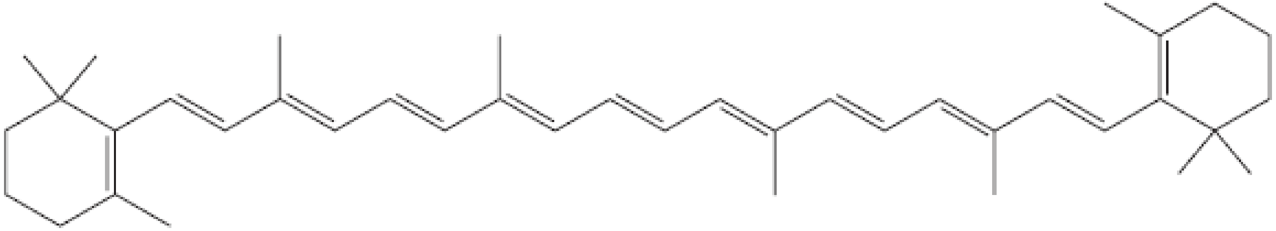
$\Delta E_{\text{conjugated}}$

HOMO — π

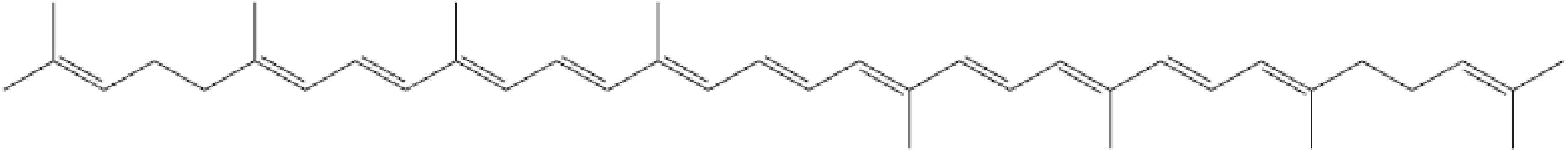
conjugated π electrons

Compound	λ_{max} (nm)	ε (M^{-1} cm^{-1})
$H_2C{=}CH_2$	165	15,000
	217	21,000
	256	50,000
	290	85,000
	334	125,000
	364	138,000

If a compound has enough conjugated double bonds, it will absorb visible light (λ_{max} > 400 nm) and the compound will be colored. *β*-Carotene, a precursor of vitamin A, is an orange substance found in carrots, apricots, and sweet potatoes. Lycopene is red and is found in tomatoes, watermelon, and pink grapefruit.

