Experiment 5 – Two-Step Synthesis of Ionones

Ionones are unsaturated ketones responsible for the characteristic fragrance of violets (**Figure 1**). Perfumes from violets are difficult to obtain and have been highly appreciated since the days of Napoleon. An old method for obtaining this perfume consisted of embedding the petals of freshly cut violets between layers of animal fat. The aromatic oils were slowly absorbed by the fat that was then used as a hair cream or ointment. The most abundant isomers, **α-** and **β-ionone**, can be synthesized in the lab in a two-step process. A concentrated mixture of ionones has a smell that resembles cedarwood.

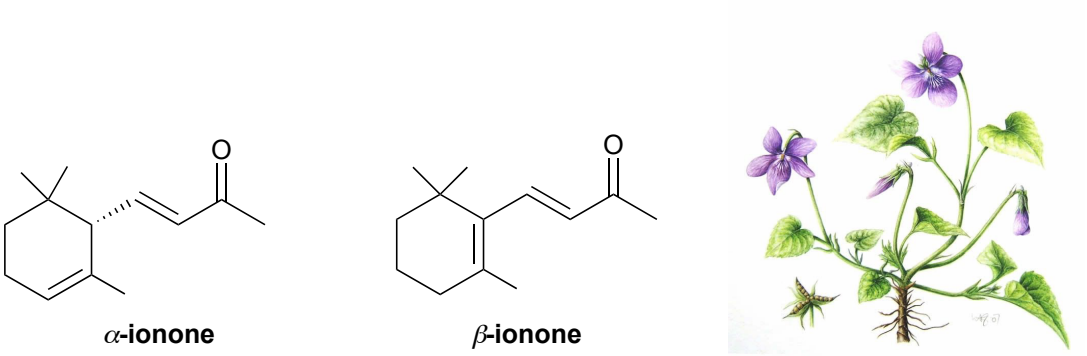


Figure 1. Structures of α- and β-ionone, present in violets

The synthesis of ionones is an important industrial process. Ionones are not only used in perfumery, but they are also key intermediates in the manufacture of vitamin A. Both ionones can be prepared *via* aldol condensation of citrals with acetone followed by acid treatment (**Figure 2**). Evaluate the structures below to identify which bond is formed by aldol condensation.

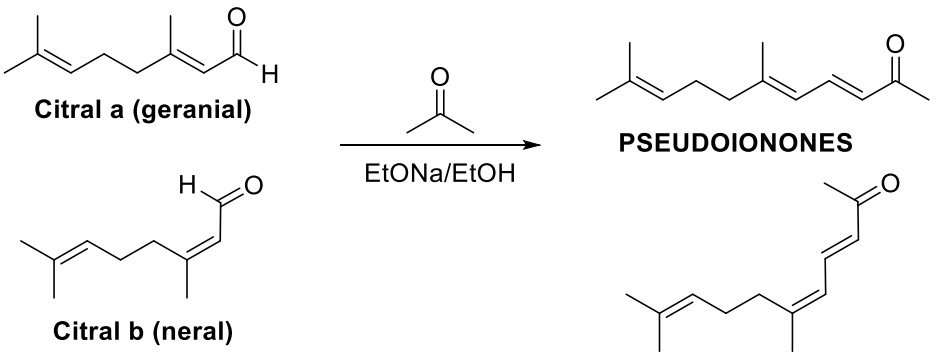


Figure 2. Synthesis of pseudoionones *via* aldol condensation

Citral is a mixture of the two *E*/*Z* stereoisomers of 3,7-dimethyl-2,6-octadienal. **Citral a** or **geranial** (*E* isomer) and **citral b** or **neral** (*Z* isomer) are the main components of lemongrass oil. The aldol condensation of citrals with acetone in the presence of sodium ethoxide gives a mixture of two products, **pseudoionones**, which differ only in the geometry of the double bond between C5 and C6 (**Figure 2**). Note that in making the **pseudoionones**, the newly formed double bond between C3 and C4 has *trans* geometry.

Treatment of **pseudoionones** with acid induces cyclization to **α-** and **β-ionones** (**Figure 3**). Depending on the nature and concentration of the acid, the formation of one isomer is preferred over the other. The products obtained in the presence of sulfuric or phosphoric acids are different; either **α-** or **β-ionone** is obtained as the major product as determined by GC.

