CHAPTER 7

SOLVED PROBLEMS

7.1 INTRODUCTION

The perennial student question: Where do we start? The instructor will be sympathetic but not rigidly prescriptive. There are, however, guidelines that do start with the prescriptive statement: *Go for the molecular formula.* Why? Simply because it is the single most useful bit of information available to the chemist and is worth the effort sometimes necessary. It provides an overall impression of the molecule (i.e., the number and kinds of atoms), and it provides the *index of hydrogen deficiency*—in other words, the sum of the number of rings and of double and triple bonds (Section 1.5.3).

Development of the molecular formula starts with recognition of the molecular ion peak (Section 1.5). We assume the usual situation: high-resolution MS instrumentation is not readily available. Let us also assume for now that the peak of highest *m/z* (except for its isotope peaks) is the molecular ion peak and is intense enough so that the isotope peak intensities can be determined accurately and the presence and number of S, Br, and Cl atoms can be ascertained. Look also at the fragmentation pattern of the mass spectrum for recognizable fragments. If the molecular ion peak is an odd number, an odd number of N atoms is present.

Difficulty often starts with uncertainty in the choice of a molecular ion peak. Many laboratories use chemical ionization as a routine supplement to electron impact, and of course, access to a high-resolution instrument is desirable for more difficult problems.

A search of the infrared spectrum for the familiar characteristic groups is now in order. Note in particular C—H stretching, O—H and/or N—H, and the presence (or absence) of unsaturated functional groups.

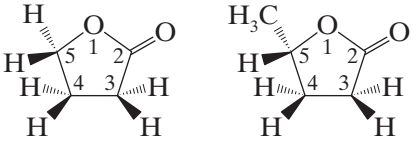
With this information in hand, search the proton NMR spectrum for confirmation and further leads. If the spectrum allows, determine the total proton count and ratios of groups of chemical shift-equivalent protons from the integration. Look for first order coupling patterns and for characteristic chemical shifts. Look at the ^{13}C/DEPT spectra; determine the carbon and proton counts and the numbers of CH_3, CH_2, CH, and C groups. A discrepancy between the proton integration and the number of protons represented in the ^{13}C/DEPT spectra represents protons on heteroatoms.

Overlap of proton absorptions is common, but absolute coincidence of nonequivalent ^{13}C peaks is quite rare with a high-resolution instrument. Now, select the most likely molecular formula(s) from Appendix A of Chapter 1 for comparison and determine the index of hydrogen deficiency for each. In addition to difficulties caused by unresolved or overlapping peaks, discrepancies may appear between the selected molecular formula(s) and the ^{1}H and ^{13}C counts because of the presence of elements of symmetry. But this information also contributes to an understanding of the molecular structure.

Students are urged to develop their own approaches. To provide practice in the use of the newer techniques, we have sometimes presented more information than needed, but other Problems should provide compensatory frustration to simulate the real world. Remember the overall strategy: Play the spectra against one another, focusing on the more obvious features. Develop a hypothesis from one spectrum: look to the other spectra for confirmation or contradictions; modify the hypothesis if necessary. *The effect is synergistic,* the total information being greater than the sum of the individual parts.

With the high resolution now available, many NMR spectra are first order, or nearly so, and can be interpreted by inspection with the leads furnished by the mass and infrared spectra. Nevertheless, a rereading of Sections 3.8 through 3.12 may engender caution.

As an example, consider two similar compounds:



Both rings exist as rapidly flexing ring conformations, but only in compound A do the protons of each CH_2 group interchange to become chemical-shift equivalent (enantiotopes). Only compound A has a plane of symmetry in the plane of the page through which the protons interchange.

From left to right in the spectrum, we predict for compound A: H-5, a two-proton triplet; H-3, a two proton triplet; H-4, a two-proton quintet (assuming nearly equal coupling constants). Given modest resolution, the spectrum is first order.

Compound B has no symmetry element in the planar conformation. C-5 is a chiral center, and the protons of each CH_2 group are diastereotopic pairs. Each proton of the pair has its own chemical shift. The H-4 proton adjacent to the chiral center is distinctly separated, but the H-3 protons are not, at 300 MHz. Each proton of a diastereotopic pair couples geminally with the other and independently (different coupling constants) with the vicinal protons to give complex multiplets.

The possibility of a chiral center should always be kept in mind; *toujours la stereochimie.*

The power of 2-D spectra will become more evident as we work through the problems in Chapters 7 and 8. It is often not necessary to examine all of the spectra in detail before proposing—tentatively—possible structures or fragments. Spectral features predicted for the postulated structures or fragments are compared with the observed spectra, and structural modifications are made to accommodate discrepancies.

These suggestions are illustrated by the following solved problems presented in increasing order of difficulty. The assigned problems of Chapter 8, again in increasing order of difficulty, will provide the essential practice.

Most students enjoy problem solving and rise to the challenge. They also begin to appreciate the elegance of chemical structure as they interpret spectra. Good sleuthing! Be wary of chirality, diastereotopes, virtual coupling, dihedral angles of about 90°, and magnetic nonequivalence.

Finally, what are the requirements for proof of structure? Ultimately, it is congruence of all available spectra with those of a pure, authentic sample obtained under the same conditions and on the same instruments. Obviously, some compromises are acceptable. Congruence with published spectra or spectral data is considered acceptable for publication, but this cannot apply to a new compound, which must then be synthesized.

Computer programs for simulation of proton NMR spectra are available.* If accurate measurements of chemical shifts and coupling constants for all of the protons can be obtained, the simulated spectrum will be congruent with the actual spectrum. In many cases, at least some of the spin systems will be first order. If not, reasonable estimates of shifts and coupling constants may be made, and the iterative computer program will adjust the values until the simulation matches the actual spectrum—assuming, of course, that the identification is valid.

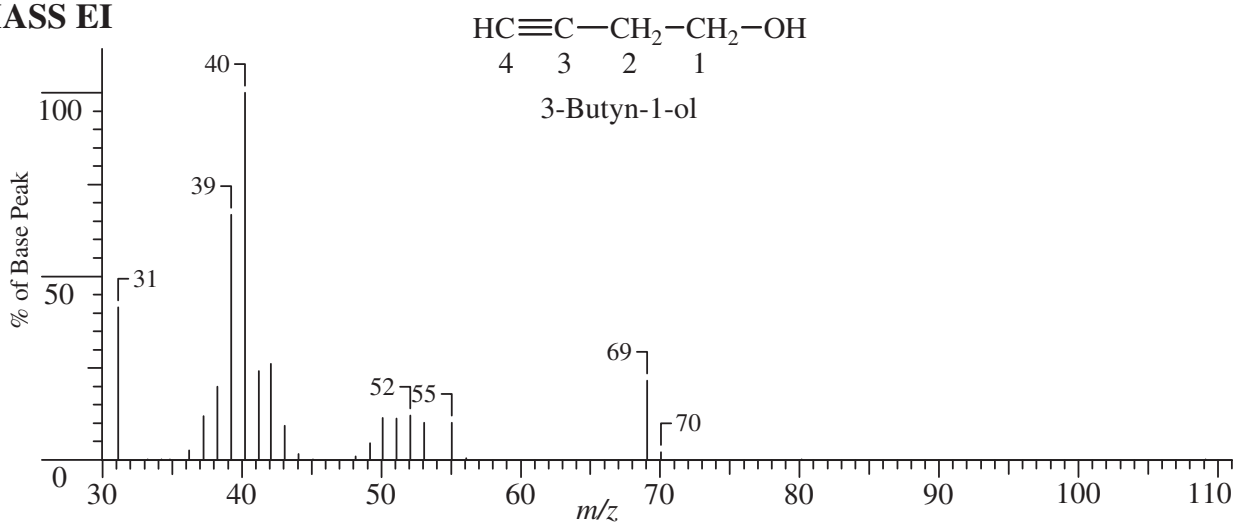
Checklist for logical and pedagogical completeness, not necessarily in order:

1. Show how the molecular formula was derived.
2. Calculate the index of hydrogen deficiency.
3. ***Assign*** diagnostic bands in the IR spectrum.
4. ***Assign all*** protons in the ^{1}H NMR spectrum.
5. ***Assign all*** carbons in the ^{13}C/DEPT NMR spectra.
6. Calculate or estimate $\Delta\nu/J$ where appropriate.
7. Explain multiplicity where appropriate.
8. Assign all correlations in 2-D spectra.
9. Show how the EI mass spectrum supports the structure.
10. Consider possible isomers.

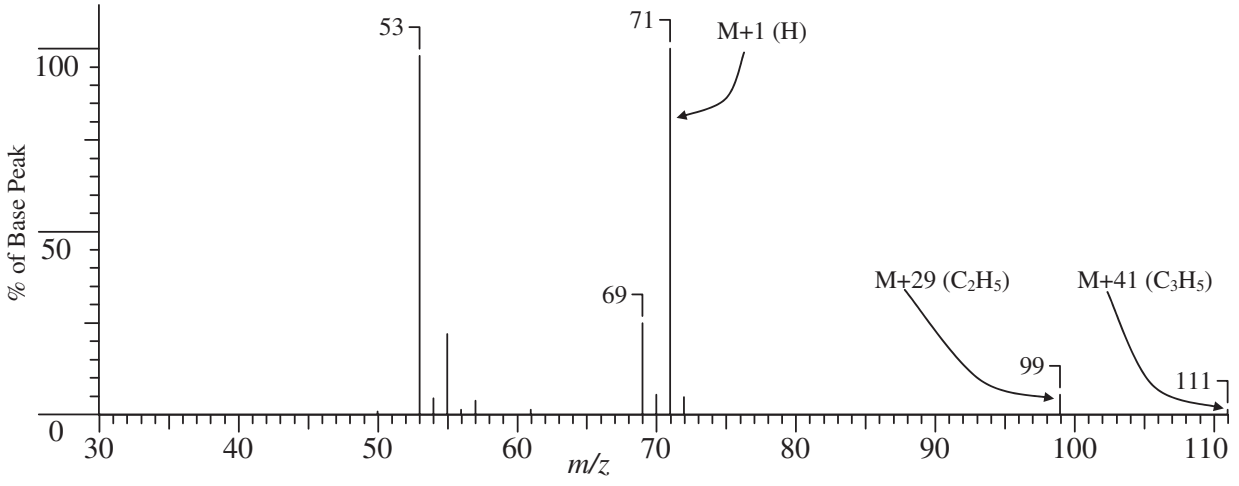
Each problem in this chapter is organized so that the molecular structure and the spectra appears first and are followed by the discussion. The molecular structure is displayed on most of the individual spectra to minimize back-and-forth page turning. The purpose of this arrangement is to encourage students to make their own tentative connections between the molecule and familiar features in the spectra. With this preparation, the subsequent discussions will be more helpful.

* Spectra can be simulated on the computer of a modern NMR spectrometer or on a PC. For example, see the NMR-SIM program, available from Bruker BIOSPIN, Billerica, MA. See also Chapter 3 (Section 3.5.3)

