Analytical Chemistry Cumulative Exam
October 20, 2007
Solutions
You were asked to "Read, and answer, all parts of all the questions."

I cannot say strongly enough that this is critically important with all exams. Read the entire question! Answer the question, and not any other question. If you simply wrote down every possible answer you could think of, about everything, with the hope that I would see something good in there, and pull it out and give you credit for it, you are wrong. I took off points for frivolous answers that had nothing to do with the question. Get in the habit of answering the question. Concise answers to just the question you are asked are always better! Period.

Note that there is not ONE correct answer for these questions. What is written below is just an example of a fully acceptable set of answers.

In 1976, The Viking Mars landers used a Thermal Vaporization-GC/MS instrument in the search for soil organic matter from the surface of Mars. It did not yield a successful observation of organic carbon on Mars. 33 years later, in 2009, NASA will launch the Mars Science Lab, which will contain a very similar experiment to search for organic matter on Mars.

All questions were worth 20 points out of 100.

1. For environmental analysis, it is often the case that sampling is as important as the nature of the instrumentation used. Discuss the limitations in the Viking soil sample acquisition, and how it may have affected the results from 1976. Discuss some of the things that should be done better (and why) the next time regarding sampling, to ensure that samples containing organic carbon (if in fact they exist) are acquired.

A major issue for sampling in the Viking experiment was that the lander acquired soil from the surface. But, the surface is exposed to high energy radiation, and to free radical oxidizing agents that would likely have consumed any organic compounds that might otherwise have been present. If you are looking to find an organic compound, you must look in an environment in which it would be expected to be stable for a very long time. Next time, they should look where remote sensing indicates there may have been water, and use a drill to sample deeper, e.g. >1m depth.

2. In the October 1 *Analytical Chemistry* story about the performance of the previously flown TV-GCMS instrument, the article mentions, in one paragraph, that the "analytical precision", the "detection sensitivity" and the "detection limit" of the instrument were in the "ppb range". To prove conclusively that there is organic carbon in Martian soil, which of these three would be most important for your instrument, if you were to design one for this task. In your answer, define the term. For example, if you think "sensitivity" is most important, define that term, and explain why it is the key performance criterion.

Detection limit is what matters here. You are simply trying to detect organic molecules. Detection limit is the minimum quantity of analyte that can be distinguished from the "noise", or

background, or blank, signal, and is often defined a $3S_{blank}$/(sensitivity), where "sensitivity" is the slope of the calibration curve. I was surprised to find many students said you need "ppb" detection limits. Why ppb? Why not ppt? ppq? Why not single molecule, wouldn't that be best?

3. Among the "prebiotic" molecules that the NASA team is interested in determining are long chain carboxylic acids. Describe some of the limitations of the TV-GCMS method, for fatty acid determination, and discuss how they might be overcome. Design a sampling and analysis procedure, with as much detail as you can, that might be more effective in the determination of fatty acids in Martian soils. Keep in mind the spacecraft limitations of low power, low mass, and small size. Explain how the sample would be processed, and how the sample would be input to the instrument.

For this one many, if not most, of you listed a large number of different approaches that "might" work. Read the question. I asked for you to design a sampling and analysis procedure, with as much detail as you can. So, don't provide me with a list of options and ask me to pick the right one.

Since fatty acids are ionizable, and can be bound rather strongly to minerals/metals, volatilizing them requires high temperatures. The high temperatures can then pyrolize the analytes, so that the structural information is lost. If the carboxylic acids retain the carboxylate group, they will be very difficult to elute/separate, using a porous polymer stationary phase like Tenax. If I were to design a sampling and analysis procedure it would have these characteristics:

1. It would involve drilling down ~1m or more into the soil, in the process grinding the soil/rock to a powder.

2. The soil/rock powder sample could then be aspirated into a sample processor.

3. In the sample processor, I would extract the organics into a mixed organic/aqueous phase (e.g. a methanol/H_2O mixture), which is made basic, to ionize the carboxylic acids, and increase their solubility. The extraction would be done with the aid of an ultrasonic source.