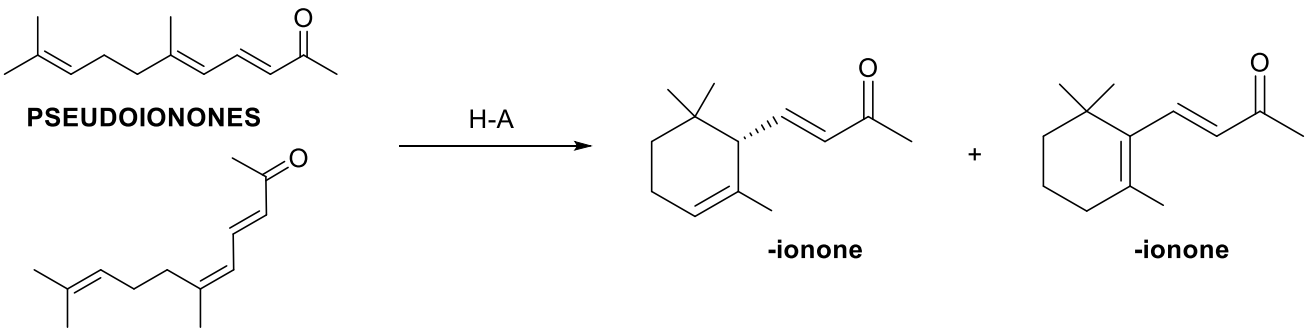


Figure 3. Acid-catalyzed cyclization of **pseudoionones**

The first step in the cyclization of **pseudoionones** is the protonation of C9 by the acid catalyst (**Figure 4**). This is followed by an intramolecular attack from C5 to form a six-membered ring with a tertiary carbocation. Both *E*- and *Z*-pseudoionones form the same carbocation intermediate.

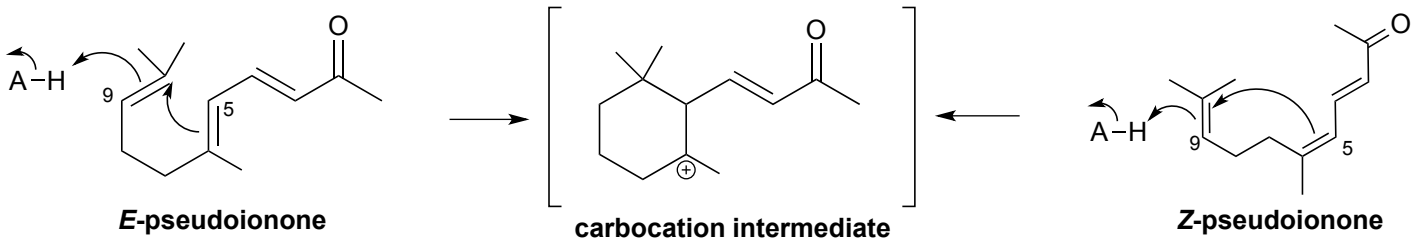


Figure 4. Carbocation formation in ionone synthesis mechanism.

Thermodynamic vs. kinetic control - One of the main objectives of the experiment is to determine which acid give which isomer as the major product and why. In the final step of the mechanism, the conjugate base of the acid catalyst removes a proton from one of the carbons adjacent to the carbocation. This reaction always gives a mixture of products. Depending on the acid catalyst used either α- or β-ionone is favored as the major product (**Figure 5**). α-Ionone is the *kinetic product*, meaning a stronger conjugate base (A^-) could quickly remove the less sterically hindered the secondary hydrogen (shown in red below). β-Ionone is the *thermodynamic product* due to the extra stability imparted by the extended conjugation. A weaker conjugate base should favor β-Ionone by removing the more acidic tertiary, allylic hydrogen shown in blue below, even though it's more sterically hindered.