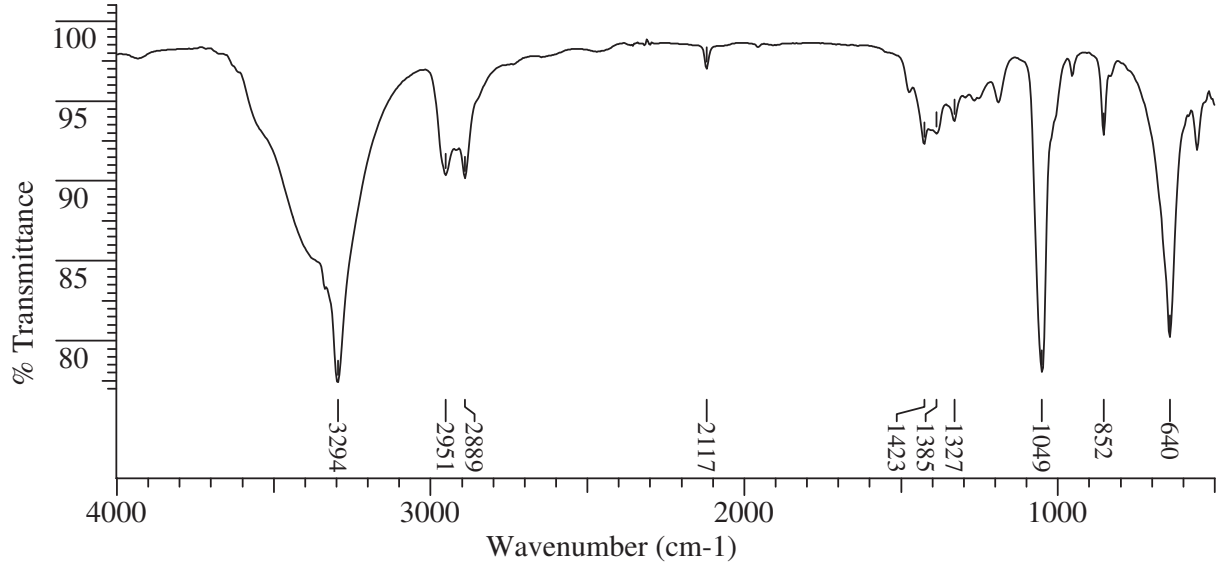
MASS EI



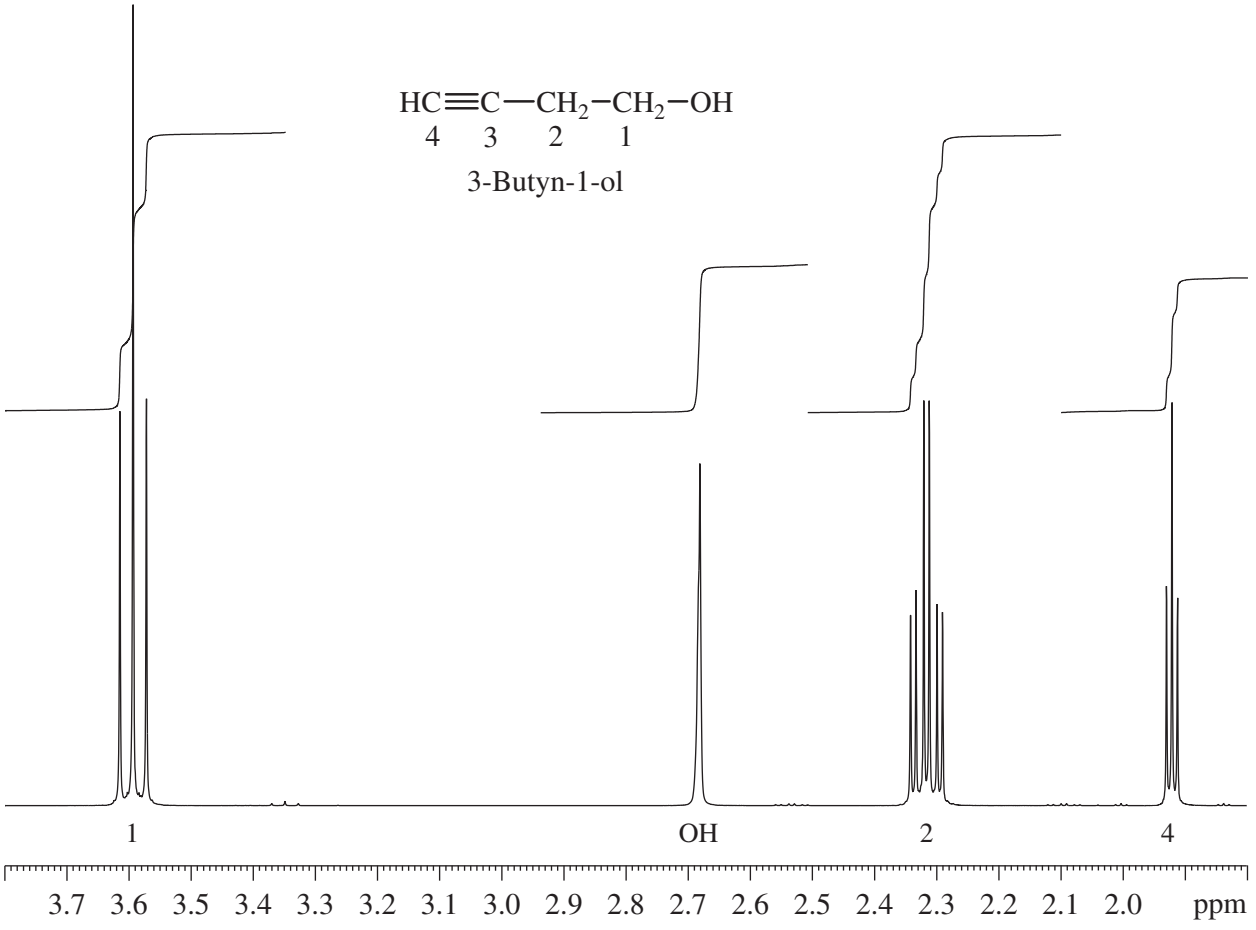
MASS CI reagent gas methane



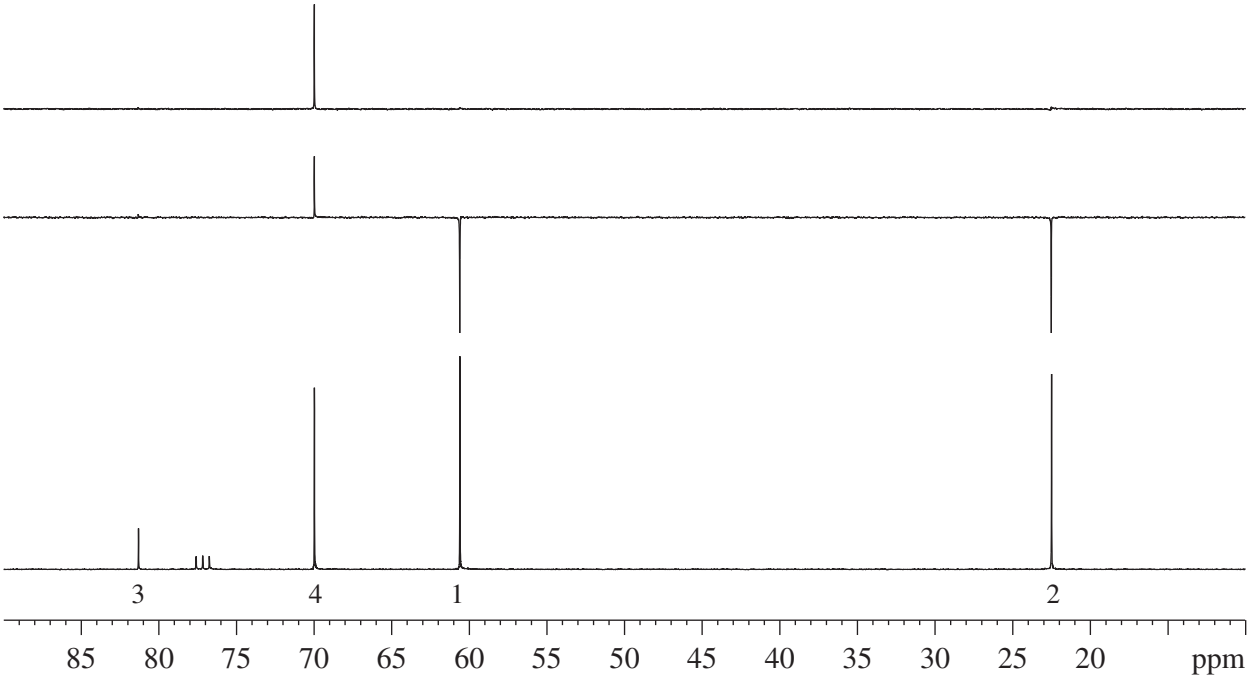
IR



^{1}H NMR 600 MHz



^{13}C/DEPT NMR 150.9 MHz



PROBLEM 7.1 DISCUSSION

Everything points to a small molecule. There appear to be no further peaks in the mass spectrum beyond *m/z* 69, but it is rejected as the molecular ion because the next peak is found at *m/z* 55, a putative loss of 14 mass units. The CI mass spectrum possesses a base peak of *m/z* 71, which represents an M + 1 pseudomolecular ion. The molecular weight of this compound is thus taken as 70 amu. The IR spectrum suggests an alcohol with a broad O—H stretching band at about 3350 cm^{-1} and a strong C—O stretching at 1049 cm^{-1}.

The proton spectrum consists of classical first-order multiplets. From left to right, the multiplicities and integrations are: triplet (2), singlet (1), doublets of triplet (2), triplet (1), which yields six hydrogen atoms. The ^{13}C/DEPT spectra provide four carbon atoms that read from left to right: C, CH, CH_2, CH_2. This discrepancy implies that one of the protons is bonded to a heteroatom. The OH proton at 2.68 ppm in the ^{1}H spectrum accounts for the difference in proton count between the ^{1}H spectrum and the ^{13}C/DEPT spectrum.

The assumption of *m/z* 70 as the molecular ion is now quite valid. The molecular formula is now assumed to be C_4H_6O with an index of hydrogen deficiency of two. The options are: two double bonds, one double bond and a ring, two rings, or a triple bond. We can consider these options seriatim.

Consider two double bonds. Do any of the proton or carbon peaks fall in the usual ranges for alkenes? Perusal of Chapters 3 and 4 eliminates the possibility. This leaves us with rings or a triple bond.

Rings are often difficult to rule out on the basis of chemical shifts alone, but the spin couplings would be difficult to explain. Let us consider a triple bond.

Yes, a triple bound would qualify on the basis of chemical shifts for both protons and carbons. The first question is whether the triple bond is terminal or internal; in other words, is there an alkyne proton?

$$H—C{\equiv}C—R \qquad \text{or} \qquad R—C{\equiv}C—R'$$

The ^{13}C spectrum is unequivocal. It shows two peaks in the range for alkyne carbons. The peak at 70 ppm is about the same height as the two CH_2 peaks, but the peak at about 81.2 ppm is distinctly less intense, indicating that it has no attached proton. Furthermore, the ^{13}C/DEPT subspectra show that the peak at about 70 ppm represents a CH group. We can now write two fragments or substructures:

$$H—C{\equiv}C— \qquad \text{and} \qquad —CH_2—OH$$

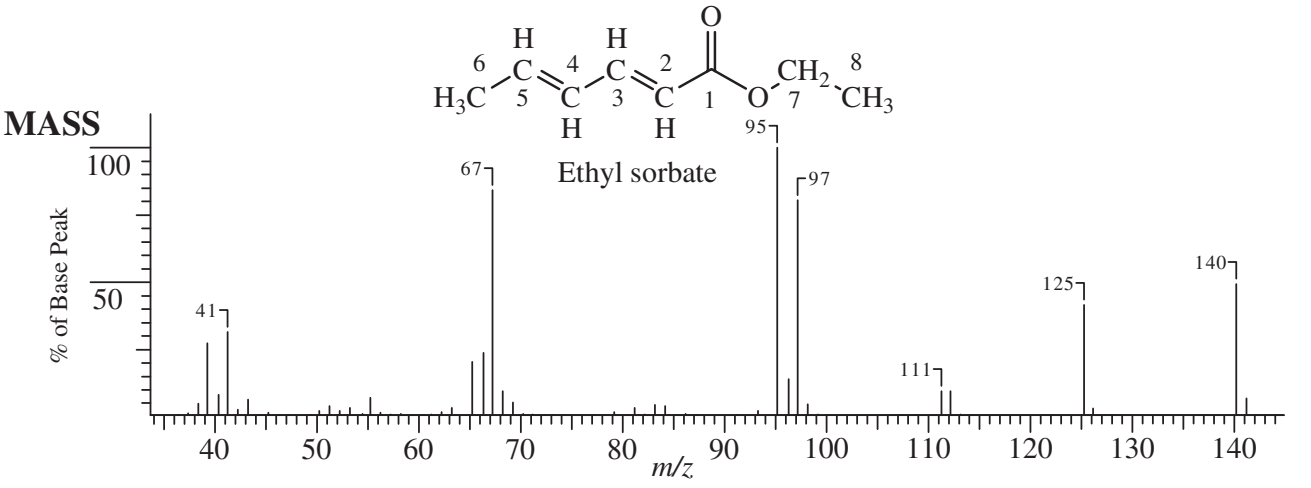
Insertion of the missing CH_2 group gives a complete molecule:

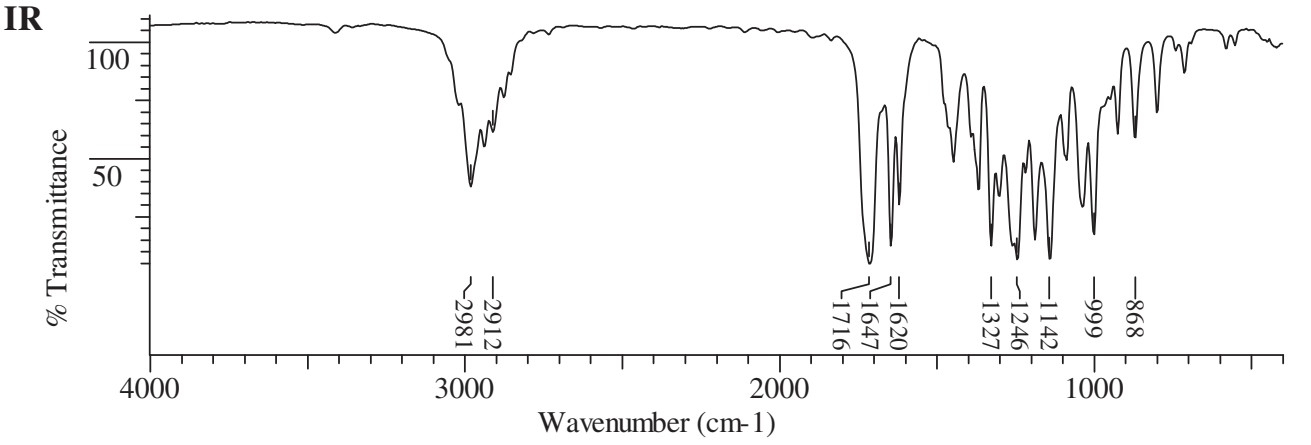
$$H—C{\equiv}C—CH_2—CH_2—OH$$

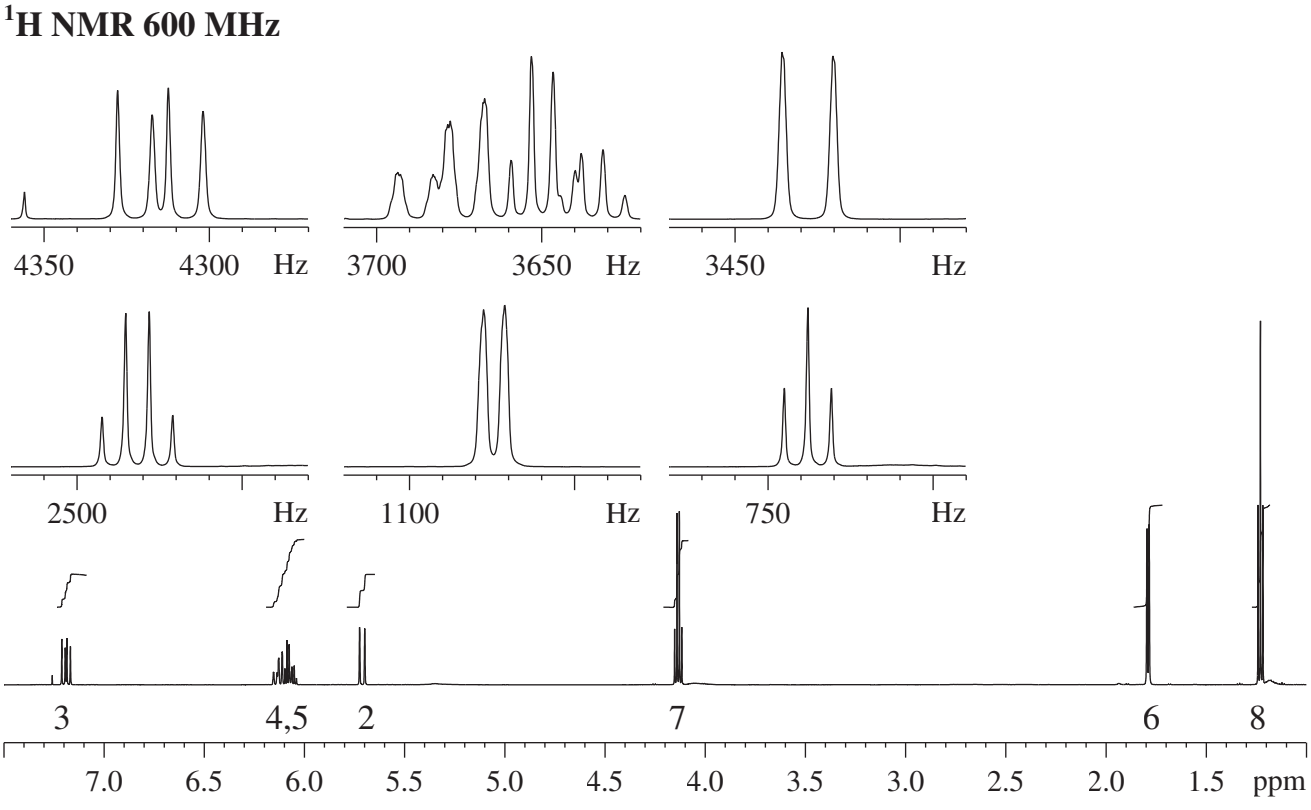
This structure is completely in accord with the ^{1}H and the ^{13}C/DEPT spectra. The ^{1}H spectrum provides a nice demonstration of long-range coupling through the triple bond (from H-4 to H-2) splitting the triplet further into doublets.

Returning with hindsight to the infrared spectrum, we may note the strong H—C≡ stretching band at 3294 cm^{-1} superposed on the O—H band. There is also a strong ≡C—H band at 640 cm^{-1}. Furthermore, there is a weak but distinctive C≡C stretching band at 2117 cm^{-1}.

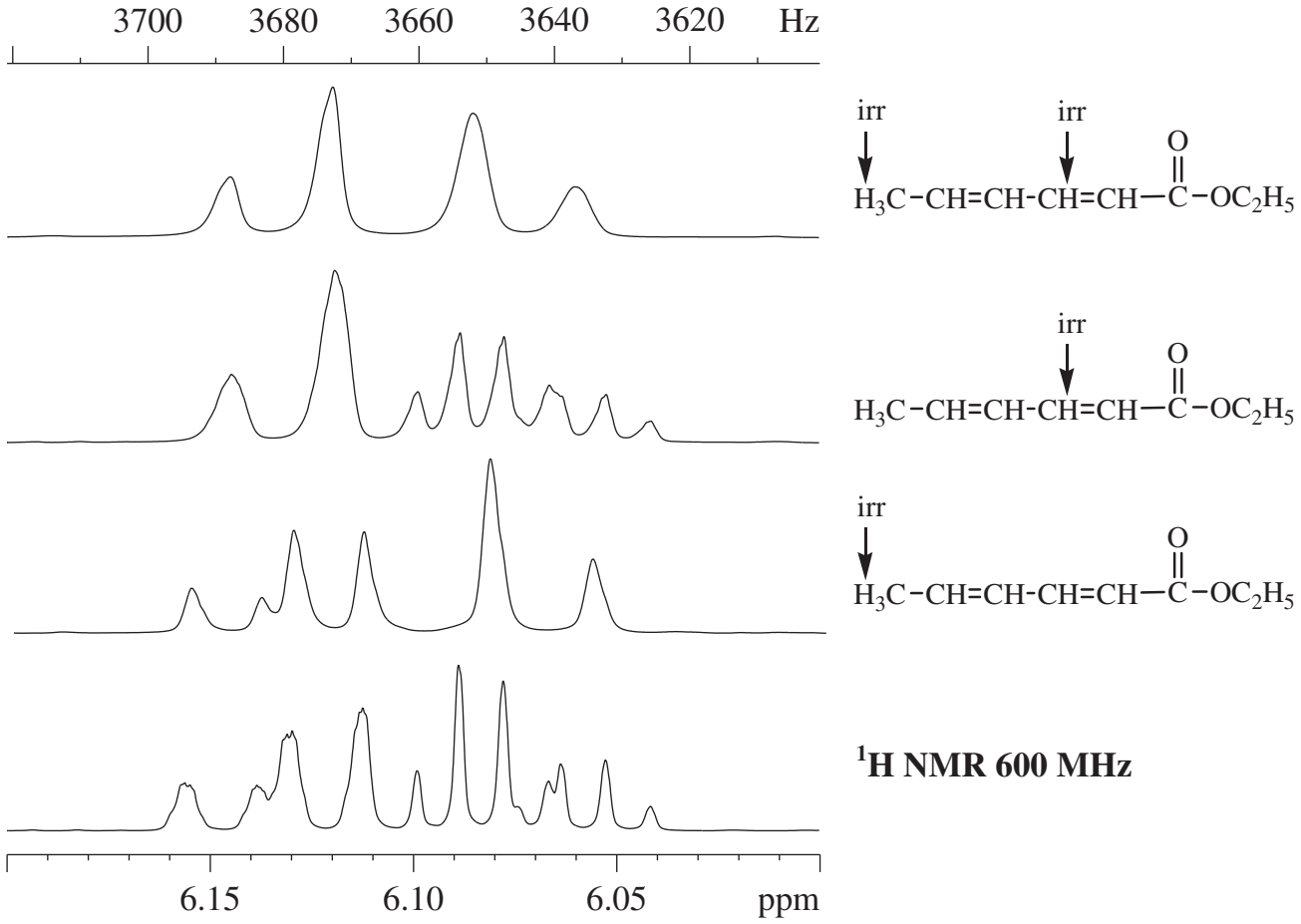
Several of the major peaks in the mass spectrum are difficult to assign since there are two closely spaced functional groups. Although trivial, verification of the assignments of the protons and their multiplicities are left as an exercise for the student. Likewise, verification of the assignments of the resonances in the ^{13}C/DEPT spectra are left for the student.


