

Third Year
17/6/2014
Time allowed: 2 hrs

Tanta University
Faculty of Pharmacy
Dept. of Pharmacognosy

Applied Pharmacognosy

Part (I): Spectroscopy and Biosynthesis

70 points

A-Spectroscopy and elemental analysis:

1-Nicotine has a molecular formula $C_{10}H_{14}N_2$, deduce the percentage composition of each element. Calculate also the unsaturation. 6 points

Molecular weight = 162

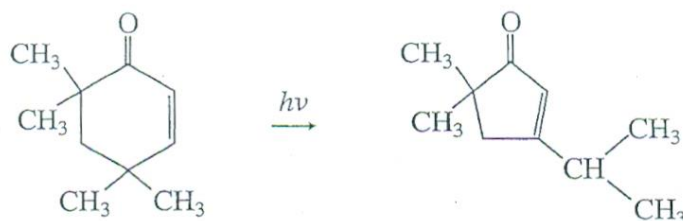
% of Carbon = $10 \times 12 \times 100 / 162 = 74\%$

% of Hydrogen = $14 \times 1 \times 100 / 162 = 8.7\%$

% of Nitrogen = $2 \times 14 \times 100 / 162 = 17.3\%$

2-Ultra violet spectroscopy:

a- Is UV spectroscopy a good way to distinguish the reactant from the product of the following photochemical reaction. 4 points



Calculation of UV λ_{\max}

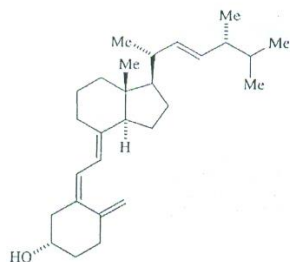
UV λ_{\max} of reactant = $215 + 12 = 227 \text{ nm}$

UV λ_{\max} of product = $202 + 2 \times 12 = 226 \text{ nm}$

Thus UV spectroscopy is not a good way to distinguish the reactant from the product of this photochemical reaction.

6-Ergocalciferol has a UV λ_{\max} at 265 nm explain based on Woodward's rules

4 points



Calculated UV λ_{\max}

Parent heteroannular diene 214 nm

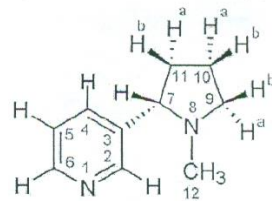
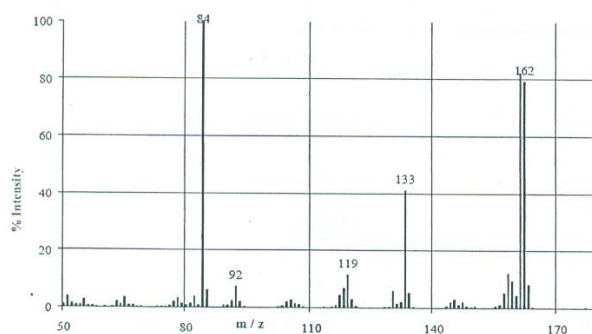
Extended conjugation + 30 nm

Four ring residues + 4x5

=264 nm,

which is consistent with the actual UV λ_{\max}

3-Mass spectroscopy

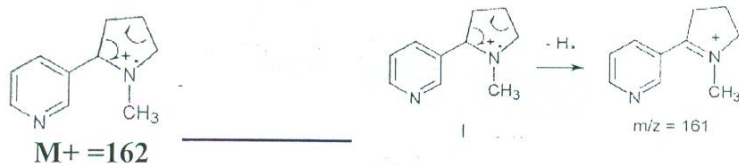


The EI MS of nicotine showed two major peaks as a result of fragmentation of its molecular ion ($M^+ = 162$): even electron ions at $m/z = 161$ and the base peak at $m/z = 84$ explain.