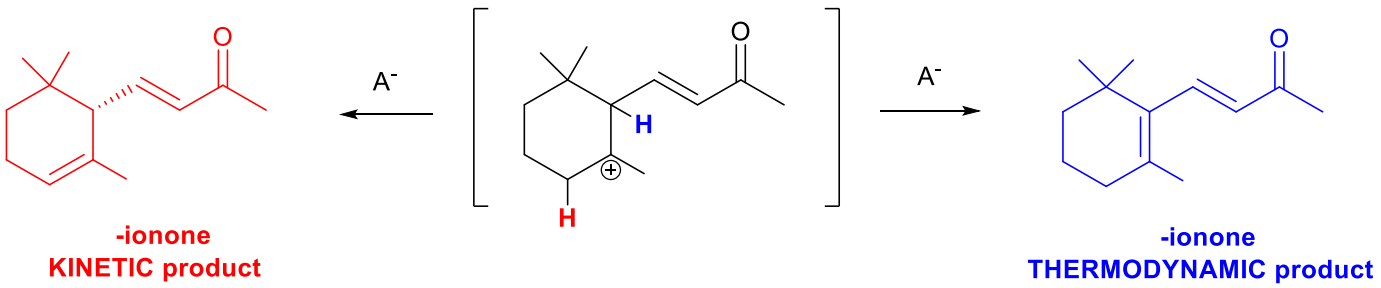
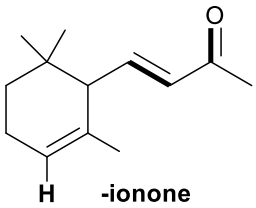


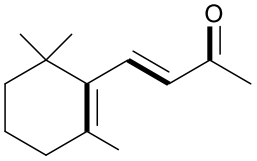
Figure 5. Formation of different alkenes in ionone isomers

In this experiment, you will be assigned either sulfuric or phosphoric acid to carry out the cyclization of **pseudoionones** and determine which ionone is the predominant product in your reaction mixture, as determined primarily by GC with UV-vis spectroscopy in a supporting role. Keep in mind that both products are formed in both reactions with a major product being present in anything greater than 50%.

The structural differences between the ionone isomers are detected by infrared (IR) and ultraviolet-visible (UV-vis) spectroscopy (**Figure 6**). All forms of spectroscopy take advantage of a molecule's ability to absorb the energy emitted by the instrument (spectrometer or "spec" for short). The remaining transmitted energy is detected by the instrument, reported to the software, and presented to the user as a graph of wavelength (λ, x-axis) vs. intensity (% absorbance or transmittance, y-axis). **IR spectroscopy** is based on the frequency of *stretching and bending of bonds* in organic molecules that fall within the IR range of the electromagnetic spectrum. The IR spectra of both isomers differ in the C-H out-of-plane bending region (600-900 cm^{-1}). α-Ionone, because of it extra vinylic hydrogen, presents several bands in this region that are absent in the IR spectrum of β-ionone (**Table 1**). They can also be distinguished by their GC elution order. In columns of medium polarity they come out in order of increasing boiling points.



-ionone
Conjugated system with **2** orbitals
Additional vinylic C-H bend: IR 600-900cm^{-1}



-ionone
Conjugated system with **3** orbitals
Strong UV absorbance at 295nm

Figure 6. IR and UV-vis characterization of ionones.

UV-vis spectroscopy is used to characterize conjugated pi (π) systems in organic compounds based on their ability to absorb energy through *resonance*. The energy emitted by the UV-vis instrument is absorbed by the conjugated ketone present in both ionone isomers, resulting in a signal at 227 nm. The additional conjugated alkene in β-ionone causes second, stronger absorbance at 295 nm. The greater number of pi orbitals in conjugation, the higher the absorbance wavelength as it translates to a lower energy.

Table 1. Physical properties of α- and β-Ionone and precursors (re-created from Palleros, D.)

	α-Ionone	**β-Ionone**	**Citral**	**Pseudoionones**
b.p. (3 mm Hg)	93 – 95 °C	101 – 103 °C	Approx. 75 °C	120 – 140 °C
λ_{max} (UV-vis spectrum)	227 nm	295 nm	-	-
IR bands, lit. (600 – 900 cm^{-1} range)	620, 738, 800, 827	-	842	-

The NMR spectra of ionones are complex on their own, let alone as a mixture of the two! It is impractical to take an NMR sample of your reaction since it will be a mixture of both isomers. Instead, literature ^{1}H NMR spectra of pure α-ionone and β-ionone are provided at the end of this document. These spectra exemplify advanced NMR analysis, including diastereotopic protons, and complex splitting patterns with long-range ^{1}H-^{1}H coupling due to the presence of alkenes.