MASS
Ethyl sorbate
% of Base Peak
100
50
41
67
95
97
111
125
140
40 50 60 70 80 90 100 110 120 130 140
m/z


IR
% Transmittance
100
50
2981
2912
1716
1647
1620
1327
1246
1142
999
868
4000 3000 2000 1000
Wavenumber (cm-1)

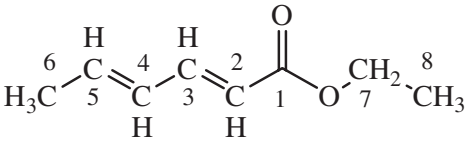

1H NMR 600 MHz
4350 4300 Hz
3700 3650 Hz
3450 Hz
2500 Hz
1100 Hz
750 Hz
3
4,5
2
7
6
8
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm

^{1}H Homodecoupled 600 MHz

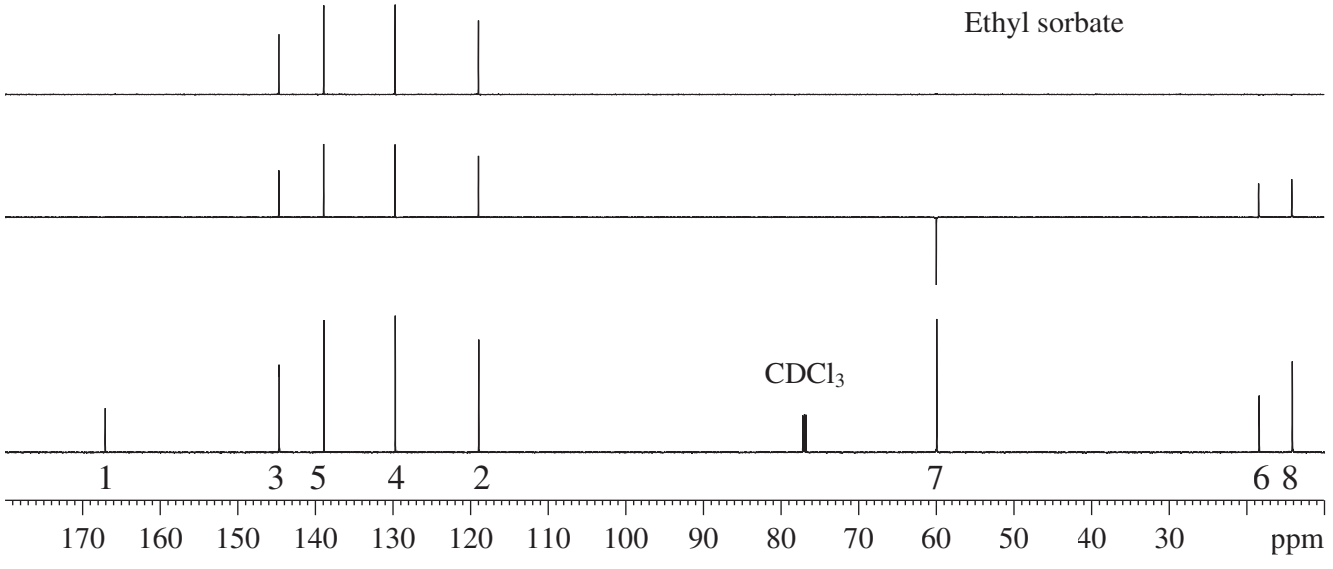


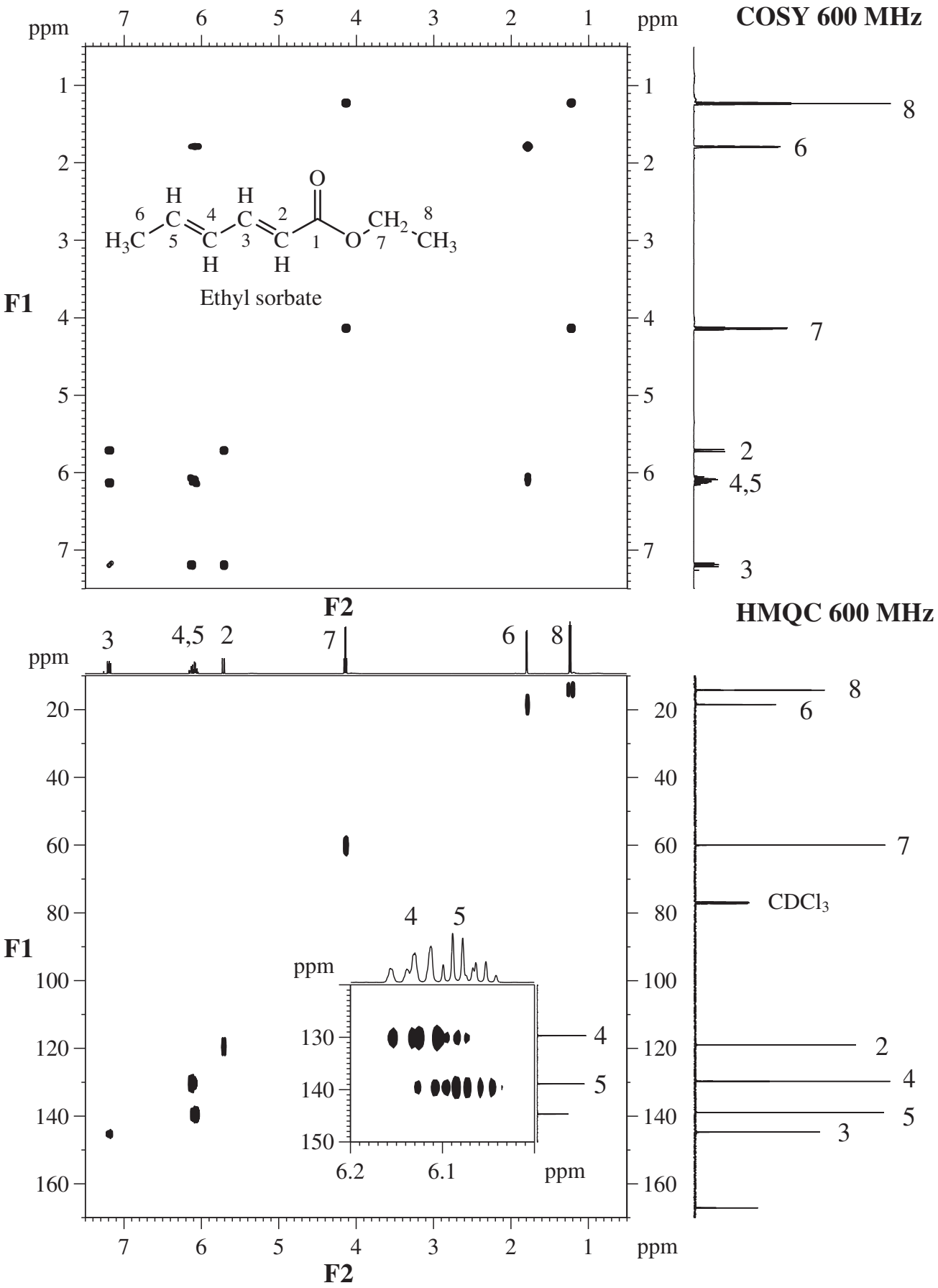
^{1}H NMR 600 MHz

^{13}C/DEPT NMR 150.9 MHz



Ethyl sorbate




COSY 600 MHz
ppm
F1
F2
Ethyl sorbate
8
6
7
2
4,5
3
HMQC 600 MHz
3
4,5
2
7
6
8
4
5
CDCl3
ppm

PROBLEM 7.2 DISCUSSION

The relatively strong peak at *m/z* 140 in the mass spectrum is a reasonable choice for the molecular ion peak, since there are no further peaks, and the fragment at *m/z* 125 represents loss of CH_3. Since 140 is an even number, there are 0, 2, 4 . . . N atoms, and we assume 0 as a starting point. The very small M + 1 and M + 2 peaks preclude S, Cl, and Br.

The strong IR band at 1716 cm^{-1} indicates a carbonyl (C═O) group. The two sharp bands at 1647 cm^{-1} and 1620 cm^{-1} indicate one or more carbon—carbon double bonds (C═C) that may be conjugated (see Section 2.6.4.1).

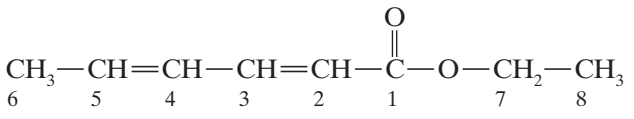
There are six different kinds of protons in the ^1H spectrum in the ratios, from left to right, 1:2:1:2:3:3 with the total of 12 protons. We now count eight peaks in the ^{13}C spectrum (assuming one carbon atom per peak), and from the ^{13}C/DEPT subspectra we read (from left): (C═O) (from IR), CH, CH, CH, CH, CH_2, CH_3, CH_3. With the present information, we write $C_8H_{12}O$ with unit mass 124, which is 16 units less than then a molecular ion peak at *m/z* 140. Is there another oxygen atom in the molecular ion?

Indeed so. The chemical shift of the CH_2 group at 60 ppm suggests a —(C═O)OCH_2— sequence (see Table 4.20). Also, the chemical shift of the carbonyl carbon in the ^{13}C (168 ppm) suggests a carboxylic acid derivative such as an ester. The partial molecular formula can now be revised to $C_8H_{12}O_2$ with a hydrogen deficiency of three.

The proton NMR spectrum immediately points out that the CH_3 triplet at the extreme right is directly attached to the deshielded CH_2 group (quartet). The COSY spectrum confirms this correlation. The sequence, above, is now one end of the molecule: —(C═O)OCH_2CH_3.

In the ^{13}C/DEPT spectra, there are four CH alkene peaks between ~119 ppm and ~145 ppm. There is also the remaining CH_3 group at ~18.5 ppm, which appears in the proton spectrum at ~1.8 ppm as a doublet—obviously attached to one of the four CH groups.

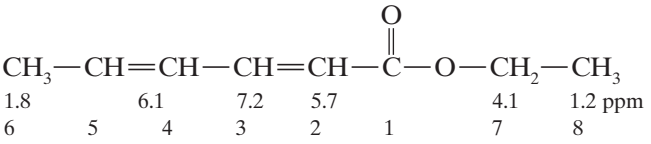
It may seem presumptuous to formulate a molecular structure at this early stage, but we do have one end of the structure, four CH groups with an attached CH_3 group, no possibility for branching, and do not forget the two remaining sites of unsaturation. With some trepidation, we offer the following structure:



The synergism between the ^{13}C/DEPT spectra and the proton spectrum should be explored. There are two aspects to a proton spectrum: The first-order multiplets can usually be resolved, whereas the higher-order multiplets are frustrating. In the present proton spectrum, there are five first-order multiplets and two overlapping multiplets that are not first-order.

The ethyl protons are represented by the triplet at ~1.2 ppm coupled to the deshielded quartet at ~4.1 ppm. The other CH_3 group is represented by the doublet at ~1.8 ppm, coupled to one of four alkene CH protons. Rather than attempting to interpret the higher-order multiplets, we turn to the 2-D spectra.

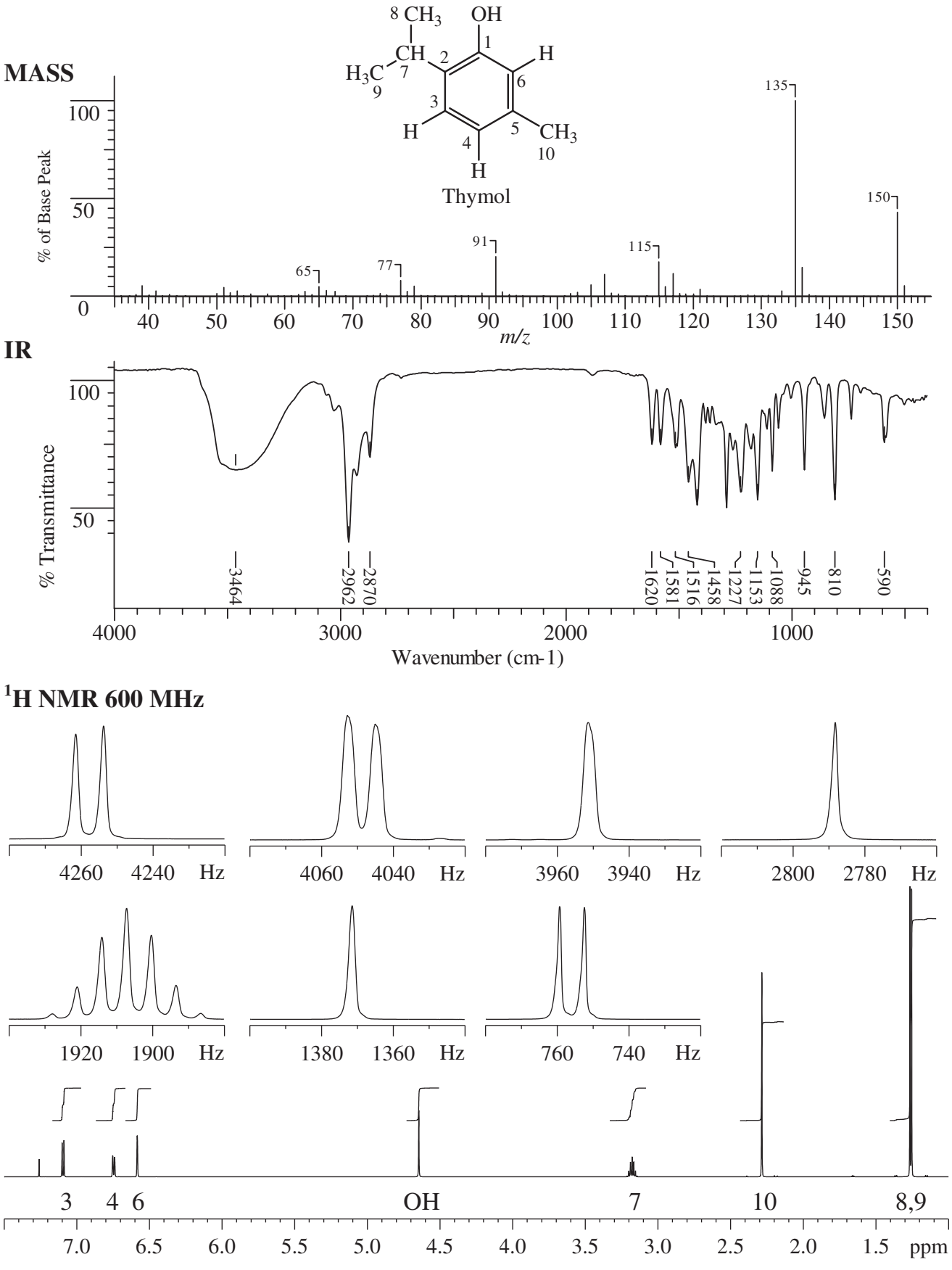
In the COSY spectrum, one of the two overlapping CH groups, which are centered at ~6.1 ppm (labeled H-5 and H-4), couples to the CH_3 doublet at ~1.8 ppm; this coupling confirms the earlier assumption that the CH_3 group is terminal. The proton labeled H-5 also couples with the other overlapping CH group (labeled H-4), which in turn couples with the neighboring CH group at ~7.2 ppm (labeled H-3). The slightly broadened doublet at ~5.7 ppm (labeled H-2) is a result of coupling to H-3 and long-range coupling. We can summarize as follows:



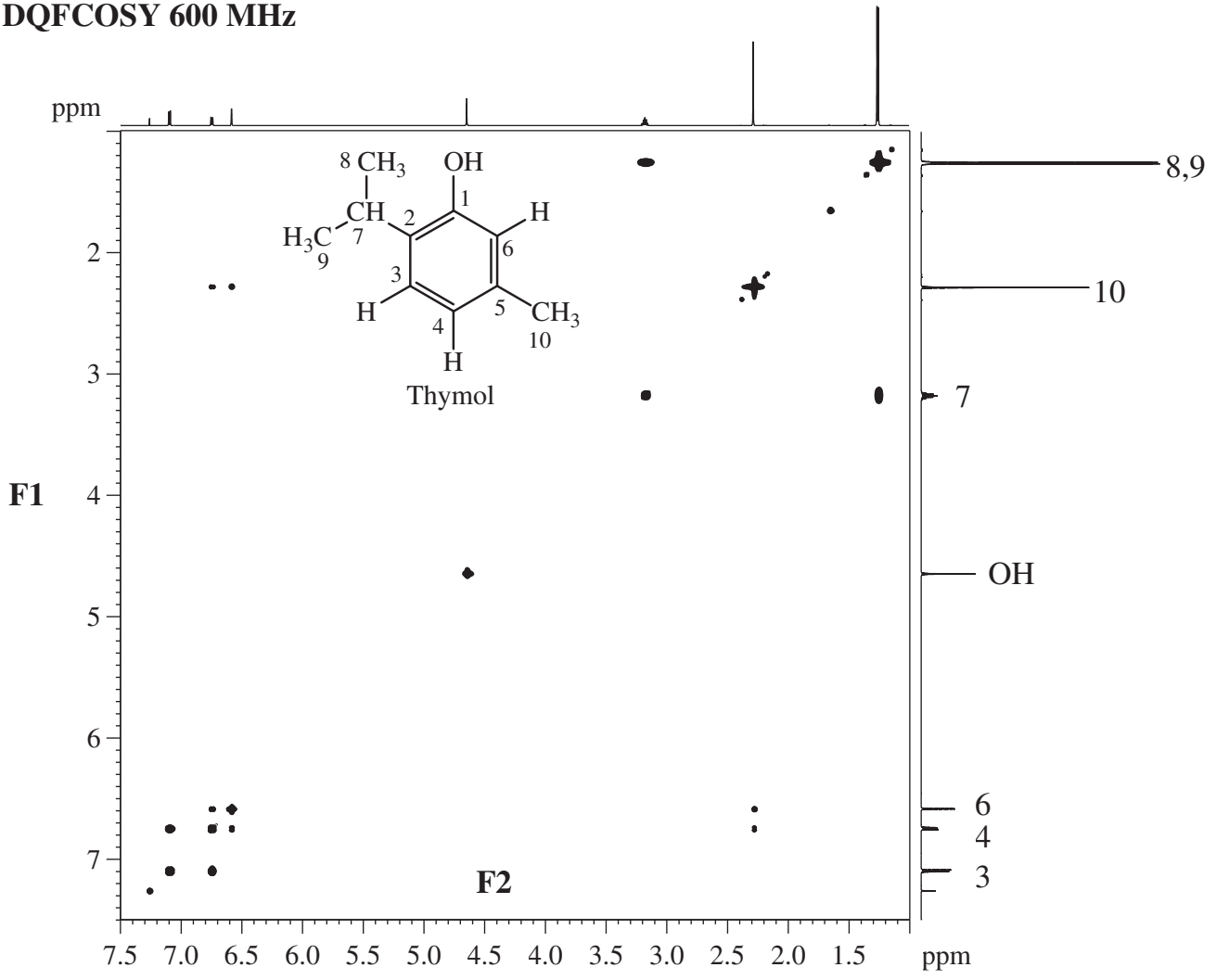
With the complete proton assignments and the direct correlations between carbons and attached protons from the HMQC, we are able to assign all of the carbon resonances, except for the quaternary carbon, which is a trivial assignment in this case. An interesting example is found in the inset of the HMQC spectrum, which shows the correlations of the two overlapped protons, H-4 and H-5. Even though they are overlapped in the proton spectrum, they are well resolved in the HMQC spectrum because the carbon resonances are not overlapped.

One important question still remains: Are the double bonds *E* (*trans*) or *Z* (*cis*)? This question can be answered if the olefinic proton *J* values can be determined. One obvious starting point is the H-2 doublet, which is the result of coupling to H-3. The *J* value is about 16 Hz; this coupling constant falls within the range given for *E*-double bonds given in Appendix F, Chapter 3.

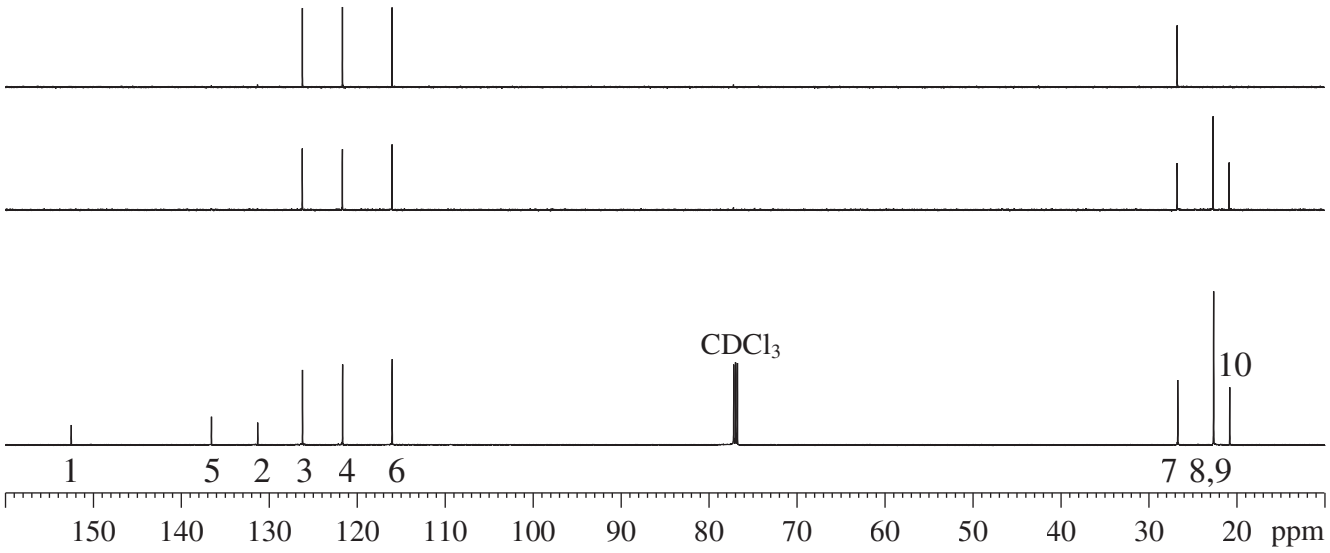
The complex, overlapping multiplets of H-4 and H-5 are not inviting. However, H-3 shows a pair of doublets as a result of the 16 Hz (*trans*) coupling to H-2 and a 10 Hz single bond coupling to H-4. Unfortunately, the coupling constant for the 4,5-double bond is not readily accessible. But spin decoupling (homodecoupling) is worth investigating (see Section 3.15). Irradiation of H-6 simplifies the overlapping H-5, H-4 complex considerably; in fact, there is a 16 Hz doublet (somewhat distorted) at the lower-frequency edge. Irradiation of H-3, individually, simplifies the complex multiplet and shows a 16 Hz doublet at the high-frequency edge. Simultaneous irradiation of H-6 and H-3 results in a pair of 16 Hz doublets. The doublet intensities are not ideal because of the small $\Delta\nu/J$ ratio. There is now no doubt that both double bonds are *E*.


MASS
8 CH3
OH
1
CH
2
H
H3C
7
6
9
3
H
5
CH3
4
10
H
Thymol
% of Base Peak
100
50
0
40 50 60 70 80 90 100 110 120 130 140 150
m/z
65
77
91
115
135
150
IR
% Transmittance
100
50
3464
2962
2870
1620
1581
1516
1458
1227
1153
1088
945
810
590
4000 3000 2000 1000
Wavenumber (cm-1)
1H NMR 600 MHz
4260 4240 Hz
4060 4040 Hz
3960 3940 Hz
2800 2780 Hz
1920 1900 Hz
1380 1360 Hz
760 740 Hz
3 4 6 OH 7 10 8,9
7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm

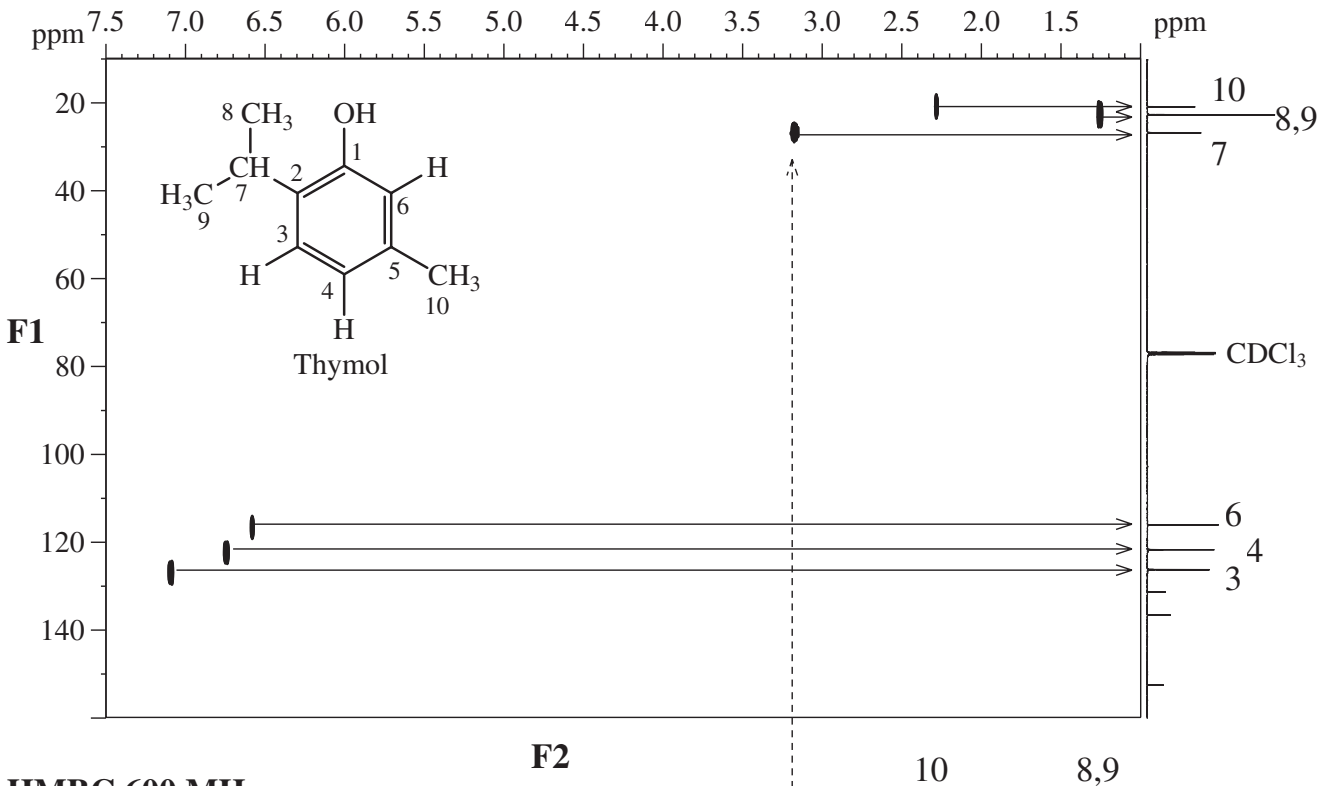
DQFCOSY 600 MHz



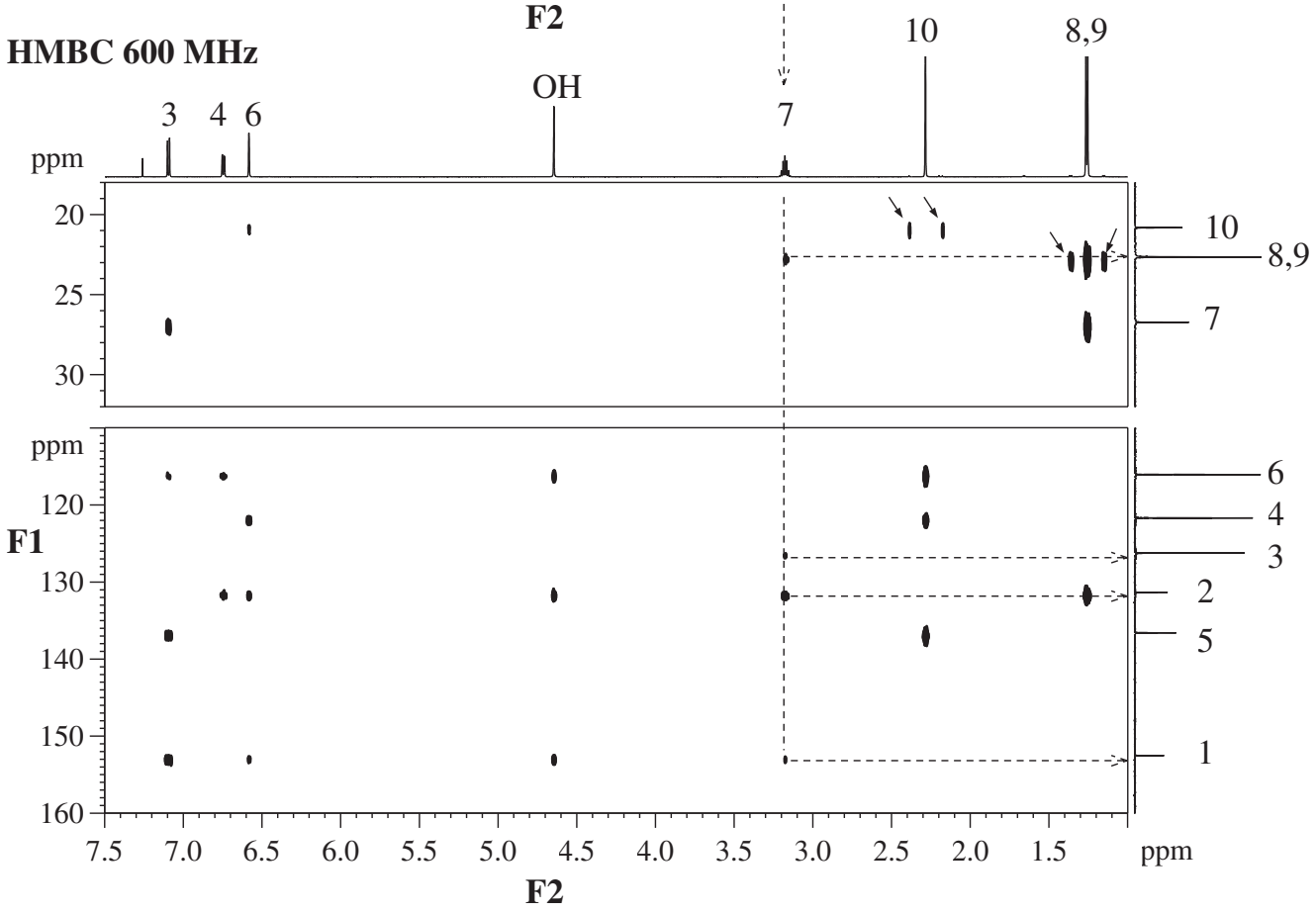
^{13}C/DEPT NMR 150.9 MHz



HMQC 600 MHz



HMBC 600 MHz



PROBLEM 7.3 DISCUSSION

The molecular ion is certainly the medium-intensity peak in the mass spectrum at *m/z* 150; there is a rational loss of a CH_3 group to give the base peak at *m/z* 135. The isotope peaks for the molecular ion do not permit the presence of S, Cl, or Br. Let us assume, tentatively, that the even-numbered molecular ion peak indicates the absence of N. If so, with the help of Appendix A (Chapter 1), the molecular formula can be limited to these possibilities: $C_6H_{14}O_4$, $C_8H_6O_3$, $C_9H_{10}O_2$, or $C_{10}H_{14}O$. The IR spectrum is notable for the intense OH peak at 3464 cm^{-1}. The immediate question is the presence or absence of aromaticity. If an aromatic ring is present, is it attached directly to the OH group to give a phenol? The ^1H and ^{13}C spectra provide answers with peaks in the aromatic regions. The strong IR peaks between 1600–600 wavenumbers suggest aromaticity and ions at 77 and 91 *m/z* serve to confirm our conclusion.

There are seven different kinds of protons in the ^1H spectrum in the ratios, from left to right, 1:1:1:1:1:3:6. Hence a total of 14 protons. The six-proton doublet at δ 1.25 probably represents two equivalent CH_3 groups of an isopropyl moiety; the one-proton septet at δ 3.2 is the corresponding methine group of the isopropyl group.

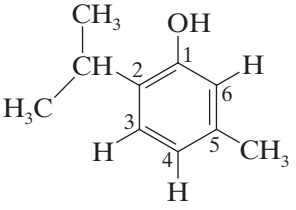
The ^{13}C spectrum shows nine peaks, but one of them (at 23 ppm) is suspiciously intense and since it correlates with the six-proton doublet in the HMQC, we conclude that there are two superposed CH_3 groups, which makes a total of 10 carbon atoms. The ^{13}C/DEPT spectra specify, from left to right, C, C, C, CH, CH, CH, CH, $CH_3(\times 2)$, CH_3, to which we add the OH group. Under unit mass 150, the most reasonable molecular formula is $C_{10}H_{14}O$, which has an index of hydrogen deficiency of four. This degree of unsaturation fully accounts for a benzene ring: i.e., three double bonds and one ring. Furthermore, the ^{13}C NMR spectrum consists of an aromatic region and an aliphatic region.

In the aromatic region, the three weak peaks represent three quaternary carbon atoms, and the three more intense peaks represent the carbon atoms with attached hydrogen atoms. The most deshielded, weak peak at 153 ppm represents the carbon atom to which the OH group is attached (see Table 4.12).

The substituents in the aliphatic region must be a methyl group and an isopropyl group. For confirmation, the aliphatic region in the proton spectrum shows (from left to right) a one-proton septet (i.e., CH), a three-proton singlet (i.e., CH_3), and a six-proton doublet. It is a doublet because it consists of two identical CH_3 groups coupled to the CH group—hence an isopropyl substituent.