4. The extract would then be filter separated from the soil particles, and reacted with a derivitizing reagent, that makes an ester that is chromatographable (i.e. they does not irreversibly bind to the column). For example, Chien et al., *ES&T, 32,* 299 (1998) describe derivitization using pentafluorobenzyl bromide, to produce pentafluorobenzyl esters as stable products. While Chien et al. and others use GC/MS to separate and detect these species, I would be inclined to use GC with an electron capture detector, because of the very low limits of detection for fluorinated species, and detector operation simplicity. With this method you maximize the chances of detection of organic acids, while retaining structure/size information from the retention times. This instrument could be small size and low power. A GC/MS would provide specific structural information, but would be higher power, and likely have poorer limits of detection. Here I will accept any answer that will solve the problems inherent in the TV-GC/MS method, while being manageable from a small spacecraft with low power requirements.

4. If your objective is to simply detect organic carbon, e.g. fatty acids, explain why you might or might not benefit from a combined GC/MS method. What might the benefits/losses be of using one of these techniques alone? Explain in detail.

There are clear benefits and costs with each approach.

a. GC alone, with some other simple detector, like the ECD, as discussed above; - this enables better limits of detection than with an MS method. It also enables lower power, lower mass, and lower complexity, except for the derivitization step. However, it does not provide compound-specific detection.

b. For MS alone – you get around the fact that fatty acids are difficult to elute/separate via direct chromatography, so you could simplify the sample preparation. There would be no need for carrier gases, and you get structural information from the spectra. However, the spectra could be complex, and thus it is likely you would need MS/MS capability to do terminal identification of individual species. That process typically degrades the limit of detection.

c. For both techniques together, you have maximum complexity (increasing possibilities of failure), except that you don't need MS/MS (although the option is there). And, making use of retention times, this method is often terminally selective. This is the relatively high power requirement method.

5. An issue for interpretation of the 1976 Viking data is the extent to which there was Fe in the soil samples, and the extent to which that Fe was Fe(III) or Fe(II). Explain exactly what the issue is with these forms of Fe, and discuss how you might avoid this problem if you were designing the experiment.

In the TV-GC/MS method the soil sample components were thermally vaporized. Because Fe^{3+} is a reasonably good oxidizing agent, at high temperatures the organic species, if they were present, could be oxidized by the Fe^{3+}. If there was a stoichiometric excess of Fe^{3+} relative to reduced carbon atoms, the oxidation could have been essentially complete, rendering the target analytes to be below the detection limit. Thus, again, if I were designing the experiment, I would sample via ultrasonic extraction.

Answers to Biochemistry Cume, October 20, 2007

1. The DNA of S *Pneumococci* was replicated in mice and then transcribed to mRNAs by the cellular enzymes. Translation of the bacterial mRNAs in mice produced the *Pneumococci* proteins that caused pneumonia in mice. **10 points**

2. The bacteriophage T2 contained ^{35}S-labeled proteins and ^{32}P labeled DNA. Briefly after exposing *E. coli* to the bacteriophage, Hershey and Chase, separated the infected cells by centrifugation. They found that the infected cells contained P32 (i.e. bacteriophage DNA) but no S35 (i.e. bacteriophage proteins). They recovered the S35 labeled material in the supernatant of the centrifugation experiment. Furthermore, they observed that P32 labeled bacteriophage DNA lead to production of bacteriophages in the infected *E. coli* cells. **20 points**

3. A cloning vector is used as a vehicle for creating recombinant DNA, to introduce "foreign" DNA in cells. **5 points**

4. A cloning vector should include: unique restriction enzyme cleavage sites, for inserting the foreign DNA into the vector; a replication origin (for amplification of the recombinant DNA); and one or more genes that would cause the recipient cells to become resistant to antibiotics. Such genes include *AmRes* and *TetRes*. They are use for selecting cells that contain the recombinant DNA molecules. **15 points**

5. In cloning experiments, it is preferable to have cohesive ends. Compatible cohesive ends can form hydrogen bonded base-pairs and would thus increase the efficiency of the ligation reactions. **5 points**

6. In cells, the genomic DNA is transcribed to mRNA precursors. These precursors are processed and modified to produce the mature mRNAs that serve as template for protein synthesis.