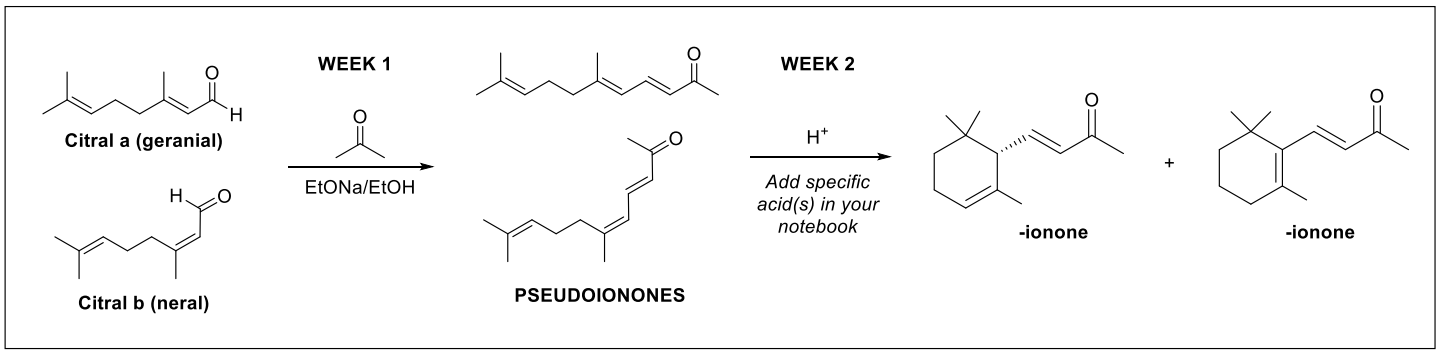


Figure 7. Reaction schemes for ionone synthesis from citrals

Notebook Preparation – refer to / copy the "worksheet" on Canvas for suggested notebook templates
** start a new notebook page each week*

- *Purpose*: Reaction schemes – **see above -** starting materials, reagents, product, other chemicals used to set up the reactions

- *Reagent table:* List the amounts (mg or mL and mmol), molar equivalents ("equiv."), and physical properties (MW, bp or mp, density, one-word hazard) of each chemical in the reaction scheme.

- *Procedure* – hand-drawn 'comic strip' with diagrams of all equipment and chemicals with amounts. Include pertinent notes from the Clean-up & Safety Table.
  - Week 1 – procedure and IR tables with expected values for the citrals & pseudoionones
  - Week 2 – include only the procedure assigned to your group based on assignment given by TA in week 1, as well as instructions for IR, GC, and UV-vis analysis.

- *Safety & Clean*-up*:* copy the pertinent information from the table that follows the procedure

EXPERIMENTAL PROCEDURE

Part A – Preparation of Pseudoionones (Week 1)

Prepare an ice-salt bath (- 8 °C) and place on a stir plate. Weigh 2.25 g of citral (1:1 mixture of citrals a & b) directly into a 25-mL Erlenmeyer flask. Add a magnetic stir bar and 11.25 mL acetone. Stir this solution in the ice-salt bath. Slowly over a period of 10 minutes, add 2.25 mL of 2.25 M NaOEt in ethanol drop-wise (solution prepared for students). Stir for an additional 20 minutes.

Slowly neutralize the reaction with 2 M HCl (approximately 3 mL). The reaction mixture should turn yellow, which is indicative of the highly conjugated product. Transfer the solution to a separatory funnel, using 15 mL of t-butyl methyl ether (BME) to aid in the transfer and to wash the flask. Add 9 mL of water and extract the product into the organic solvent. Separate the layers and extract the aqueous layer with two more portions 5 mL portion of BME. Separate once again and wash the combined organic extracts with a NaCl solution (10% w/v).

Dry the organic layer over $MgSO_4$, gravity filter with a glass funnel into a pre-weighed RBF, and concentrate using a rota-vap. Weigh the product and calculate the percent yield of the synthesis of pseudoionones. Obtain the IR spectrum of the product and compare to the provided IR spectrum of citrals (in lab). Analyze the citrals and products by GC to determine percent conversion to product. Save the remaining product in a labeled vial for next week.