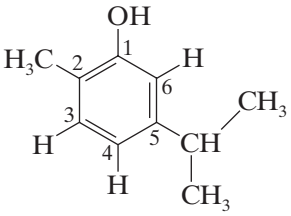
At this point, we distribute the two alkyl substituents with reference to the OH group and do so somewhat indirectly by considering the chemical shifts and coupling constants of the three ring protons. We can assume that the proton peak at δ 6.6 is *ortho* to the OH group (see Chart D.1, Chapter 3). Since this peak is a broadened singlet, there is no adjacent hydrogen atom, but there is a hydrogen atom *meta* to it with a coupling constant too small to delineate. Furthermore, since the spectrum shows only one proton *ortho* to the OH substituent, the other *ortho* position must be attached to either the methyl or the isopropyl group.

The sharp doublet at δ 7.1 with a *J* value of about 8 Hz represents an aromatic hydrogen atom with one *ortho* coupling. Since the peaks are sharp, there is no *meta* coupling. Its chemical shift places it *meta* to the OH group, the alkyl groups having little effect on the chemical shift (see Chart D.1, Chapter 3). The broad doublet at δ 6.75 *para* to the OH group, the coupling being *ortho* and weakly *meta*. The choice is between *I* and *II*.



Thymol

I

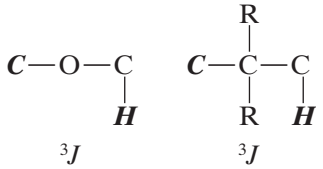


II

The COSY spectrum confirms the previous findings and shows that the protons of the methyl substituent are long-range coupled (^4J) to H-4 and H-6. Interestingly, the isopropyl CH proton does not show long-range coupling to H-3 possibly due to the high multiplicity of the CH absorption, which would produce a very diffuse (not visible) cross peak. As expected, the aromatic protons show meta coupling (^4J) between H-6 and H-4, and ortho (^3J) coupling between H-4 and H-3. Structure I (thymol) is now heavily favored. Note that the definitive long-range coupling between the CH_3 substituent and H-4 and H-6 was not resolved in the ^1H spectrum.

The HMQC shows $^1J_{CH}$ coupling. Table 4.12 in Chapter 4 allows us to arrange the aromatic unsubstituted carbon atoms as C-6, C-4, C-3 from top to bottom. The HMQC spectrum confirms the same sequence for H-6, H-4, H-3. The aromatic, unsubstituted carbon atoms can now be correlated with the firmly assigned aliphatic protons. The substituted aromatic carbon atoms cannot yet be assigned.

The HMBC spectrum permits correlation between isolated proton spin systems—i.e., bridging such "insulating" atoms as O, S, N, and quaternary carbon atoms.

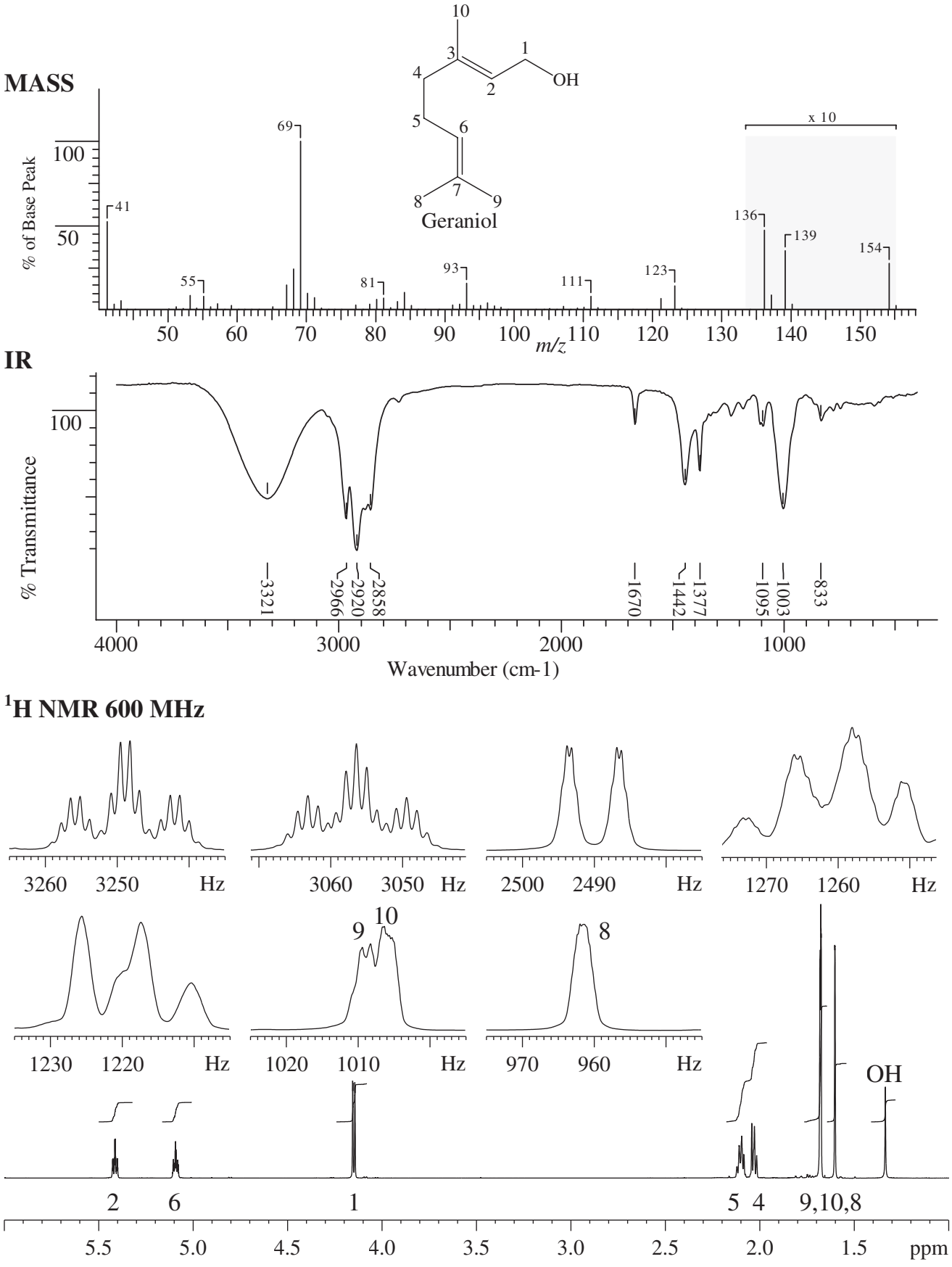


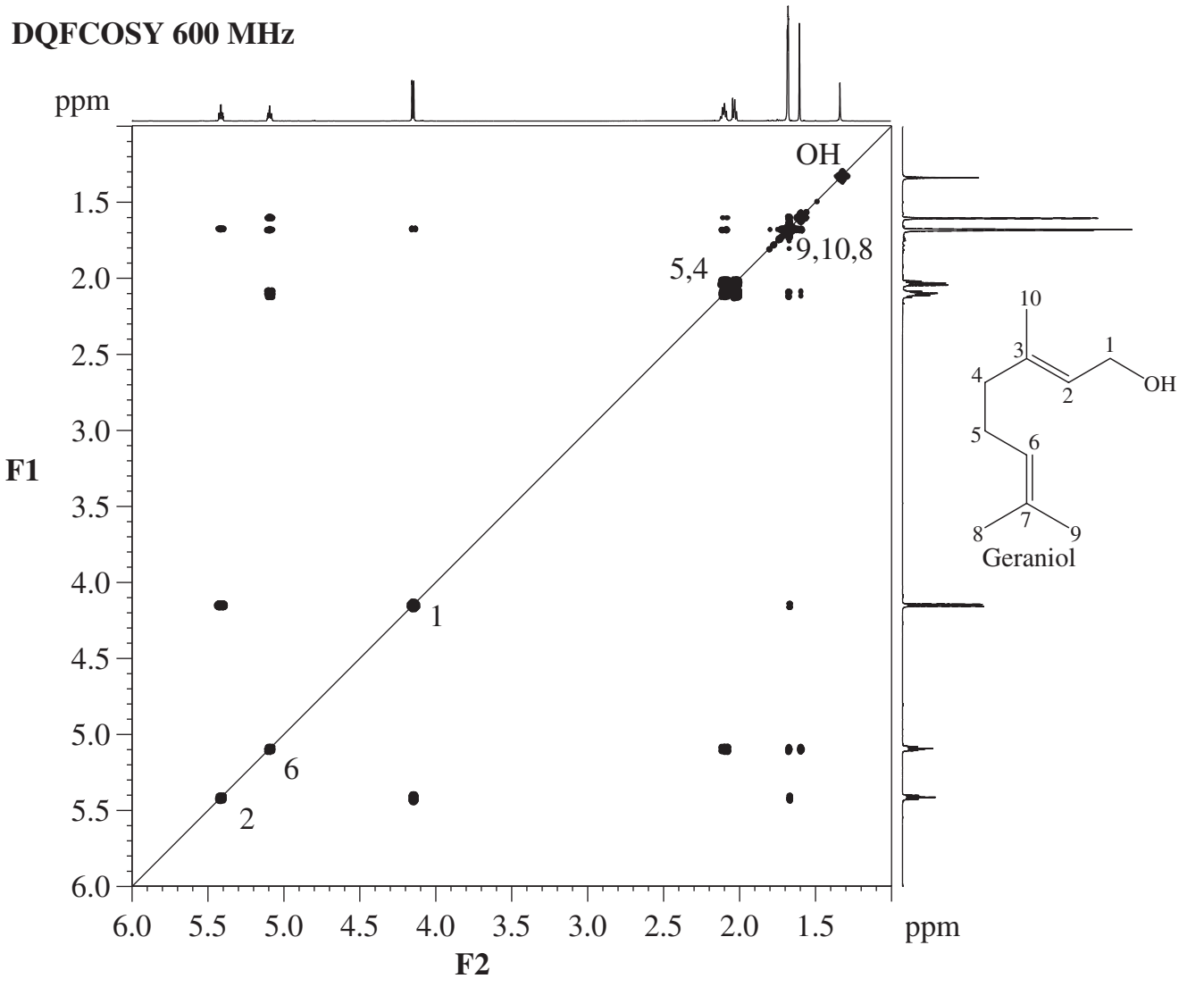
Even in a molecule of modest size, the number of $^2J_{CH}$ and $^3J_{CH}$ couplings can be daunting. Where to start?

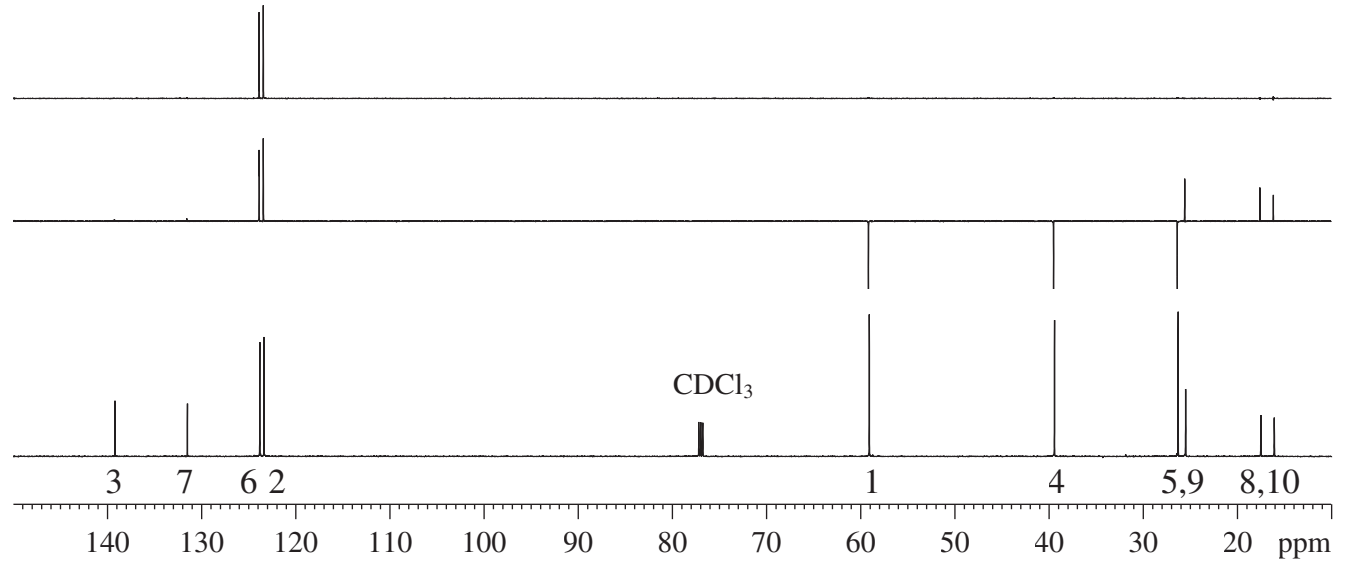
Well, simply pose an important question: How do we fully confirm the positions of alkyl substituents? The COSY spectrum did detect the long-range coupling for the methyl substituent but not for the isopropyl substituent. Confirmation can be found by looking down from the CH isopropyl septet in the HMBC spectrum and observe four cross peaks that correlate this CH proton with C-8,9 (^2J), C-2 (^2J), C-3 (^3J) and C-1 (^3J) in the thymol structure. Certainly convincing. As overkill, note that in the HMBC spectrum, the protons of the methyl substituent correlate with C-6 (^3J), C-4 (^3J) and C-5 (^2J). Further, note that the six methyl protons of the isopropyl group correlate with C-7 (^2J) and with C-2 (^3J). Interesting to note that the correlations of H-8 to C-9 (^3J) and H-9 to C-8 (^3J) exist.

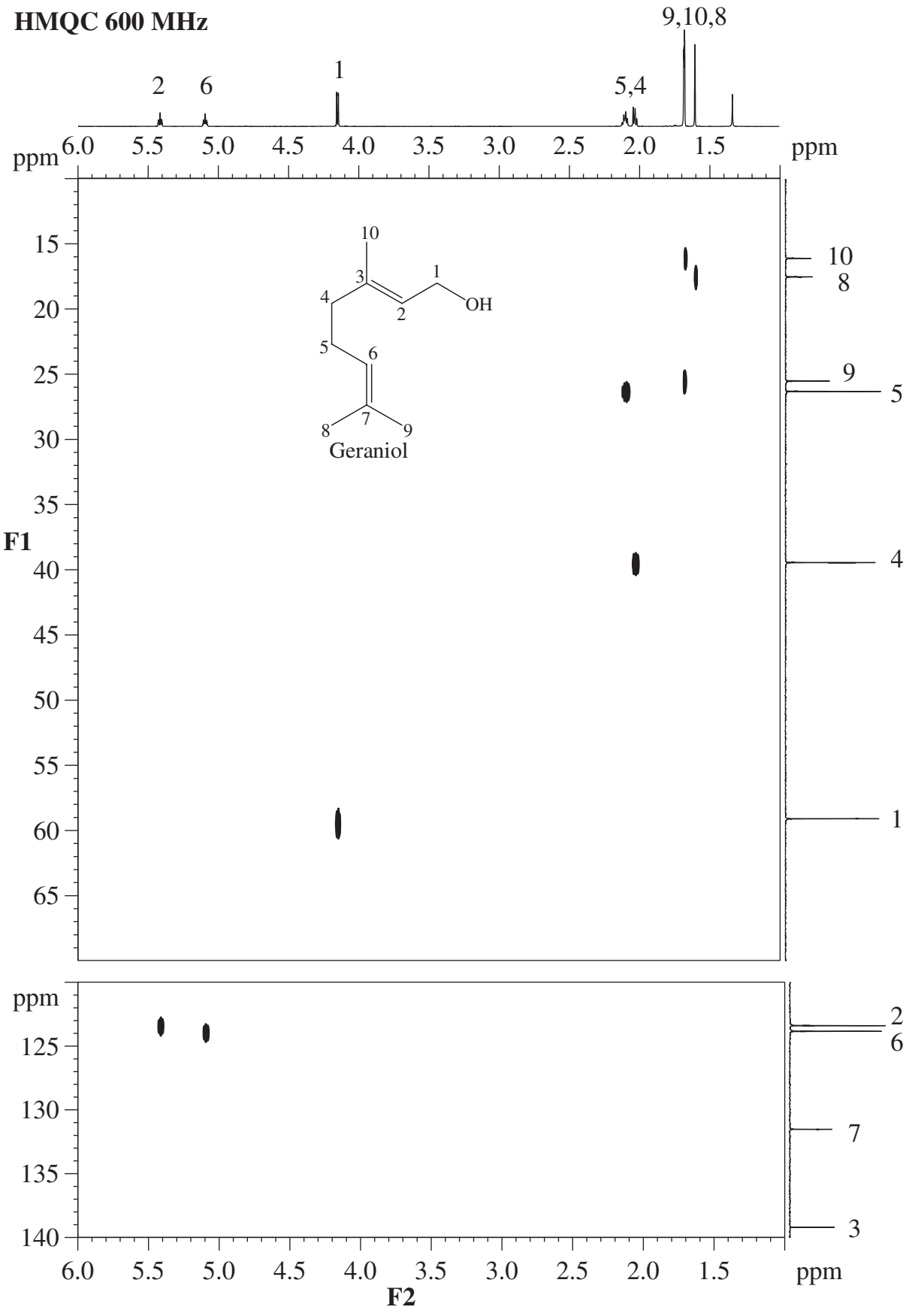
The utility of HMBC in correlating quaternary carbon atoms with assigned protons can be shown by working out the correlations of C-1, C-5, and C-2. The assignment earlier of C-1 on the basis of its chemical shift is sound, but the assignment of C-5 and C-2 on the basis of chemical shift alone should be affirmed by correlations. This exercise is left to the student.