   Libraries of cDNAs are produced from reverse transcription of mature mRNAs isolated from cells. The cDNAs are copied to create double-stranded DNA for cloning in appropriate vectors. Therefore, the sequence of cDNAs would correspond to the sequence of mRNAs present in a given cell-type. **10 points**

   To produce genomic libraries, the genomic DNA is fragmented and then cloned in appropriate vectors. **5 points**

7. No question 7, the information was part of question 8

8. Yes, one can use a portion of the coding region for the human β–globin gene as probe to screen either a human cDNA library or a human genomic DNA library. In screening genomic libraries, the probe will hybridize to the bacterial colonies that contain the exons of the gene. In screening cDNA libraries from erythroid cells, the probe will identify colonies that contain the cDNA corresponding to human β–globin mRNA. **10 points**

9. A gene containing 3 introns would contain 4 exons. In contrast, a complete cDNA would contain only the exons. Therefore the cDNA would be shorter than the genomic DNA. **10 points**

10. Given the sequences of both the cDNA and the genomic DNA, we can identify both the introns and the exons of a gene. This achieved by using sequence alignment programs. The sequence of the cDNA would produce a match with the genomic DNA regions that contain the exons. The sequence between the exons in the genomic DNA would correspond to the intronic regions. **10 points**

No Inorganic crib available

October 20, 2007

Written by Professor Wilker

Department of Chemistry
Organic Cumulative Examination
October 20, 2007

You may choose to answer any exam from any area covered in the examination booklet. Each exam may contain multiple parts. You may answer more than one exam but each exam is scored separately and is treated as an individual examination result. Thus, answering parts of two exams with a score of 50% *would not* yield a 100% grade for this cumulative exam. Instead you would receive 50% on each examination attempted.

This booklet contains ***one*** examination.

1) Organic Cumulative Examination, Page 1-7

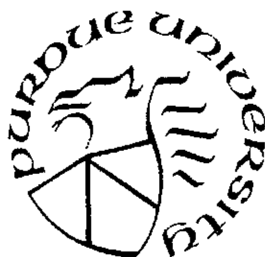
Please answer on these examination pages:

1) Print your student ID number. Crib

2) Exam Booklet number: 158

Do not write your name anywhere on the examination. Each exam will be scored anonymously. If you attempt more than one exam, *you must use a separate examination booklet for each examination*.

When you complete this examination, return it to the proctor. Exam results will be posted on bulletin board #2B on the north side of the hall near BRWN 2124.