Part B.1 – Cyclization with Sulfuric and Acetic Acids (Week 2)

Prepare an ice-water bath and place on a stir plate. In a 50-mL Erlenmeyer flask equipped with stir bar, add 1.95 mL of glacial acetic acid then slowly add 2.55 mL of concentrated sulfuric acid (98% w/w). Be especially careful when using these solutions in the fume hood and change your gloves immediately after, regardless of whether you think they’re contaminated.

Weigh 1.4 g of pseudoionones (week 1 product) into a test tube. If the week 1 product yield was less than 1.4 g, use the entire product. Add the pseudoionones to the acidic solution drop-wise over a period of 20 minutes. Record observations, including color changes and visible changes to the viscosity of the mixture. After the addition is complete, stir at room temperature for 20 minutes.

Prepare a mixture of 30 mL of cold water and 6 mL of BME in a flask. Swirl, then transfer to the reaction mixture, mix, and transfer it to a separatory funnel. Extract the product into the organic layer. Separate the layers and extract the aqueous layer with an additional 6 mL of BME. Wash the combined organic layers with 2 x 12 mL of an aqueous solution containing $NaHCO_3$ (5% w/v) and NaCl (10% w/v).

Check the pH of the aqueous solution to ensure it is basic before disposing. If necessary, adjust the pH with a $NaHCO_3$ solution and wait for bubbling to subside before transferring to waste. Dry the organic layer over Na_2SO_4, filter into a pre-weighed RBF, and concentrate with a rota-vap. Proceed to IR, GC, and UV-vis analysis.

Part B.2 – Cyclization with Phosphoric Acid (Week 2)

In a 25-mL Erlenmeyer flask equipped with stir bar and immersed in a water bath (30 °C), place 4.0 mL of concentrated phosphoric acid (85% w/w). Be especially careful when using this solution in the fume hood and change your gloves immediately after, regardless of whether you think they're contaminated.

Weigh 1.4 g of pseudoionones (week 1 product) into a test tube. If the week 1 product yield was less than 1.4 g, use the entire product. Add the pseudoionones to the acidic solution drop-wise over a period of 20 minutes. Record observations, including color changes and visible changes to the viscosity of the mixture. After the addition is complete, stir in the water bath for 20 minutes.

Add 30 mL of aqueous NaCl (10% w/v) and transfer the mixture into a separatory funnel. Wash the flask with 15 mL of BME and transfer the wash to the separatory funnel. Mix and separate the layers. Extract the aqueous layer again with 15 mL of BME. Wash the combined organic layers first with 15 mL of an aqueous solution containing $NaHCO_3$ (5% w/v) and NaCl (10% w/v), followed by 15 mL of aqueous NaCl.

Check the pH of the aqueous solution to ensure it is basic before disposing. If necessary, adjust the pH with a $NaHCO_3$ solution and wait for bubbling to subside before transferring to waste. Dry the organic layer over Na_2SO_4, filter into a pre-weighed RBF, and concentrate with a rota-vap. Proceed to IR, GC, and UV-vis analysis.

Analysis

Weigh the product and calculate the percent yield of ionones. Obtain the IR of product and analyze the product by GC. Do not inject thick products as this may damage the column. Instead, dilute a small sample of your product with acetone a few drops at a time in a vial or test tube until the solution is freely flowing, then inject the solution. Compare your sample to the provided 1:1 standard of α- and β-ionone, using boiling points for peak identification. Determine the percent conversion to product as well as percent composition of α- and β-ionone.

Dissolve 5 mg ($\pm$0.1 mg) of the ionones product in a 10-mL volumetric flask. Dilute to 10 mL with 100% ethanol. Perform a 1:20 dilution by dissolving 500 μL of the solution with 100% ethanol in a separate 10-mL volumetric flask. Follow TA instructions to obtain the UV-vis spectrum of this solution in the range 200-400 nm. If the absorbances at 227 and 295 nm are greater than 2, dilute the solution with ethanol and measure again.

Table 2. Clean-up & safety guidelines

Clean-up	**Safety**
Solid waste: pipets, filter paper, drying agent	$MgSO_4$, $NaHCO_3$, and citrals are irritants
Liquid Waste: aqueous layers & contents of rota-vap trap	Concentrated sulfuric acid is corrosive; take only what you need with the pipet provided; do not remove from hood
Return equipment to proper place – keep those ring stands organized please!	Acetic acid, phosphoric acid, HCl, and sulfuric acid are corrosive and irritants.
Clean up any snow storms of solids!	Acetone, ethanol, and BME are flammable
Quartz cuvettes for UV-Vis must remain in matched pairs – DO NOT MIX Quartz cuvettes are very expensive. There are no extras. Please be careful!	