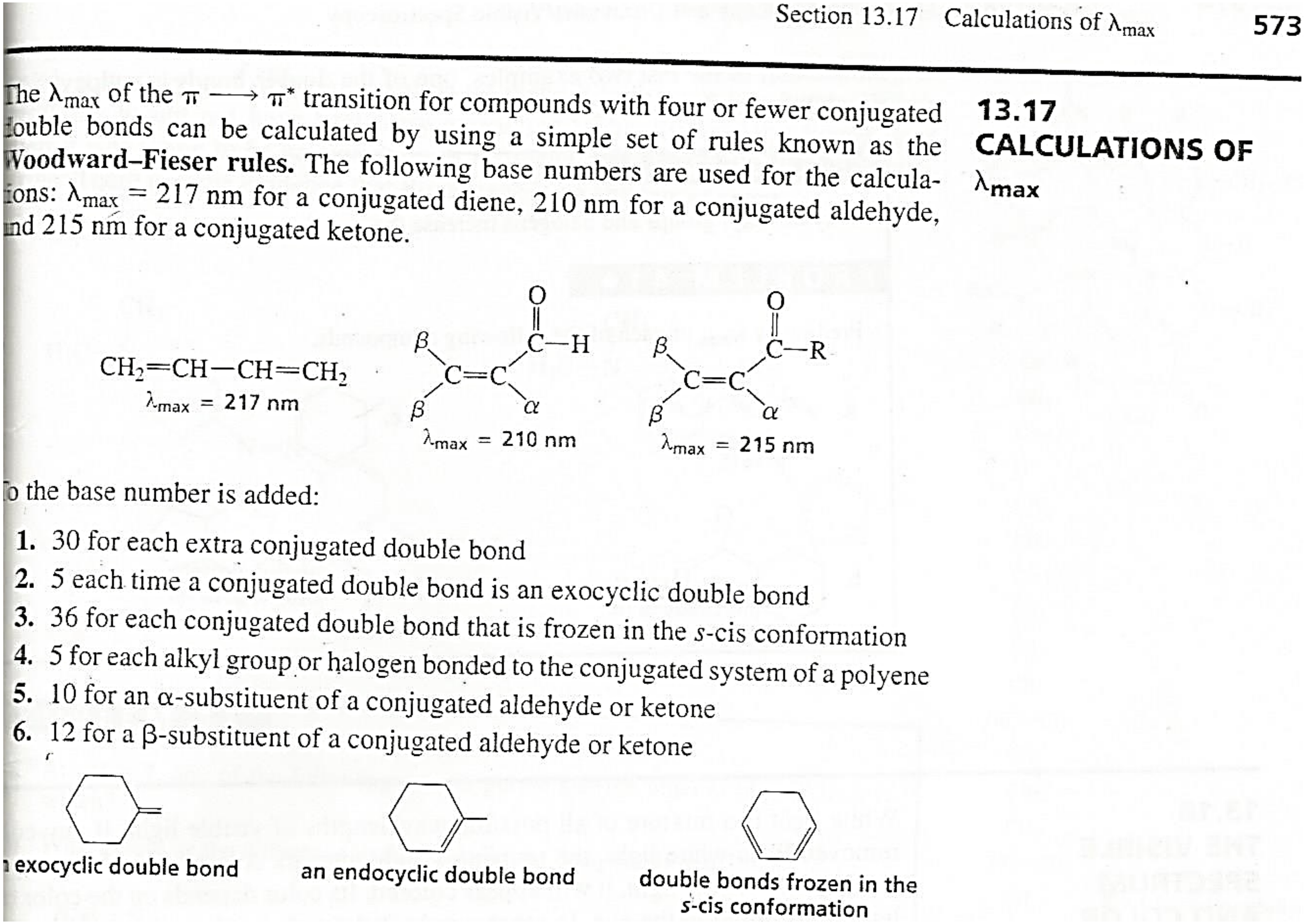


***β*-carotene**
λ_{max} = 455 nm



lycopene
λ_{max} = 474 nm

The Woodward-Fieser Rules:



Section 13.17 Calculations of λ_{max} 573

13.17 CALCULATIONS OF λ_{max}

he λ_{max} of the $\pi \longrightarrow \pi^*$ transition for compounds with four or fewer conjugated ouble bonds can be calculated by using a simple set of rules known as the **Voodward–Fieser rules.** The following base numbers are used for the calculaons: λ_{max} = 217 nm for a conjugated diene, 210 nm for a conjugated aldehyde, nd 215 nm for a conjugated ketone.