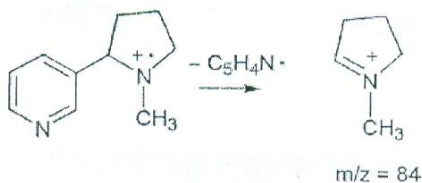
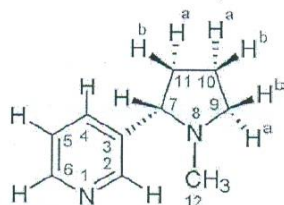
8 points

The fragment ions are formed by cleavage β to heteroatom to form resonance stabilized carbonium ion:

a-



b-

**4-NMR spectroscopy:**

Nicotine

Considering alkaloid nicotine, it showed the following NMR spectral data:

^1H -NMR spectrum (CDCl_3) δ_{H} ppm:

9 points

a-8.54 (1H, s).	b-8.49 (1H, d, $j = 4.8$ Hz)
c-7.69 (1H, d, $j = 7.9$ Hz).	d-7.26 (1H, dd, $j = 4.8$ Hz, 7.9 Hz)
e-3.08 (1H, t, $j = 7.0$ Hz)	f-3.00 (2H, t, $j = 7.4$ Hz)
g-2.70 (3H, s)	h-2.00 (2H, m)
i-1.80 (2H, m)	

heavily mark the position of each signal in answer sheet 1.

ii- ^{13}C NMR spectrum (CDCl_3) δ_{C} ppm (multiplicity indicated in parenthesis):

10 points

a-149.6 (d)	b-148.7 (d)
c-138.8 (s)	d-134.8 (d)
e-123.6 (d)	f-68.9 (d)

g-57.0 (t)
i-35.3 (t)

h-40.4 (q)
j-22.6 (t).

Heavily mark the position of each signal using answer sheet 2.

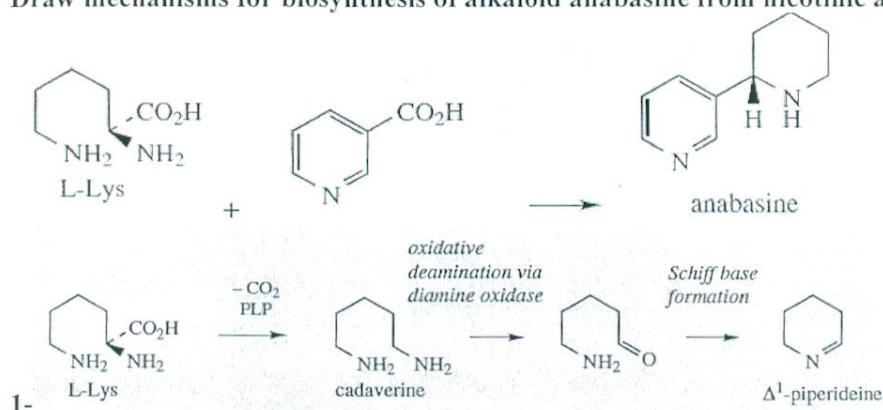
iii-What are the factors that affected your assignment of both proton and carbon-13 NMR signals? 2 points

In proton NMR : chemical shift, multiplicity, integration and coupling constants.
In carbon-13 NMR: chemical shift and multiplicity

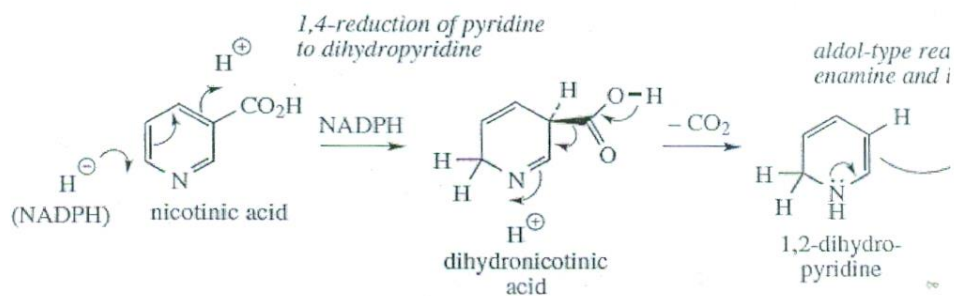
B-Biosynthesis:

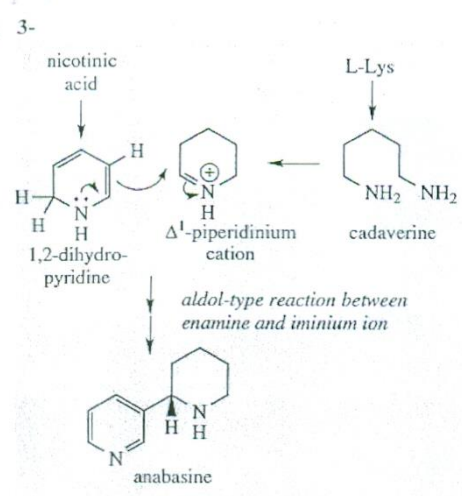
12 points

Draw mechanisms for biosynthesis of alkaloid anabasin from nicotinic acid and l- lysine



2-





C-General:

15 points

Match the number of the following statements with a correct answer in answer sheet 3 by writing the number of the sentence in front of the correct answer:

- 1-A cleavage of a molecular ion, which produces an odd electron ion and a neutral molecule.
- 2-Reagent gases used for CIMS. $3-\gamma = 2 \pi \mu / h I$
- 4-A UV shift reagent used to detect C-4'-hydroxyl group in flavonoids.
- 5-Abundant ions observed in mass spectra of natural products, when anion (-ve mode) ESI-MS is used.
- 6-A peak encountered in mass spectra of aromatic compounds with alkyl substitution.
- 7-A factor, which contributes to increased abundance of fragment or molecular ion
- 8-Fragmentation of carbonyl compounds with alkyl substitution longer than 2 carbons.
- 9-Larmor equation
- 10-Chemically and magnetically different methylene protons.
- 11-A factor, which causes shielding of acetylene protons and deshielding of aromatic protons.
- 12-Enzyme and reaction name, which catalyses conversion of chrosmic acid to prephenic acid.
- 13-Mechanism of aromatic hydroxylation & experiment used to prove itf.
- 14-The reason for use of deuterated NMR solvents.
- 15-The reason (s) for increased sensitivity of modern NMR machines.
- 16-the reason for downfield location of carboxylic acid protons.
- 17-An cofactor, which mediates decarboxylation and transamination reactions.
- 18-Compounds, which show long range coupling.
- 19-Assessment of incorporation of radioactive precursors into natural products.
- 20-An NMR experiment, used to determine carbon multiplicity.
- 21-A biosynthetic reaction, which links carbonyl, amino group and electron rich carbon and pivotal for alkaloid biosynthesis
- 22-One way for detection of NH or OH protons.
- 23-Natural products with mixed biosynthetic origin (shikimate and acetate).
- 24-Precursor for plant steroids
- 25-Amino acid precursors of pyrrolidine and piperidine alkaloids, respectively
- 26-Intermediate cation in the biosynthesis of hygrine, cuschygrin, atropine and nicotine.
- 27-precursor (s) of nicotinic acid in most plants.
- 28-A factor, which affects 3J coupling constant.
- 29-A change, which happens in the formation of sesquiterpenes.
- 30-Detection of metabolites with contiguously labeled ^{13}C atoms.

Answer sheet (1): ^1H NMR assignment

Position	a	b	c	d	e	f	g	h	i
2	X								
4			X						
5				X					
6		X							
7					X				
9						X			
10									X
11								X	
12							X		

Answer sheet (2): ^{13}C NMR assignment

Position	a	b	c	d	e	f	g	h	i	j
2	X									
3			X							
4				X						
5					X					
6		X								
7						X				
9							X			
10										X
11									X	
12								X		

Answer Sheet (3)