References

- Hibbert, H.; Cannon, L. T. *J. Amer. Chem. Soc*. **1924**, *46*, 119-130.
- Kimel, W., *et. al*. *J. Org. Chem*. **1957**, *22*, 1611-1618.
- Krishna, H. J. V.; Joshi, B. N. *J. Org. Chem.* **1957**, *22*, 224-226.
- Palleros, D. R. *Experimental Organic Chemistry*; Wiley: New York, **2000**; pp. 520 – 530.
- Royals, E. E. *Ind. Engineer. Chem.* **1946**, *38*, 546-548.

Pre-Lab Questions – incorporated into auto-graded Canvas quiz, due Monday before WEEK 2 of this lab

1. Calculate the theoretical yield of pseudoionones from the amount of citrals given in the procedure. Show your work (including calculations for mmoles of starting materials).

2. How does the addition of salt lower the temperature of the ice bath?

3. Why is the NaOEt solution added drop-wise?

4. What is the purpose of HCl and NaCl in the reaction work-up of pseudoionones?

5. Calculate the theoretical yield of ionones from pseudoionones. Show your work.

6. The cyclization reaction to form ionones is catalyzed either by phosphoric or sulfuric acid. Report the pKa of both acids. Which acid has a stronger conjugate base?

7. Predict the IR spectra of citrals, pseudoionones, and ionones. What *changes* do you expect in the IR spectra after each reaction?

8. Predict the ^1H NMR spectra of both citral isomers.

LAB REPORT

See Technical Writing Guidelines on Canvas for general format.

Abstract one paragraph for the entire experiment in the following order

- 1-2 sentences on the **purpose** of the entire experiment
- 2-3 sentences describing the **methods** for each part
  - Include reagents used for each **reaction set up**, not for the workup
- 2-3 **results** sentences
  - Brief description of products (ex. Clear / yellow / brown oil), yields of each reaction (mg and % yield), and "confirmation *via*…" each technique used for analysis without listing the data itself (no numbers aside from yields)
- 1-2 conclusion sentences
  - Briefly comment on **success of each reaction.**
  - Restate which **acid** was used in the cyclization step, identify the **major product** and whether the reaction took place under **kinetic or thermodynamic control** without lengthy discussion of how you came to that conclusion (save that the in-lab questions)

In-Lab Questions

1. Draw the mechanism for the aldol condensation of geranial (citral a) *or* neral (citral b) with acetone and sodium ethoxide. Why is the *trans*-isomer preferred for the *newly formed* alkene?

2. Draw the cyclization mechanism for either the *E*- or *Z*-pseudoionone.

3. Report the yield (mg) and % yield for both steps.

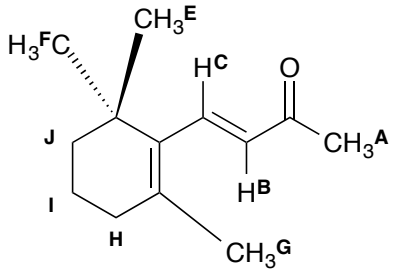
4. Report the corrected GC retention times, integration, and percent composition for each chromatogram. Be sure to identify the peaks. Report your data in table format and briefly comment on the success of reaction and purity of products. Report whether the cyclization was performed with acetic or phosphoric acid. Discuss your GC results with a neighboring group to collect percent composition data for the reactions done with both acids. How can the acid strength (sulfuric or phosphoric) be used to explain why different cyclization products are favored?

5. Interpret the IR spectra of citral, pseudoionones, and the ionones. Report your analysis in table format. What are the distinguishing peaks in each? Attach the IRs to the back of the report or refer to your lab partner's report.

6. Report the observed peaks and absorbances in the UV-vis spectra. Briefly explain why there is a difference in the absorbance UV-vis max (λ_{max}) of α- and β-ionone.

7. Interpret the ^{1}H NMR of α- and β-ionone on the following pages. Assign as many peaks as possible using the tables provided (please reproduce these or similar tables in your word processing document).