$CH_2{=}CH{-}CH{=}CH_2$
λ_{max} = 217 nm

λ_{max} = 210 nm

λ_{max} = 215 nm

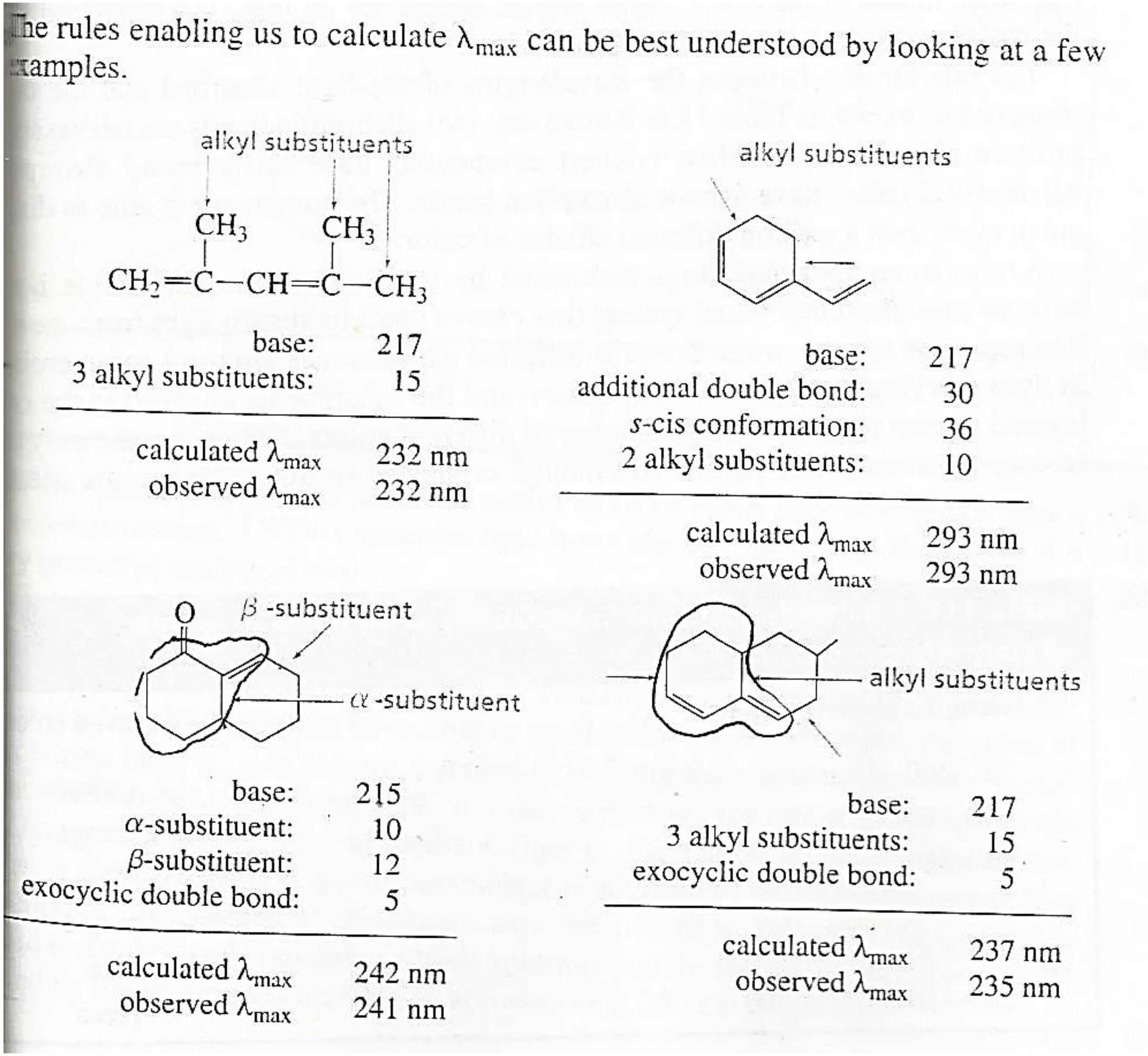
o the base number is added:

1. 30 for each extra conjugated double bond
2. 5 each time a conjugated double bond is an exocyclic double bond
3. 36 for each conjugated double bond that is frozen in the *s*-cis conformation
4. 5 for each alkyl group or halogen bonded to the conjugated system of a polyene
5. 10 for an α-substituent of a conjugated aldehyde or ketone
6. 12 for a β-substituent of a conjugated aldehyde or ketone

exocyclic double bond

an endocyclic double bond

double bonds frozen in the *s*-cis conformation



he rules enabling us to calculate λ_{max} can be best understood by looking at a few xamples.

base:	217
3 alkyl substituents:	15
calculated λ_{max}	232 nm
observed λ_{max}	232 nm

base:	217
additional double bond:	30
s-cis conformation:	36
2 alkyl substituents:	10
calculated λ_{max}	293 nm
observed λ_{max}	293 nm

base:	215
α-substituent:	10
β-substituent:	12
exocyclic double bond:	5
calculated λ_{max}	242 nm
observed λ_{max}	241 nm

base:	217
3 alkyl substituents:	15
exocyclic double bond:	5
calculated λ_{max}	237 nm
observed λ_{max}	235 nm