Answer	Statement Number	Answer	Statement Number
Benzylic or tropolonium cation	6	Methyl & hydride shifts	29
Liquid scintillation counting.	19	Pyridoxal phosphate	17
Sodium methoxide (NaOMe)	4	Dihedral angle	28
Satellites in ^{13}C NMR spectra	30	As a signal to stabilize magnetic field	14
Diastereomeric protons	10		
Use of FT and High field NMR	15		
Mannich like reaction	21		
Aspartic acid + Glyceraldehyde -3-P	27		
N-methylpyrrolinium salt	26		
Stability of production	7		
(M-H) $^+$, (M $^+$ anion) $^-$	5		
Distortionless Enhancement by polarization transfer (DEPT)	20		
Unsaturated & rigid bicyclic systems	18		
Addition of D $_2$ O	22		
Methane, ammonia & Isobutane	2		
$\square = \text{KH}_0$	9		
Ornithine & lysine	25		
Formation of arene oxide, NIH shift	13		
Cycloartenol	24		
Stilbenes and phenanthrenes	23		
Gyromagnetic ratio	3		
Magnetic anisotropy	11		
Claisen rearrangement (mutase)	12		
Electronegativity & Resonance	16		
Retro Diels Alder	1		
McLafferty rearrangement	8		

- 17- Regarding to Gradient elution
 a- Shortening of the time of analysis
 b- Is used for separation of compounds of different polarity
 c- Can be used with refractive index detector
 d- All of the above e- Both (a) and (b)
- 18- The shape of the peak depends on mass transfer which is
 a- Increased by decreasing the thickness of the liquid film
 b- Cause radial and axial transport of the solute
 c- Cause radial transport of solute in opposite direction in both phases
 d- Considered as minor transport
 e-Both (a) and (b)
- 19- For separation of mixture composed of 50% NaOH, 25%Ca (OH)₂ and 25%Al (OH)₃ we can use cationic exchange resin and
 a- Al (OH)₃ is separated first c- Ca(OH)₂ separated later
 b- NaOH is separated first e- Both (a) and (c)
 d- Both (b) and (c)
- 20- Penetration of the substances through the gel depend on all of the following except
 a- Molecular weights b- Pores size of the gel
 c- Aromatic or heterocyclic compounds
 d- Branched or straight chain
 e- Arrangement of the atoms in the space

Answer sheet

No.	a	b	c	d	e	No.	a	b	c	d	e	No.	a	b	c	d	e
1						8						15					
2						9						16					
3						10						17					
4						11						18					
5						12						19					
6						13						20					
7						14											

B- Complete the following sentences and write your answers in the answer sheet;

- β ray in electron capture detector emit → ..(1)... electrons which react with the carrier gas to give ..(2)... electrons.
- The dissolved gas in mobile phase in HPLC is removed by... (3)... and...(4)...
- ...(5)... is a detector which make continuous scanning of the absorbance spectrum of the eluant
- The highly sensitive detector used in HPLC is ... (6) ...
- The separation of compounds by ion exchange depends on ... (7)... and ... (8)...of compound of low affinity,
- The amount of charged function groups in the resin is called ... (9)
- Polymerization of vinyl pyridine with acrylic acid gives (10) ... resin
- Quaternary ammonium resin is(11) resin.
- Carbohydrates are changed to volatile compounds by ... (12) ... and to ionic compounds by (13)

- Pyrolysis GC is used for the identification of ... (14) ...
- The hydroxyl propylation of sephadex G-25 give ... (15) ... where the water regain value of it is equal to(16)... ml/gm
- ... (17) ...detector is used for determination of organic compounds only
 - Centrifugal desalting is used for desalting of ... (18)...
 - In gel filtration Kd equation is ... (19) ...
 -(20) ... is suitable for separation of very high molecular weight compounds

Answer sheet:

No.	Correct word (s)	No.	Correct word (s)
1	Highly energetic	11	Strong anionic.
2	Thermal	12	Silylation
3	ultra sonication	13	Porate complex.
4	Filtration under vacuum	14	Non vol. polymers
5	photodiode array	15	LH ₂ O
6	Fluorimetric	16	2ml/gm
7	Size of charge	17	FID
8	Conc. of sub.	18	Viscous soln.
9	resin capacity	19	$K_d = \frac{V_e - V_0}{V_i}$
10	Amphoteric resin	20	Sepharose gel.