8. Comment on the differences and similarities of the ^{1}H NMR of α- and β-ionone. Which peaks would best distinguish α- and β-ionone from each other?



^{1}H NMR of β-ionone **β-ionone**

Signal	Integration (#H's)	Splitting (exp/obs)	Chemical Shift, Expected	Chemical Shift, Observed (Fig 20.3)
A	3			
B	1			
C	1			
D	-- N/A --			
E	3			
F	3			
G	3			
H	2			
I	2			
J	2			

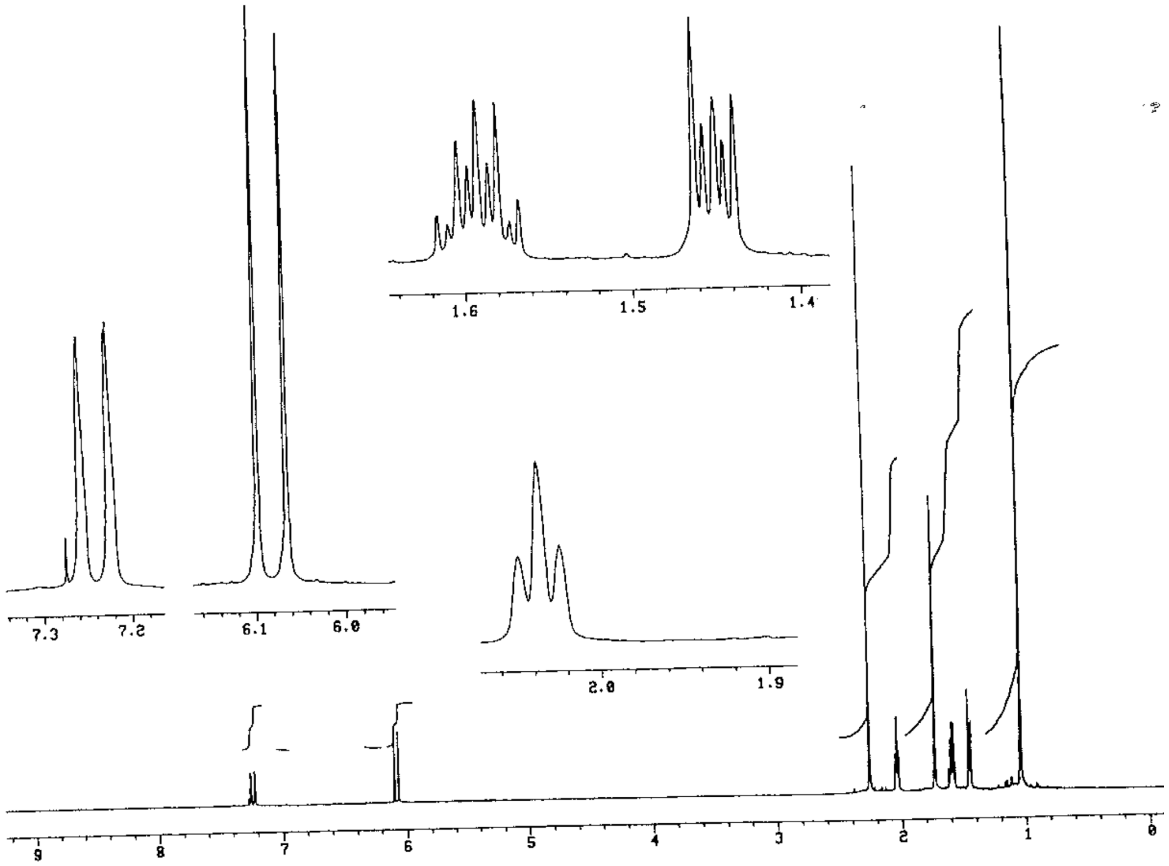
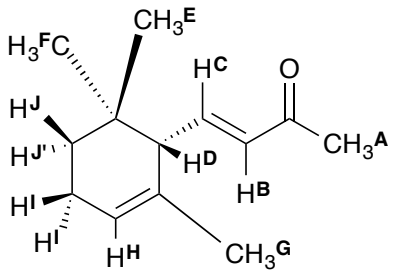


Figure 23.15 500-MHz ^{1}H-NMR spectrum of β-ionone in $CDCl_3$.