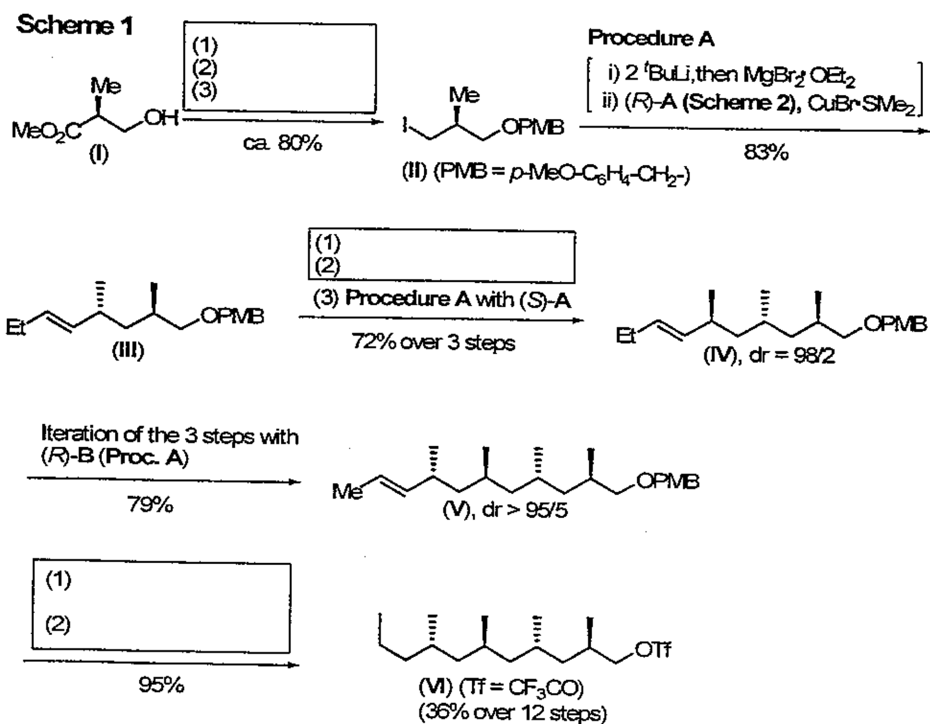


Student ID Number______________________________

Organic Cumulative Examination

10-20-2007

Schemes 1-3 show some details of the synthesis of 4, 6, 8, 10, 16, 18-hexamethyldocosane (*X*) by B. Breit (*ACIE* **2005**, *44*, 5267. See also *ACIE* **2004**, *43*, 3790 and a related work by K. Burgess *OL* **2007**, *8*, 1391).



Scheme 2

Novozym 435 (enzymatic catalyst)

OAc

55% conversion

E (selectivity factor) = 52

(S) 45% recovery 99% ee

o-DPPBOH DCC, DMAP CH_2Cl_2 88%

(S)-(-)-A >99% ee, >99% E

(R) 55%, 82% ee

Novozym 435 buffer (PH 7)

73% yield 96% ee

o-DPPBOH DCC, DMAP CH_2Cl_2

(R)-A >96% ee, >99% E

(R)-B = >99% ee, >99% E

o-DPPBOH = PPh_2 COOH

Scheme 3

(L) OTf

(R) Br Me

(1)

(2)

(3)

(X)

Questions

[1] (15 pts.) How would you achieve the **(I)**-to-**(II)** conversion? Just show **appropriate reagents without using names or nature of transformation**, such as Wittig olefination or oxidation.

5 pts each

(1) PMBCl, NEt_3 or another base

(2) DIBAL-H or its equivalent

(3) I_2, PPh_3

[2] (10 pts.) Do the same for the **(III)**-to-**(IV)** conversion.

5 pts each

(1) O_3, $NaBH_4$

(2) I_2, PPh_3

[3] (10 pts.) Do the same for the **(V)**-to-**(VI)** conversion.

5 pts each

(1) H_2, Pd catalyst (Pt and others may work for the C=C → C–C conversion)

(2) Tf_2O, NEt_3

[4] (15 pts.) Judging from the results shown in **Scheme 2**, the Novozym 435-catalyzed acetylation with vinyl acetate is clearly R-selective. Why then the (S)-isomer,which was not acetylated, was obtained as an essentially pure single enantiomer of >99% *ee*, whereas the selectively acetylated (S)-isomer was obtained as a much less pure compound of 82% *ee*? **Your answer must be less than 50 words.**

R

15 pts

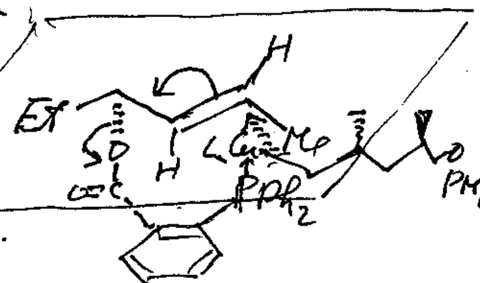
(1) The (S)-alcohol/(R)-alcohol ratio keeps increasing with time of reaction. So, (S)-alcohol of any desired enantiomeric purity can be obtained.

(2) On the other hand, the (R)-acetate/(S)-acetate ratio may initially be reasonably high. However, it will eventually become 1 (provided that the starting material is racemic).

[5] (15 pts.) Knowing the structures of (*R*)- and (*S*)-**A**, describe **very succinctly** the nature of the chain homologation steps, e.g., (*III*)-to-(*IV*), in terms of (1) stereochemistry, (2) regiochemistry, (3) possible mechanistic factor (or origin) of the observed stereochemistry.

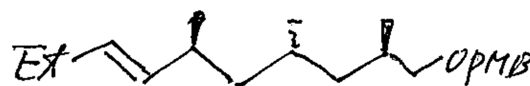
5 pts each

(1) Cu-catalyzed syn-allylation of an isoalkylmagnesium derivative



(2) The reaction proceeds with allylic rearrangement, as shown.

