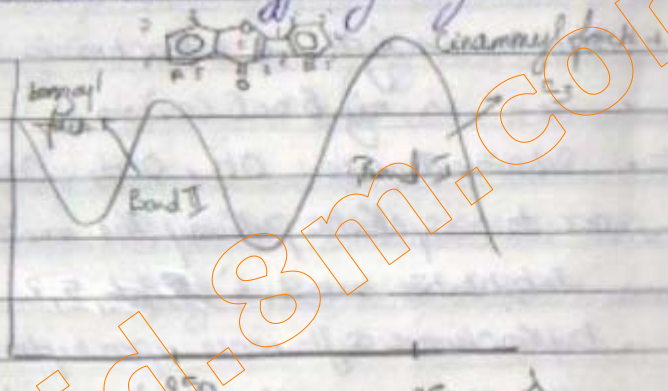
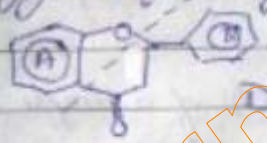
Lect (4)

Detectors of Flavonoids:

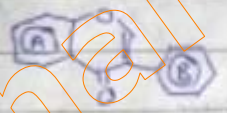
Flavonoidal cpds may be characterized through investigation of their U.V. spectra in methanol & diff. shift reagents



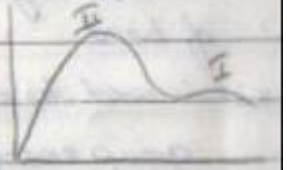
if conjugation of cinnamoyl fraction ↓



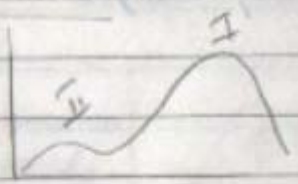
Conjugated flavone → only band II appears



isoflavone →



band I



Chalcone & aurone

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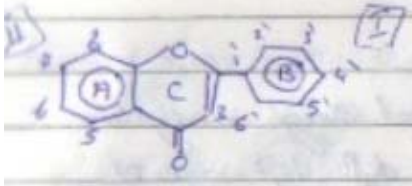


- Substitution on ring A → bathochromic shift in band II
- " " ring B or C (c-2) → " " " " I
- Substituents are: OH, OCH_3, Oxygen
- acc. to biosynthetic pathway the most common position for substitution are 3, 4', 5, 7
- bathochromic shift occur upon hydroxylation due to inc. in polarity 3-OH > 4'-OH > 5-OH > 7-OH (in polarity)

Shift reagents:

* reagents when added to the methanolic soln. of a sample causes a shift of λmax & it may affect intensity & position of band.
* it is used to determine the position of OH group

group example:- 1) NaOMe (4'-OH, 7-OH, 6,8 ↑, 3,6 ↑, 3',4' ↑)
2) $AlCl_3$ (3-OH, 5-OH & ortho dihydroxy gp.)
3) HCl (3-OH, 5-OH & orthodihydroxy gp.)
4) NaOAc (7-OH)
5) H_3BO_3 (ortho dihydroxy gp. i.e. 3', 4')

Practical steps:

1- prepare the methanolic solution of flavonoid & measure λmax. Then add few drops of NaOMe to the solution & remeasure
2- another methanolic solution + few drops of $AlCl_3$ & measure λmax. then add few drops of HCl to the solution & remeasure
3- dissolve crystals of NaOAc in another MeOH solution till formation of a saturated solution & measure λmax, then add few drops (or solid) of H_3BO_3 to the solution & remeasure

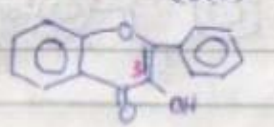
[1] MeOH
MeOH + NaOMe
[2] MeOH
MeOH + $AlCl_3$ + HCl
[3] MeOH + NaOAc
MeOH + NaOAc + H_3BO_3

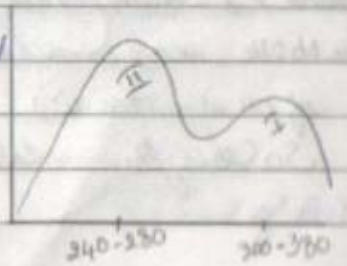
UV spectra:

1- methanol:-

2 bands appear but we are only concerned
a) band I (even if band II appeared, we don't want it)

a) λmax > 350 nm ⇝ ∴ it is Flavanol (3-OH)





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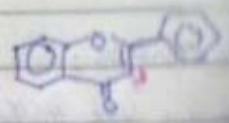
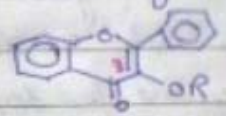
b) λmax < 350 nm ⇒ it is Flavone (3H) 

c) λmax 330-350 nm ⇒ it's a substituted flavone



(where R = CH_3, sugar, ...)

2. NaOMe:

* concerned by band I, it indicates the presence of 4' OH
* it forms salt with 4' OH, makes it more polar,
  ⇒ it makes a strong bathochromic shift ≈ 50 - 70 nm

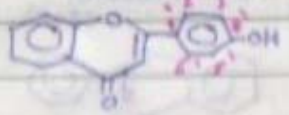
N.B: NaOMe has a small effect on OH groups at all positions but not of interest

	band II	band I
MeOH	255	348
NaOMe	263	394

(in methanol we are only concerned by band I, (348 nm) ⇒ means that there is one ortho position)

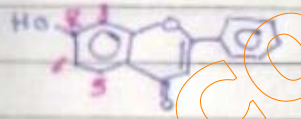
(in NaOMe, we are concerned also by band I (394 nm) ⇒ indicating presence of OH at position 4')

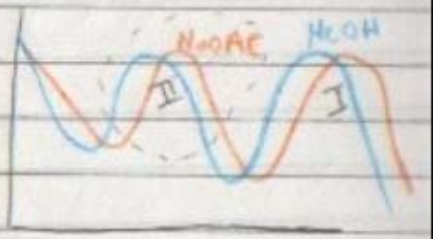
So this flavonoidal cpd has the following structure



3. NaOAc:

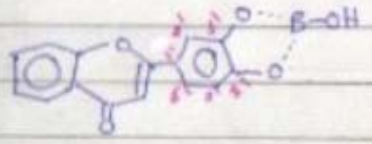
* concerned by band II, it indicates the presence of free 7-OH, it causes bathochromic shift (7-20)

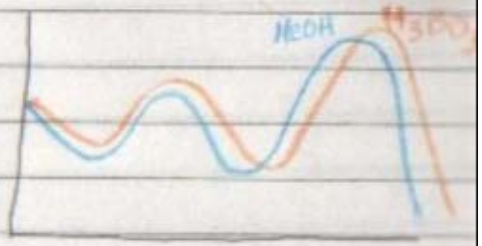




4. H_3BO_3:

* indicates the presence of O-dihydroxy group (especially 3',4')
* we are concerned by band I & II, but we are concerned more with I





The end of lect 4

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