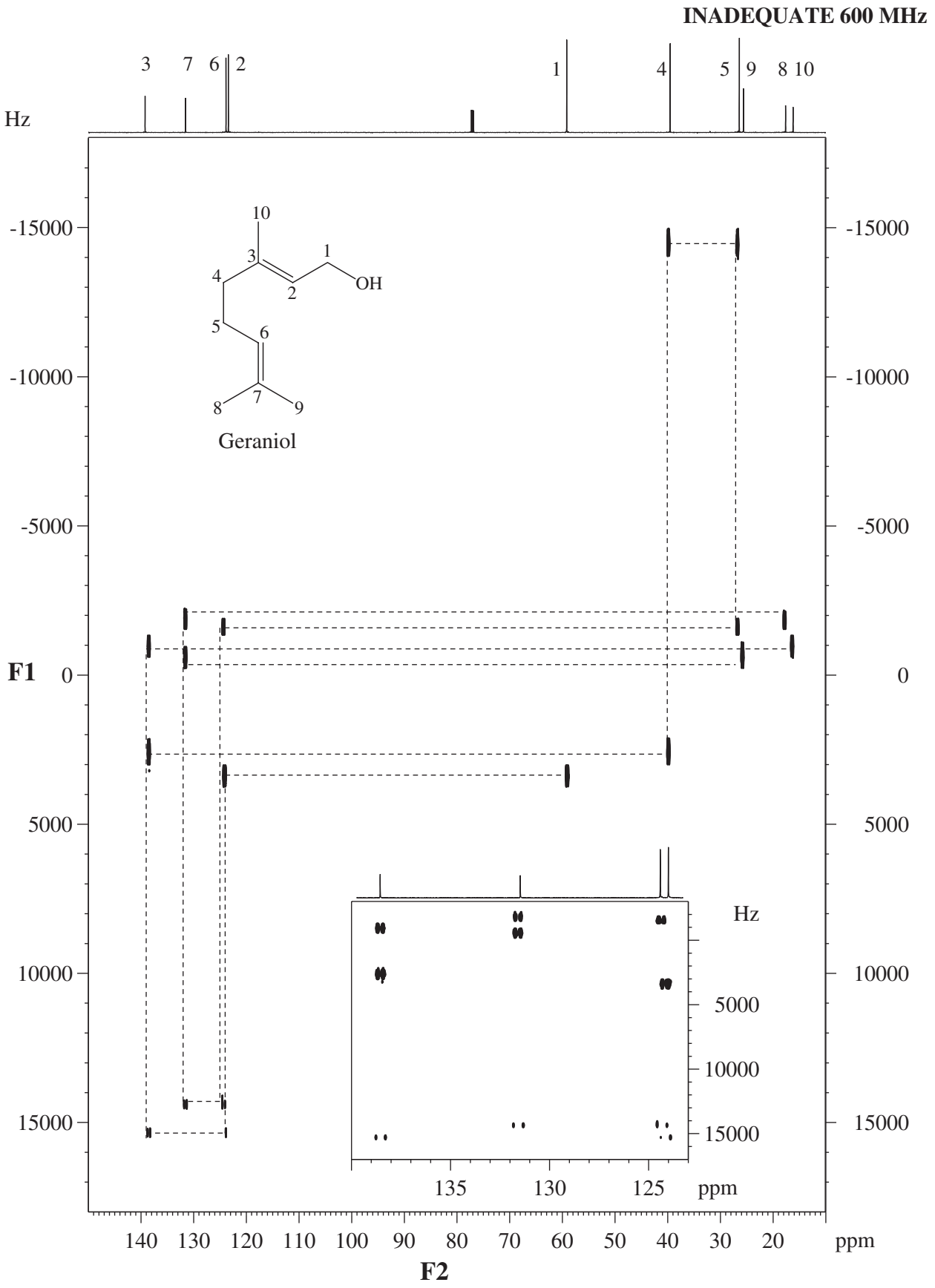
Bridging across quaternary carbon atoms has been demonstrated in the course of the above correlations. Two final points: (1) There are four contours, designated by arrows, that represent $^1J_{CH}$ couplings (large) that have not been completely suppressed. These CH doublets are obvious since they straddle the proton peaks. They can be ignored. (2) The correlations of the OH proton with C-6, C-2, and C-1 should be noted. Correlations to OH protons can be very useful, but are rarely seen in an HMBC because they are typically too broad to detect.

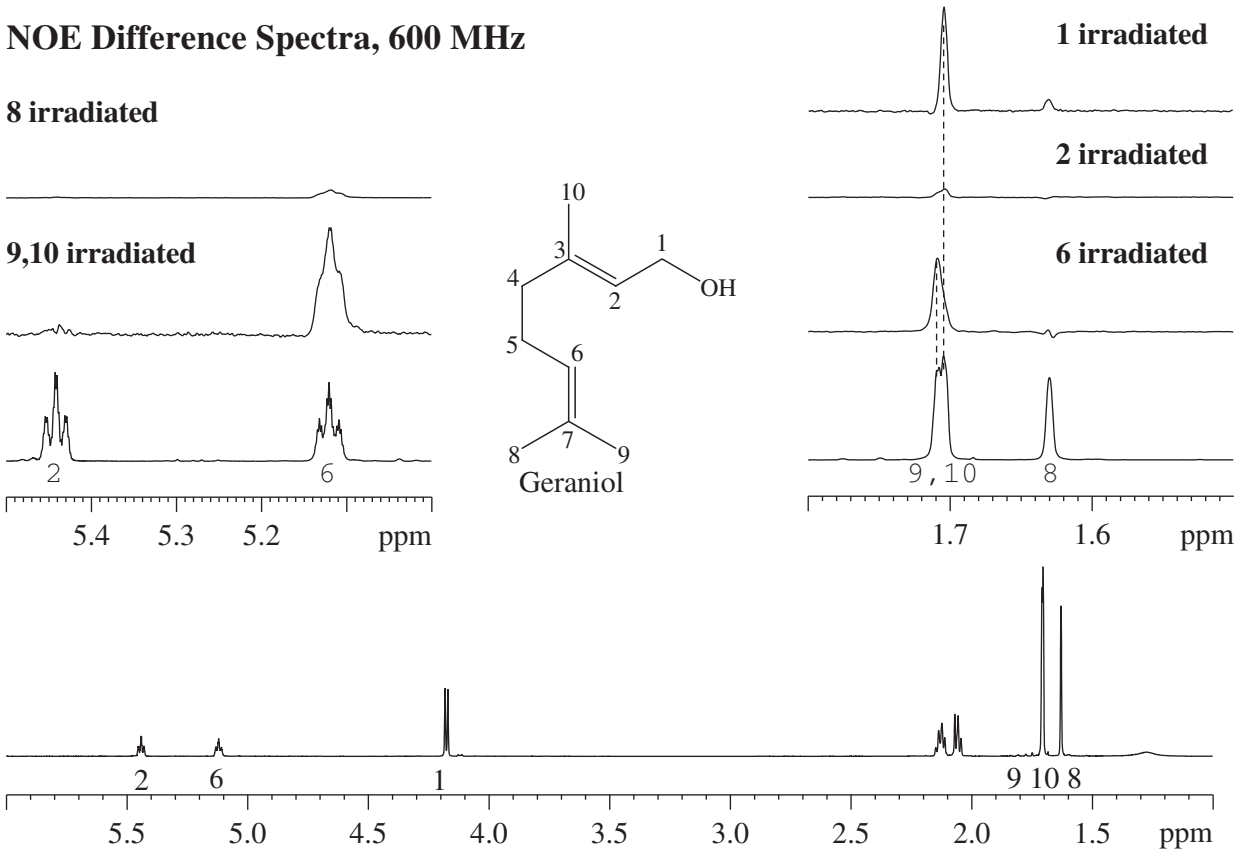

MASS
Geraniol
% of Base Peak
m/z
x 10
IR
% Transmittance
Wavenumber (cm-1)
3321
2966
2920
2858
1670
1442
1377
1095
1003
833
^{1}H NMR 600 MHz
Hz
ppm
OH
2
6
1
5 4
9,10,8

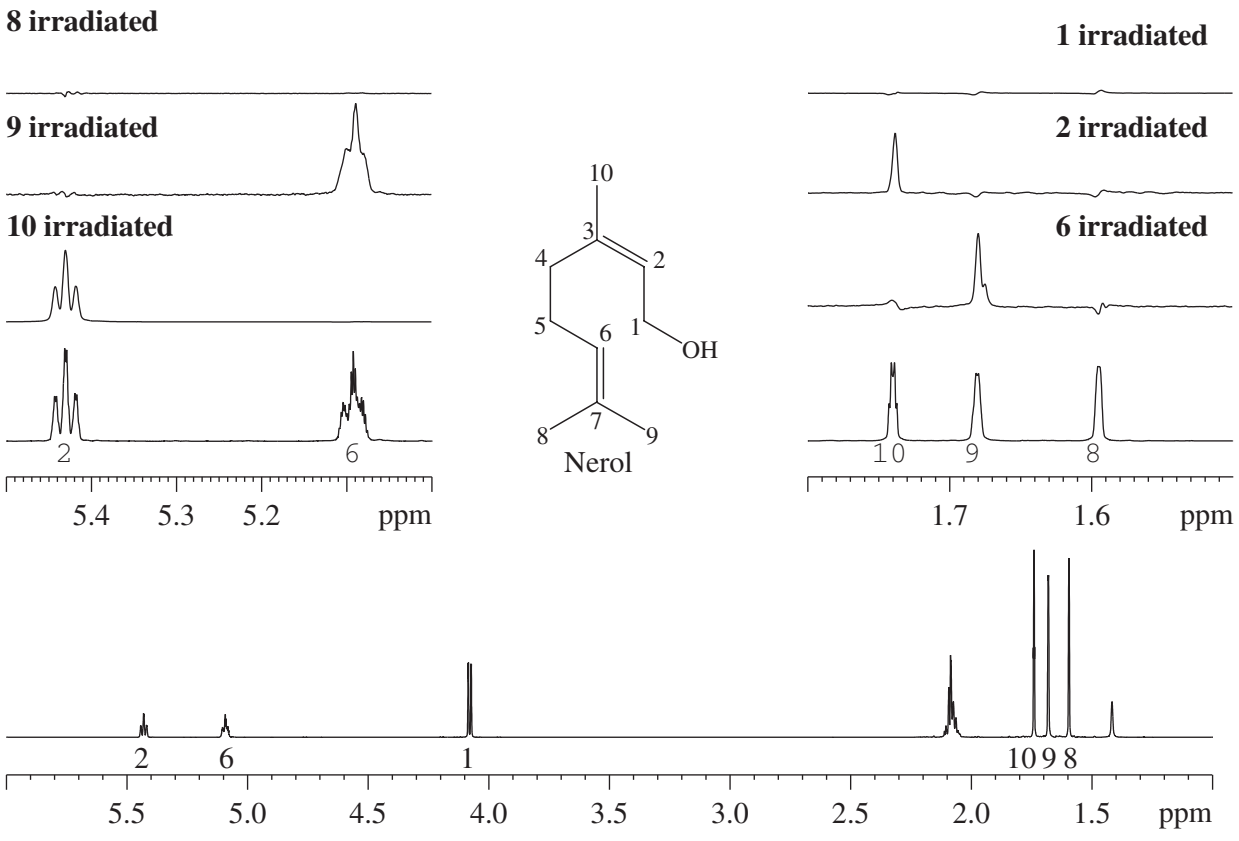

DQFCOSY 600 MHz
ppm
OH
9,10,8
5,4
1
6
2
F1
F2
ppm
10
3
1
4
2
OH
5
6
8
7
9
Geraniol


13C/DEPT NMR 150.9 MHz
CDCl3
3 7 6 2 1 4 5,9 8,10
ppm


HMQC 600 MHz
9,10,8
2
6
1
5,4
ppm
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
10
3
1
4
2
OH
5
6
8
7
9
Geraniol
F1
15
20
25
30
35
40
45
50
55
60
65
10
8
9
5
4
1
ppm
125
130
135
140
2
6
7
3
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
F2


INADEQUATE 600 MHz
3 7 6 2 1 4 5 9 8 10
Hz
10
3
1
4
2
OH
5
6
8
7
9
Geraniol
-15000
-10000
-5000
0
5000
10000
15000
F1
Hz
5000
10000
15000
135 130 125 ppm
140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
F2


NOE Difference Spectra, 600 MHz
8 irradiated
9,10 irradiated
1 irradiated
2 irradiated
6 irradiated
10
3
1
4
2
OH
5
6
8
7
9
Geraniol
2
6
9,10
8
5.4
5.3
5.2
ppm
1.7
1.6
ppm
2
6
1
9 10 8
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm


8 irradiated
9 irradiated
10 irradiated
1 irradiated
2 irradiated
6 irradiated
10
3
4
2
5
6
1
OH
8
7
9
Nerol
2
6
10
9
8
5.4
5.3
5.2
ppm
1.7
1.6
ppm
2
6
1
10 9 8
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

PROBLEM 7.4 DISCUSSION

It is quite likely that the *m/z* 154 peak, though small (the gray area is multiplied by ten), is the molecular ion peak. The *m/z* 139 peak, also small, results from rational loss of a methyl group. The alert interpreter also notes the M-18 peak at *m/z* 136 and promptly finds the intense, broad OH peak in the "neat" IR spectrum at 3321 cm^{-1} for confirmation; the intense band at 1003 cm^{-1} is probably C—O stretching. Again, as in Problem 7.3 we ask: alcohol or phenol; aromatic or not?

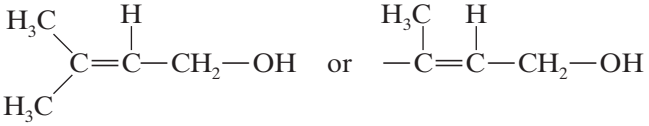
The very weak molecular ion peak in the present problem, together with loss of H_2O, suggests, but does not prove, an alcohol rather than a phenol. It may be worthwhile at this point to entertain the possibility that the base peak (*m/z* 69) represents the fragment $C_5H_9^+$ and results directly from the molecular ion peak by a strongly favored mechanism. If so, the intact molecule probably contains at least one double bond.

The ^{13}C/DEPT spectra provide ten distinct carbons and seventeen hydrogen atoms arranged thus from left to right: C, C, CH, CH, CH_2, CH_2, CH_2, CH_3, CH_3, CH_3. The first four are very likely olefinic. If the hydroxylic hydrogen atom is added, the tentative molecular formula is $C_{10}H_{18}O$, in accord with the molecular ion, *m/z* 154. The index of hydrogen deficiency is two, which would allow two double bonds, supported by the four olefinic carbon atoms.

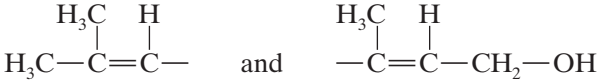
At this point, it is possible to solve the overall structure by using the wealth of information in the 1-D NMR spectra. This (traditional) approach will be explored first, followed by modern use of 2-D NMR spectra. Let us note at this time that the stereochemistry of this molecule cannot be ***proved*** using simple ^1H and ^{13}C NMR spectra.

Beginning with the ^1H NMR spectrum, the integration from left to right reads: 1:1:2:2:2:6:3:1, in conformity with the 18 hydrogen atoms in the molecular formula. It can also be read: (CH, CH olefinic), (CH_2, deshielded by OH), CH_2, CH_2, CH_3, CH_3, (almost superposed), CH_3, OH. Recall from the ^{13}C/DEPT spectra that there are two carbon atoms that have no attached hydrogen atoms. Recall also that the ^{13}C/DEPT spectra showed three distinct CH_3 groups, whereas the ^1H NMR spectrum showed H-9 and H-10 peaks apparently superposed even at 600 MHz. However, they are not completely superposed when expanded; they are partially overlapped with some long-range coupling.

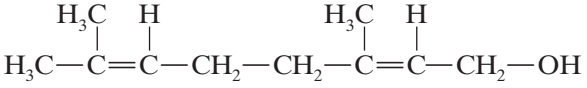
The carbinyl carbon is a methylene group (from the ^{13}C/DEPT) and it is a doublet (with some long-range coupling from H-10) at δ 4.15 in the ^1H NMR spectrum. Since the only methine groups in the structure are olefinic (also from the ^{13}C/DEPT), the compound must be an allylic alcohol. The three methyl groups are relatively deshielded and show no vicinal coupling forcing us to place them on olefinic carbon atoms. We consider two possible resulting allylic alcohol structures:



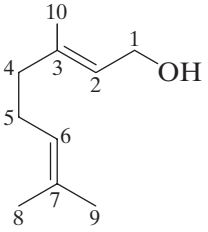
The structure on the left is in fact a complete molecule with no open valences; hence, it is rejected as the alcohol "fragment." The fragment on the right however seems plausible.