(3) It is very likely that the active species of the reaction is a phosphine complexed isoalkylcopper which should undergo a syn addition



(IV)

to give (IV) with the regio- and stereochemistry shown in the structure (IV).

[6] (15 pts.) The final coupling of **L** and **R** to produce **(X)** was achieved in one pot. Indicate the ~~required~~ reagent(s), if any, in the appropriate boxes.

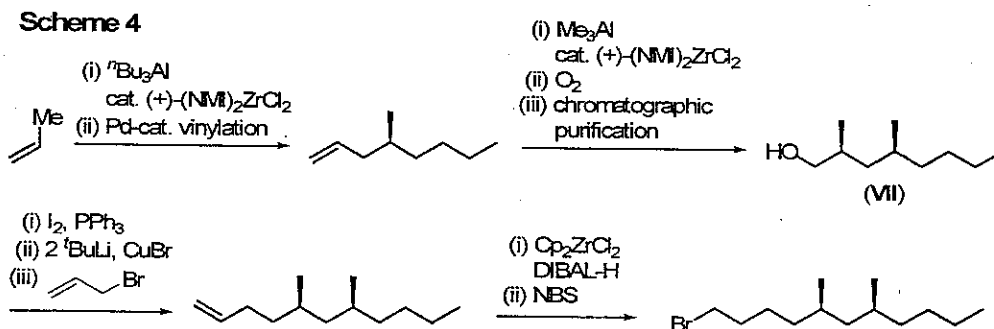
(Instead) ~~(1)~~ None needed

(2) Mg (and $BrCH_2CH_2Br$ added as a promoter of the Grignard reagent formation) ← optional

(3) A catalytic amount of Li_2CuCl_4 called Kochi's catalyst.

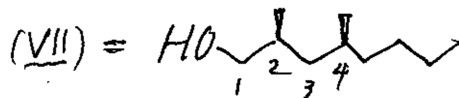
Note: Despite recent developments with Ni, Pd, Fe, and others, there is none better than the Cu-catalyzed Grignard reaction described above.

The Breit synthesis of **(R)** required 7 steps from **(I)**, which must be prepared in one or more steps. Using the Zr-catalyzed asymmetric carboalumination (ZACA reaction) discovered and developed recently, however, **R** can be prepared in 4 steps (cf. *PNAS* **2004**, *101*, 5782-5787), as shown below **(Scheme 4)**.

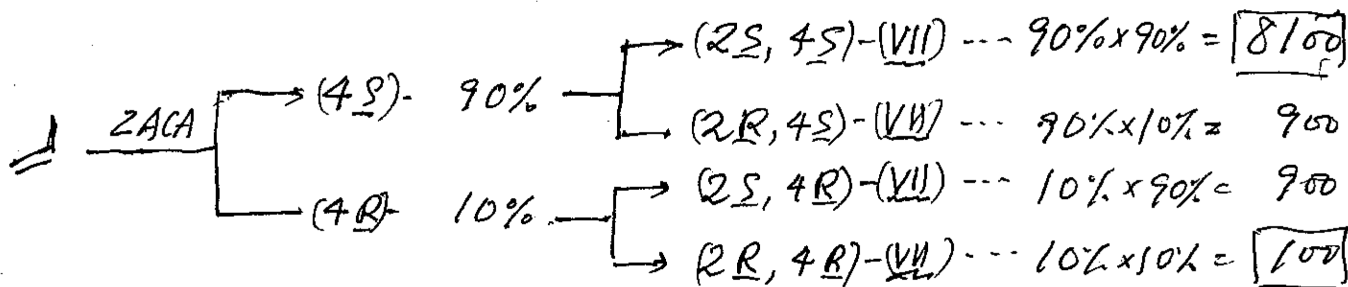


[7] (10 pts.) Assuming that both of the two ZACA processes are 80% *ee* producing a mixture of the *S* and *R* isomers in a 90:10 ratio, what are:

5 pts (i) overall enantiomeric excess of **(VII)** in *ee*?



Show a brief process of calculating your answer below. Use 2 significant digits.



Enantiomeric excess for (2S, 4S)-isomer $= \frac{8100-100}{8100+100} \times 100 = \frac{8000}{8200} \times 100 = 97.6\% \simeq$ 98% *ee*

5 pts (ii) overall distereomeric ratio of **(VII)**?