α-ionone

^{1}H NMR Analysis of α-ionone

Signal	Integration (#H's)	Splitting (exp/obs)	Chemical Shift, Expected	Chemical Shift, Observed (Fig 20.3)
A	3			
B	1			
C	1			
D	1			
E	3			
F	3			
G	3			
H	1			
I	2			
J	1			
J'	1			

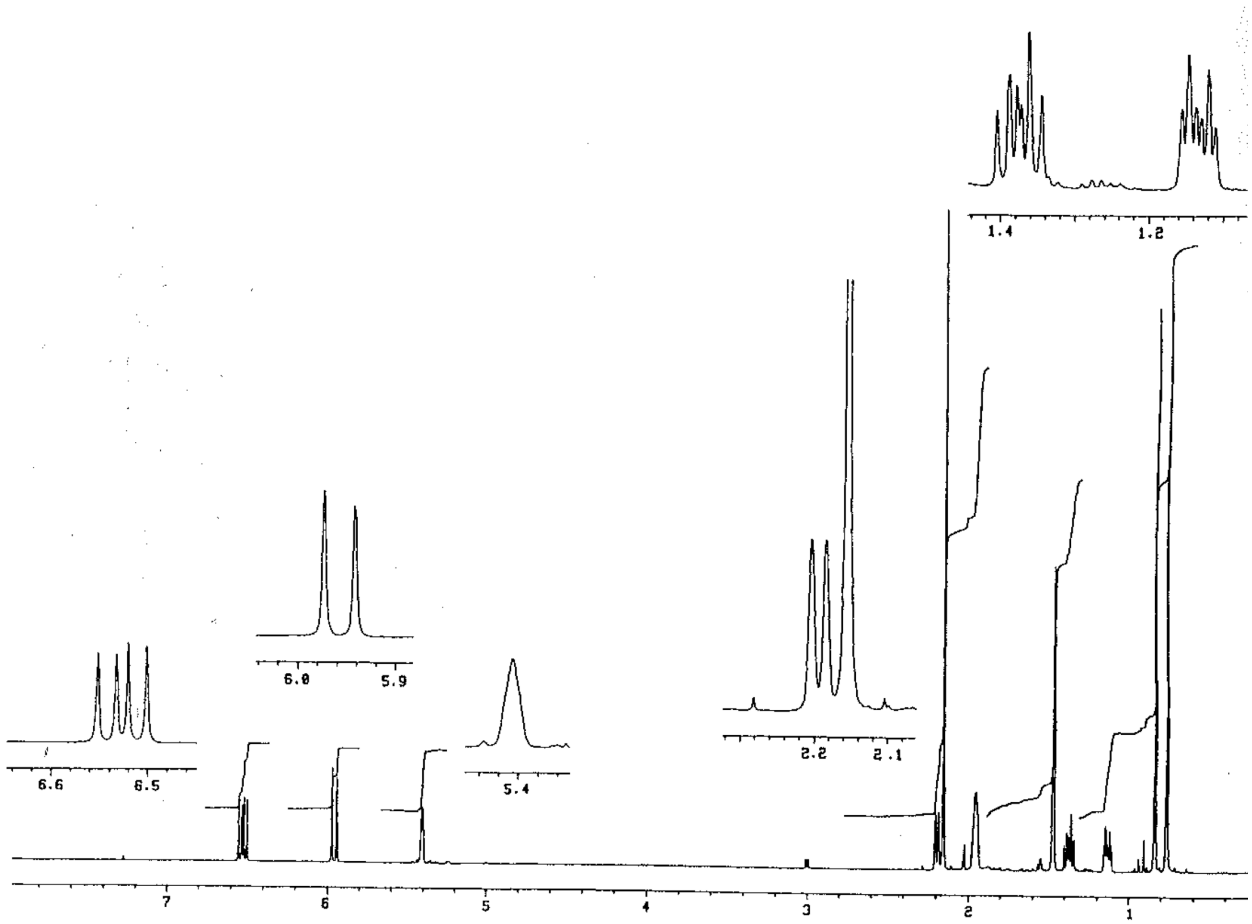


Figure 23.12 500-MHz ^{1}H-NMR spectrum of α-ionone in $CDCl_3$.