Another "fragment" can be constructed by considering that we have another double bond with two methyl groups that have no vicinal coupling (i.e., they are geminal) and an olefinic methine, shown at left above. If we consider the two fragments that we now have and realize that the two remaining pieces that have not been used are methylene groups, it is a simple matter of inserting them between the two fragments to arrive at the structure below:



This is a doubly unsaturated terpene alcohol. The stereochemistry is more accessible (and more obvious) with the structure of a conventional terpene:



The structure has no chiral center. There is a plane of symmetry in the plane of the page; thus, the protons of each methylene group are interchangeable (enantiotopic). The H-4 protons show a distorted triplet by coupling to the H-5 protons, which show a distorted quartet by coupling to the H-4 protons and to the H-6 proton with a slightly different coupling constant. The small $\Delta\nu/J$ ratio for H-4, H-5 also contributes to the distortion. The methyl groups, H-8 and H-9, are in the symmetry plane, thus not interchangeable.

Certainly the story is convincing, but the evidence is based only on the chemical shifts and on coupling patterns. It is unwarranted to base an analysis on chemical shifts and coupling patterns when a detailed analysis can be done unambiguously with 2-D experiments.

A better approach for solving structures relies less on the 1-D spectra and taps the wealth of information in the 2-D spectra. We obtain the molecular formula as we did above, noting also the presence of the alcohol function from the IR with confirmation in the ^{13}C/DEPT and ^{1}H NMR spectra. Next, we turn to the 2-D data. Evidence of diastereotopic protons is quickly ascertained in the HMQC by noting if there are two protons with different chemical shifts that are correlate to the same ^{13}C peak. No such diastereotopic correlations are seen.

The connectivity data of the COSY spectrum are most reassuring and a good place to start. The peaks along the diagonal are numbered for convenience. A good entry point for the COSY data is the carbinyl methylene at δ 4.15 (H-1). (If you need convincing that this peak is the carbinyl methylene, confirmation can be found in the HMQC and ^{13}C/DEPT.) Correlation by way of vicinal coupling is found to the olefinic methine (H-2) at δ 5.41 and correlation to a methyl group at δ 1.68 (H-10) by way of long range coupling is also evident. How do we know that the proton multiplet at δ 5.41 is a methine? By using the natural interplay of spectra, the proton multiplet at δ 5.41 correlates with a carbon resonance at 123 ppm in the HMQC; this information is fed back into the ^{13}C/DEPT spectra and we find that the carbon resonance at 123 ppm is a methine. Likewise, the proton absorption at δ 1.68 is correlated to a methyl carbon atom in the HMQC.

We can continue the connectivity pattern with the COSY to H-4 because there is a weak long range coupling from methyl H-10 to methylene H-4 at δ 2.11. (The student is encouraged to confirm that the multiplet at δ 2.11 is a methylene group by switching from the COSY to the HMQC to the ^{13}C/DEPT.) The only other correlation to H-4 is to H-5; this correlation is difficult to discern because the cross peaks are nearly on the diagonal. The H-5 methylene group shows a correlation to H-6 (the other olefinic methine) at δ 2.03. H-6 shows two other correlations, both long range, to the methyl groups H-8 and H-9. There are no other correlations in this COSY spectrum. The OH proton, of course, shows no cross peak because of rapid exchange.

At this point, we have assigned all of the protons but still cannot differentiate between the methyl groups at H-8 and H-9. Since we know all the ^{1}H assignments, it is a trivial task to transfer assignments to the ^{13}C signals through the HMQC spectrum. The quaternary carbons C-3 and C-7 have no attached protons and cannot be correlated in the HMQC spectrum. An HMBC spectrum could be used to correlate the quaternary carbon atoms, but for this problem we use carbon connectivities instead.

The INADEQUATE spectrum delineates the connectivities between adjacent ^{13}C atoms. It is a most powerful tool; after all, organic chemistry consists mainly of chains and rings of carbon atoms. Lines showing connectivities between and among correlated carbons have been added. As a starting point, consider the three methyl group carbons C-8, C-9, and C-10. We note that C-10 is connected to C-3 whereas C-8 and C-9 are both connected to an olefinic carbon (C-7). These connectivities confirm our assignment of C-10; however, we are unable to distinguish between C-8 and C-9. These assignments are made in the next section. If we continue from C-3, we see two more connectivities, one to an olefinic carbon (C-2) (see inset) and the other to an aliphatic carbon (C-4). The rest of the connectivities are left as an exercise for the student to transform the correlations into a carbon skeleton.

There are still two remaining tasks: assignment of stereochemistry of the C-2, C-3-double bond and assignment of the C-8 and C-9 methyl groups. NOE difference spectrometry is described in Section 3.16. It is a 1-D experiment that reveals ^{1}H—^{1}H proximity through space because of enhancement by the nuclear Overhauser effect. The "difference" spectrum is obtained by subtracting a standard ^{1}H spectrum from the NOE spectrum; this leaves only the enhanced peak(s).

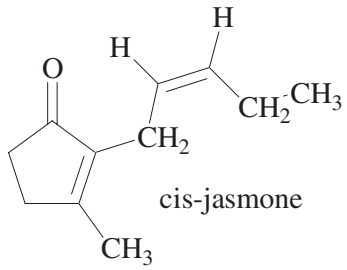
The task we face with the present molecule—distinguishing between a trisubstituted (*E*) double bond and the corresponding (*Z*) double bond—is not a trivial assignment. Nor is the task of distinguishing H-8 and H-9 methyl groups, as has been mentioned earlier. For conclusive results, we examine both the (*E*) isomer (geraniol) and the (*Z*) isomer (nerol) at the C-2 double bond.

In the top half of the NOE Difference Spectra, the ^{1}H NMR spectrum of geraniol, along with the NOE difference subspectra resulting from irradiation of key proton groups, and, in the bottom half of the page, the ^{1}H NMR spectrum of nerol (the geometric isomer of geraniol) along with the corresponding NOE difference subspectra are given. In geraniol, irradiation of olefinic methine H-2 shows no NOE enhancement of the H-10 methyl group; the reciprocal irradiation of the H-10 methyl group shows no NOE enhancement of the H-2 methine group. We conclude that these two groups are on opposite sides of the double bond and assign geraniol an *E*-double bond. This assignment is confirmed by irradiation of the H-1 allylic methylene group and the concomitant NOE enhancement of the H-10 methyl group thereby proving their disposition on the same side of the double bond. Since methyl groups H-9 and H-10 overlap in the proton spectrum,

they are irradiated together and we see an NOE enhancement of olefinic methine H-6. Check the result of irradiation of methine H-6.

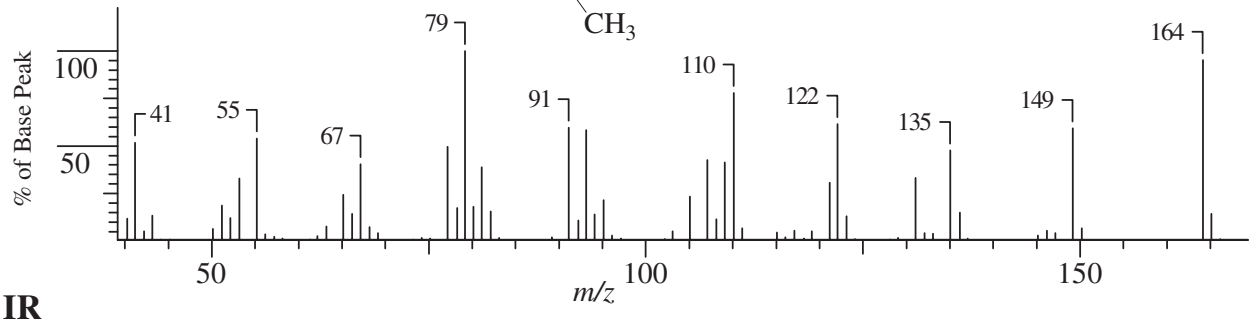
Quite often in "real life" problems, especially those involving natural products, the geometric isomer is not available (although, in principle, it could be synthesized). For pedagogical purposes, the results from nerol are presented. In this case, irradiation of olefinic H-2 does result in NOE enhancement of methyl group H-10 and we conclude that nerol has a Z-double bond. The assignments of methyl groups H-8 and H-9 are left to the student.

With hindsight, we can now recognize the fragment peak at *m/z* 69 (the base peak) as the result of the allylic cleavage of an olefin. Ordinarily, reliance on this cleavage for location of a double bond is dubious, but in geraniol, cleavage of the bis-allylic bond between C-4 and C-5 results in the stabilized fragment, *m/z* 69. See Section 1.6.1.2 for the analogous allylic cleavage of β-myrcene into fragments *m/z* 69 and 67.