Diastereomeric ratio of (VII) $= \frac{8100+100}{900+900} = \frac{8200}{1800} =$ 4.6

[8] (10 pts.) Regarding stereoisomeric detection and/or purification, indicate the applicability, in principle, of the following methods. Just enter either Applicable or Not Applicable.

(1 pt each)

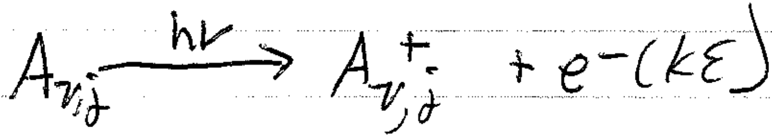
Method	Diastereomers	Enantiomers
• ^{1}H NMR (without added chiral reagent or solvent)	A	NA
• ^{13}C NMR (without added chiral reagent or solvent)	A	NA
• Chromatographic separation (without chiral chemicals)	A	NA
• Recrystallization	A	A in some cases
• Enzyme-catalyzed kinetic resolution	A in some cases	A

A = Applicable.
NA = Not applicable.

Physical Crib

2503

1 a) Photoelectron Spectroscopy

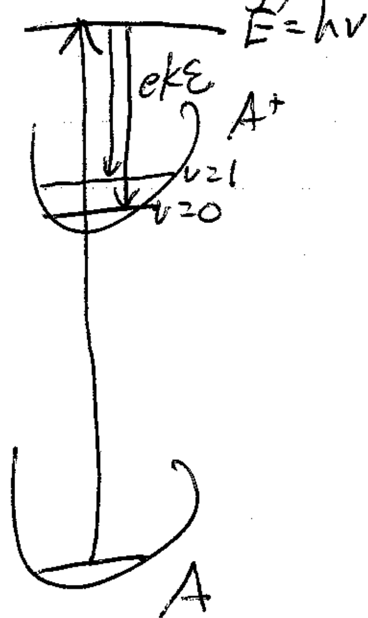


$$A_{v,j} \xrightarrow{h\nu} A^{+}_{v,j} + e^{-}(KE)$$

Molecule A (which can be neutral or anionic) is detached by monoenergetic photons $E = h\nu$, where E is above the ionization (detachment) threshold. The experiment involves measuring the kinetic energies of the detached electrons (eKE)

For the band from $A_{v=0} \rightarrow A^{+}_{v=0}$, the relationship is $E = h\nu = IE + eKE$

From an energy diagram



The energy difference between $v=0$ and $v=1$ (and $v=2$ etc) indicates vibrational levels in the upper state.

<u>Hot Bands</u> give vibrational levels in the lower state

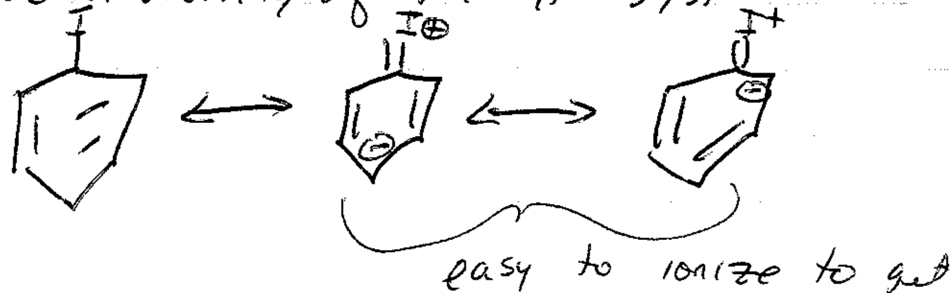
A plot of # of electrons vs kinetic energy is the <u>Photoelectron Spectrum</u>

8 + 1

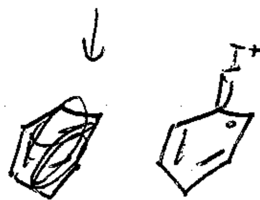
1b) Substituents can ~~cause~~ affect system by
1) inductive effects (σ)
2) resonance effects (π)
3) polarizibility (size)
4) electronegativity (normally similar to inductive effects, but ~~gen~~ technically separate)