PART (III): Quality Control and Complementary and Alternative Medicine

(40 Marks, 32 min.)

Complete the following sentences and put your answer in the Answer Table below:

- Going on a special nutrition program instead of receiving chemotherapy for cancer is an example of(1)...
- When live microorganisms administered in adequate amounts confer a health benefit to the host, this is called.....(2).....
-(3) uses a variety of techniques designed to enhance the mind's capacity to affect bodily function and symptoms.
- Illness is the result of negative states of mind and mental disharmony. This is the basic concept of.....(4)...
- Beneficial effects of soy on menopausal symptoms and in the prevention of osteoporosis, atherosclerosis, and breast and prostate cancers is an example of.....(5).
-(6).... is a health care discipline and profession that emphasizes diagnosis, treatment and prevention of mechanical disorders of the musculoskeletal system.
-(7).... is based on the belief that an energy, or 'life force', flows through the body in channels called meridians.
-(8).... is the best deep tissue massage available being free from side effects.
- Sickness, stress, impurities alter the natural frequency of the body causing some areas of the body to be unable to receive the necessary energy. This is the basic concept of.....(9).....
- (10).... is a natural healing art based on the principle that there are reflexes in the feet, hands and ears and their referral areas, which correspond to every part, gland and organ of the body.
- Acupuncture may stimulate the release of (11) ... from nerves and muscle tissue.
- Chiropractic is absolutely contraindicated in case of.....(12).....
- ... (13)can conveniently be employed for determining the percent of foreign matter in whole or cut plant materials.

-(14)..... is employed for drying of some volatile oil containing drugs e.g chamomile flowers.
- A high acid-insoluble ash in drugs such as senna and licorice indicates... (15)
-(16).....is used to detect excessive amounts of woody matter in powdered drugs.
- Determination of tannin content is based on polyphenols adsorbed by hide powder and giving a color reaction with.... (17).....
- The bitterness value is determined organoleptically by comparison with(18)..... which acts as a standard.
- The presence of.....(19).... in plant material can be hazardous to health if absorbed even in very small amounts.
-(20)... is an example for standards applicable to volatile and fixed oils.
-(21)..... is a test that uses antibodies and color change to identify a substance.
- Measurements of some cell types such as stomata, vessels, stone cells, fibers, pollen grains or cell contents is called.....(22)...
- Stomatal number varies considerably with the age of the leaf, while(23)..... is highly constant for a given species.
- A magnified image of the object under the microscope may be traced on paper and determined quantitatively by the use of.....(24)....
- Determination of the percentage of a drug present in a mixture could be achieved by using.....(25)....

- Summarize the principle equations for Karl Fischer procedures(2.5 Marks):

.....

.....

.....

.....

.....

.....

.....

Answer Table

No.	Answer	No.	Answer
1	Alternative/nutraceuticals	14	Distillation method / <i>clavanger</i>
2	Probiotic	15	Contamination with earthy material (sand and silica). / <i>adulteration</i>
3	Mind-Body Medicine / <i>prayer</i>	16	Crude fibre determinations
4	Flowers Remedies.	17	Sodium phosphomolybdotungstate reagent.
5	Functional foods / Nutraceuicals / <i>Biologically based</i>	18	Quinine hydrochloride solution
6	Chiropractic	19	Aflatoxins
7	Acupuncture	20	Refractive index , Optical rotation , Quantitative chemical tests
8	Cupping Therapy	21	ELISA
9	Crystal Therapy - <i>Energy medicine</i>	22	Microscopical linear measurements.
10	Reflexology	23	Stomatal index
11	endogenous opioids / <i>serotonin</i>	24	Camera lucida
12	Bone Weakening / Neurological Disorders / <i>Pregnancy</i>	25	Lycopodium spore methods / <i>Quantitative microscopy</i>
13	Macroscopic examination		

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