8 pts

1c) i) I is a good π donor and increases the electron density of the π system



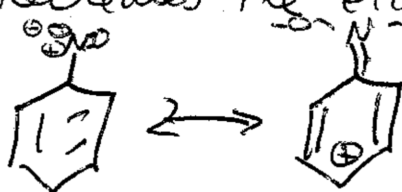
easy to ionize to get



IOW, the π charge can be delocalized to I

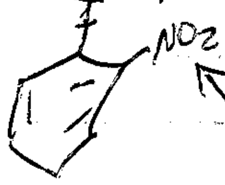
6 pts

1c ii) NO_2 is π withdrawing (acceptor) and decreases the electron density of the ring



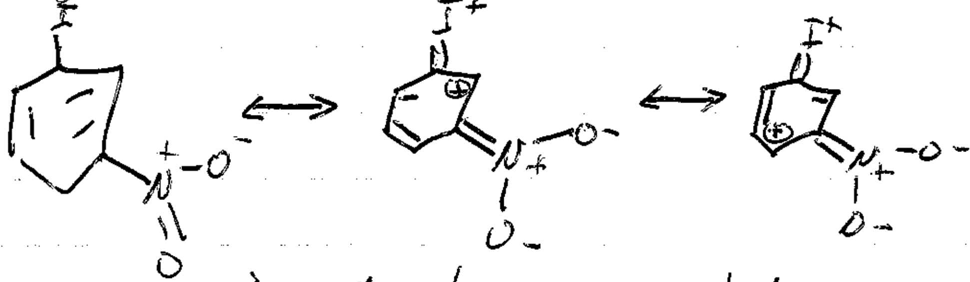
Electron deficient ring is harder to ionize

1c) iii) 6 pts Size problem → steric effects



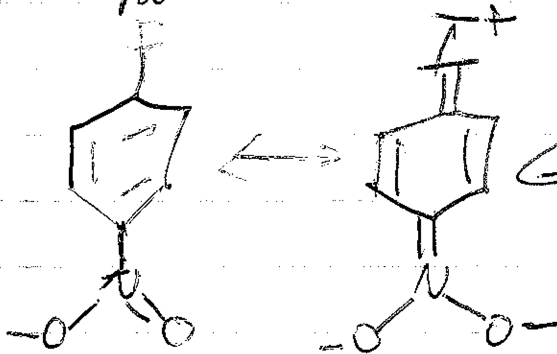
↖ the nitro can't adopt a planar arrangement with the ring, so NO_2 can't act as a π withdrawing group

iv) 5 pts My guess — compare m & p resonance structures



In m, the excess electrons are always in the ring

In para



← combination of 2 effects

Any reasonable answer is considered

1d) 6pts $E \geq IE$

$E = h\nu = hc/\lambda$

$\lambda \leq hc/E \quad hc = 1240\ eV \cdot nm$

E has to be greater than 9.40 eV

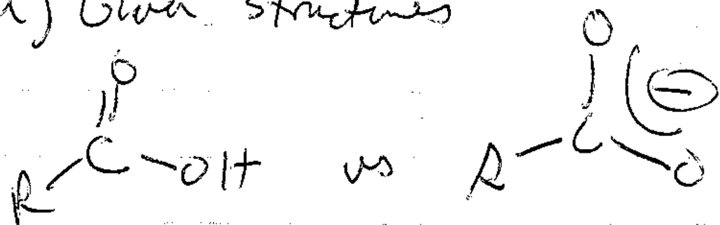
$\lambda \leq 1240/9.40 = 131.9\ nm$

1e) 3pts a) VUV = vacuum UV
UV that is too short of wavelength to pass through air

b) 8pts Photoionization is similar to PES in that $A \xrightarrow{h\nu} A^+ + e^-$

The differences are
1) Photoionization uses tunable wavelength
PES is fixed frequency
2) PI usually monitors ion formation as function of λ
PES detects electrons

2) a) [15 pts] Given structures



The neutral should have 2 distinct Oxygens, while carboxylate has 1 type

Much of the delocalization in the anion is already present in the neutral

b) [15 pts] Resonance is not the only (main) reason why carboxylic acids have enhanced acidity. The inductive effect of the carbonyl is a big reason for it (~70% due to inductive effects)