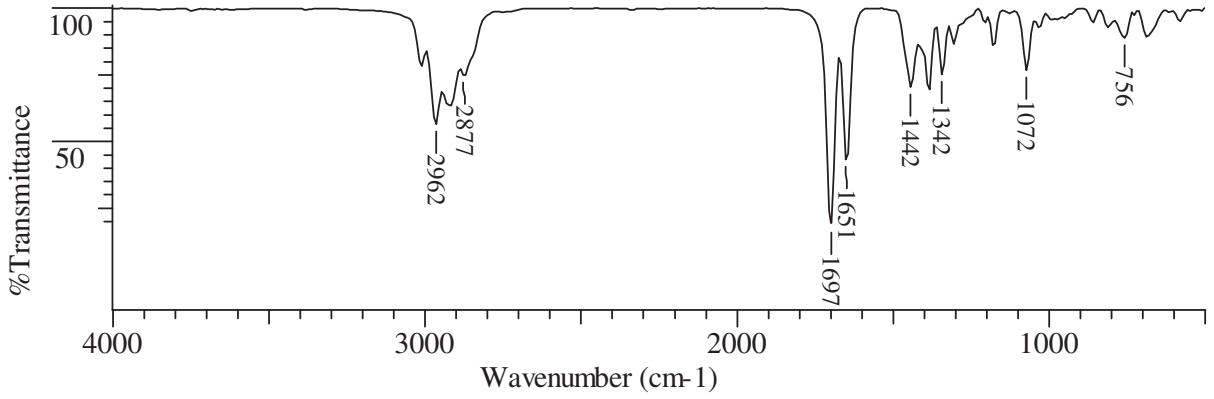


cis-jasmone

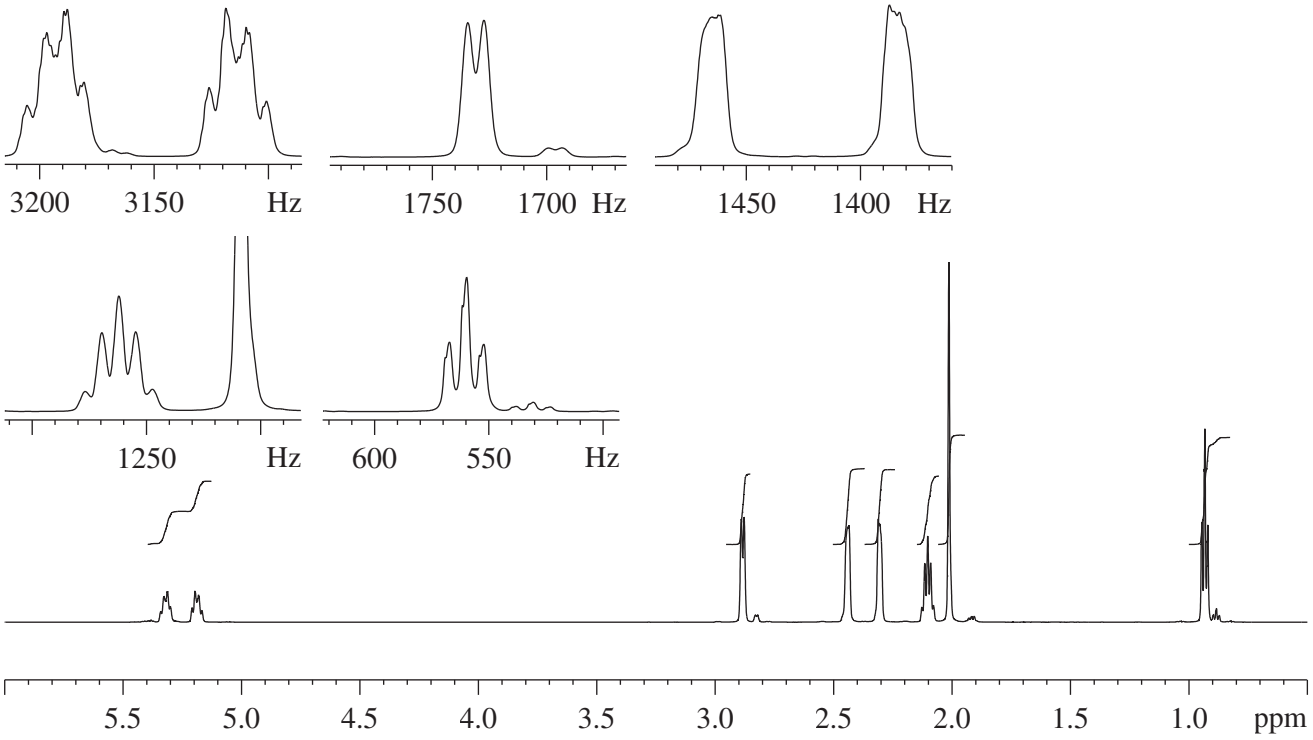
MASS



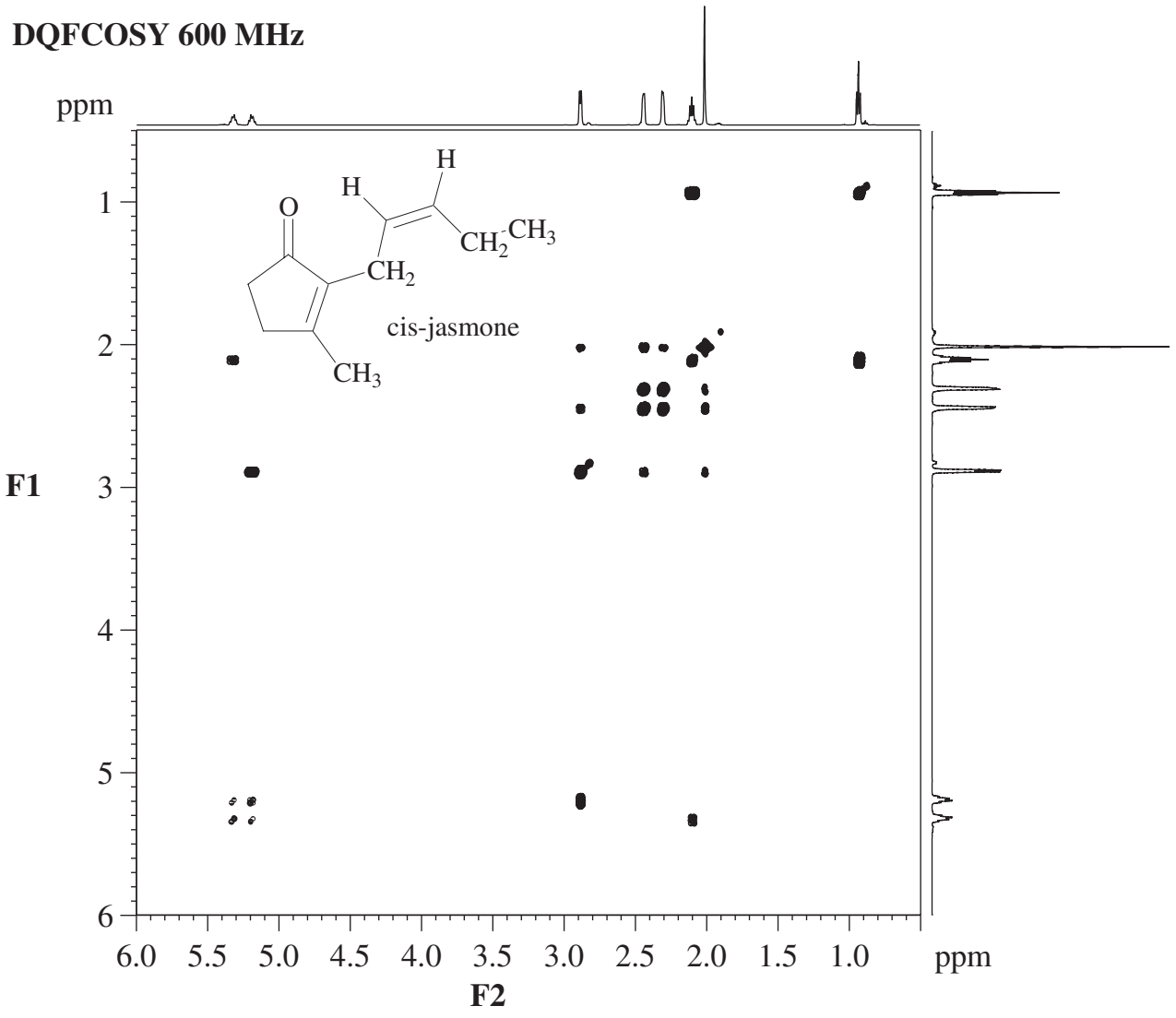
IR



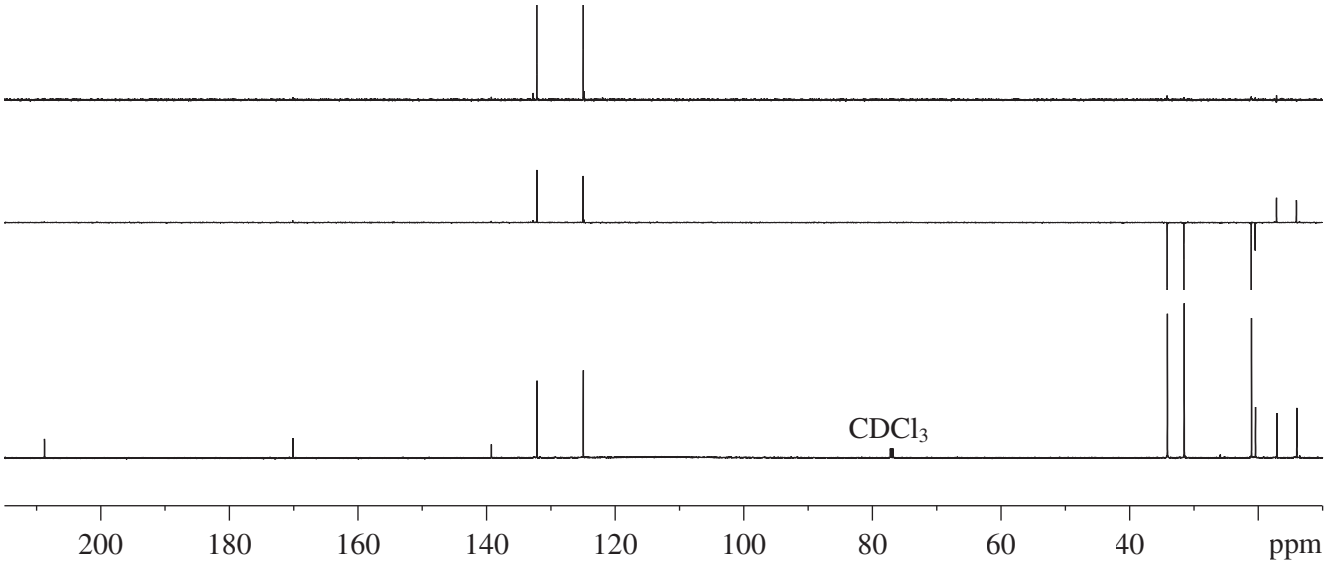
^{1}H NMR 600 MHz

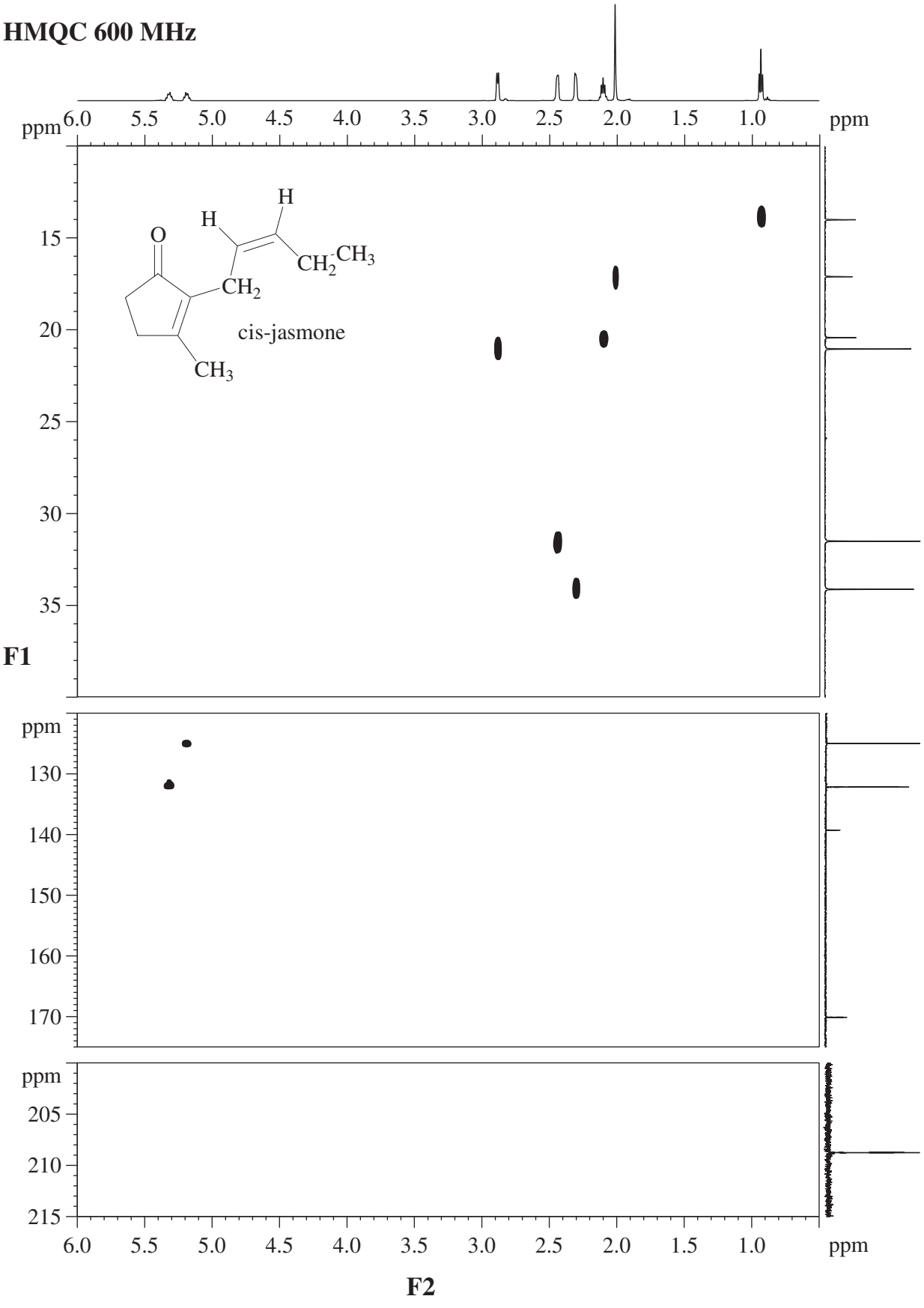


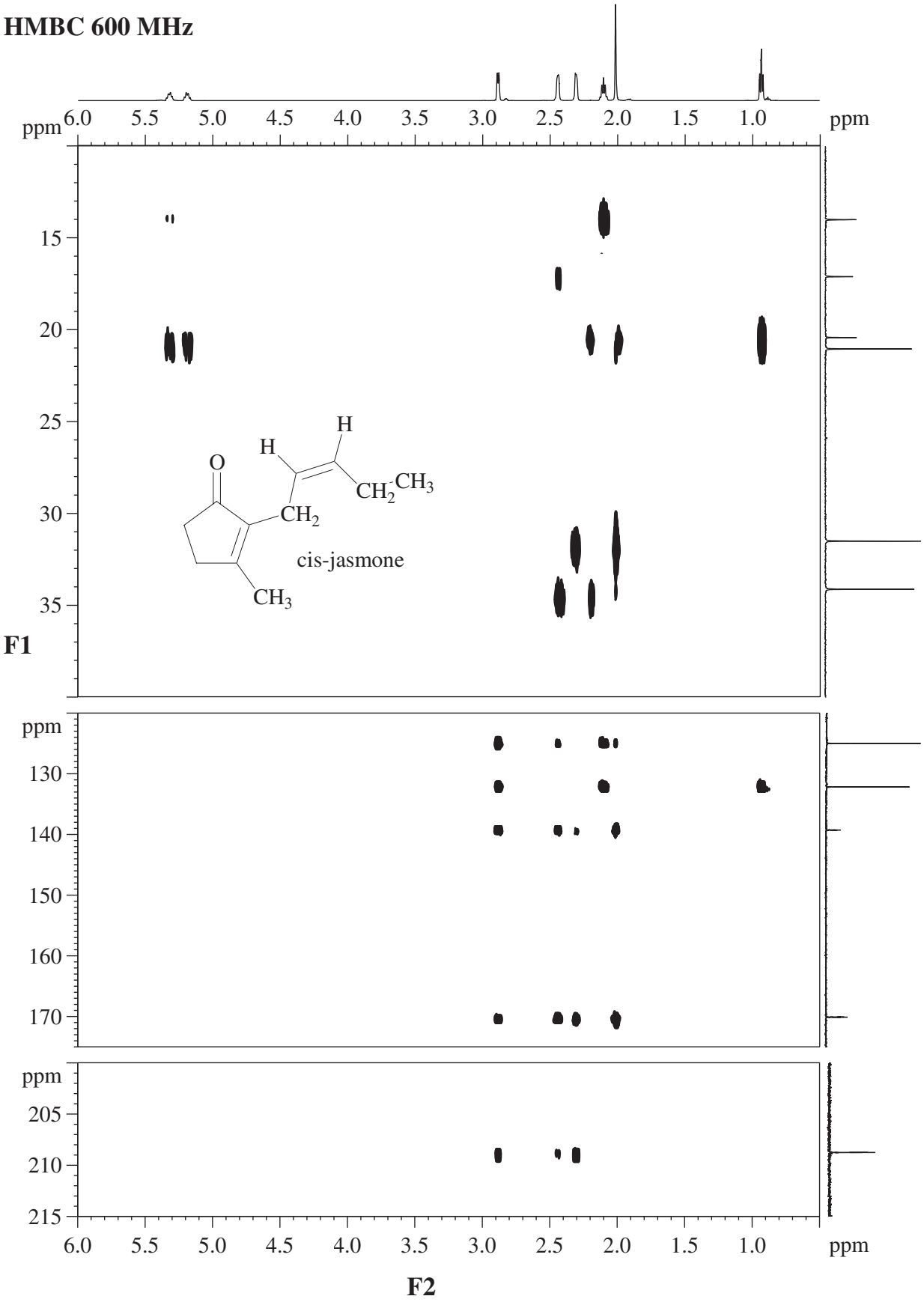
DQFCOSY 600 MHz



^{13}C/DEPT NMR 150.9 MHz




HMQC 600 MHz
ppm
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm
15
20
25
30
35
F1
O
H
H
CH2
CH2
CH3
CH3
cis-jasmone
ppm
130
140
150
160
170
ppm
205
210
215
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm
F2


HMBC 600 MHz
ppm
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
15
20
25
30
35
F1
O
H
H
CH2CH3
CH2
CH3
cis-jasmone
ppm
130
140
150
160
170
ppm
205
210
215
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
F2

PROBLEM 7.5 DISCUSSION

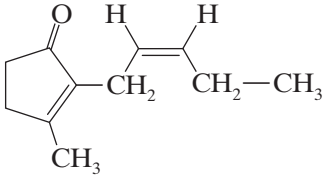
In the mass spectrum, the peak at *m/z* 149 represents a rational loss of a CH_3 group (M-15) and indicates that the *m/z* 164 peak is the molecular ion peak. The absent M + 2 indicates the absence of Cl, Br, and S. The IR spectrum shows an intense peak at 1697 cm^{-1}, suggestive of a C═O group, which is confirmed by the peak in the ^{13}C NMR spectrum at 208 ppm (in the range of ketones). In the ^{1}H NMR spectrum, the integration steps are: 1:1:2:2:2:2:3:3 (16 protons). The ^{13}C NMR spectrum indicates 11 carbon atoms. Thus, the tentative molecular formula is $C_{11}H_{16}O$, which agrees with the molecular ion peak of *m/z* 164. The index of hydrogen deficiency is four. From left to right in the ^{13}C/DEPT spectra, the number of protons attached to each carbon are: C, C, C, CH, CH, CH_2, CH_2, CH_2, CH_2, CH_3, CH_3.

Three of the four degrees of unsaturation can be dealt with directly in the ^{13}C NMR spectrum. We have already noted the carbonyl peak and because of its chemical shift we associate it with a ketone. There are four olefinic carbon resonances, which account for two more degrees of unsaturation. By inference, the fourth degree of unsaturation is attributed to a ring.

The ^{13}C/DEPT spectra have provided the molecular contents, which is a large step toward deciphering the structure. We turn to the 2-D spectra to assemble the pieces. As needed, we will jump from one spectrum to another. The more shielded of the two methyl groups at δ 0.93, which correlates with the methyl carbon resonance at 15 ppm in the HMQC, is a good place to start. This methyl group is coupled to a methylene group at δ 2.12 as evidenced by the strong cross peak in the COSY spectrum. The carbon resonance associated with this group is found at 21 ppm in the HMQC spectrum. This methylene group also correlates with one of the olefinic methines in the COSY at δ 5.33. The methylene group at δ 2.12 shows a nearly first order quintet.

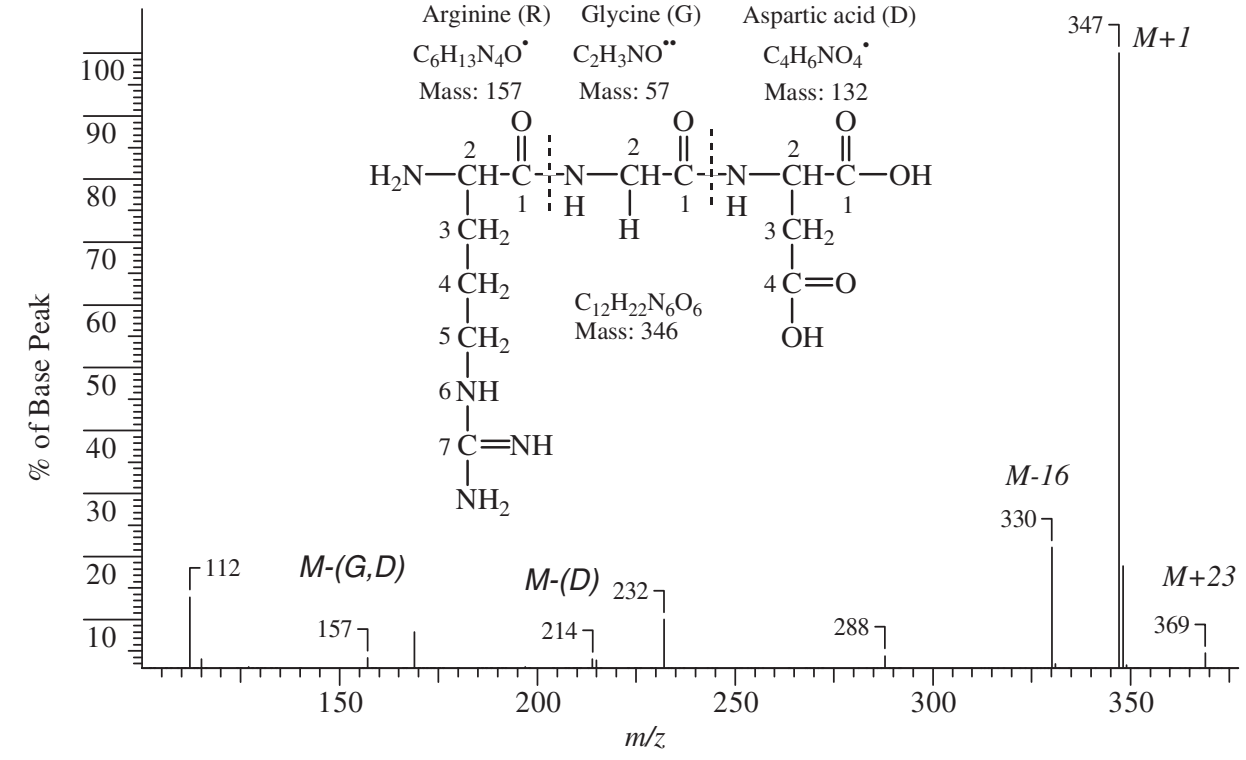
The other olefinic methine at δ 5.18 shows a correlation in the COSY to the first methine (δ 5.33) strongly suggesting a disubstituted double bond. In addition, the methine at δ 5.18 is coupled to a rather deshielded methylene group at δ 2.88. This methylene is a broadened doublet and shows no other vicinal coupling. According to the COSY, this methylene group shows long-range coupling to two other groups not yet used (one of the two unused methylene groups and the other methyl group.) So far we have a 2-pentenyl group, which must be attached to one of the quaternary olefinic carbon atoms. The broadened methyl singlet at δ 2.01 must be attached to the other quaternary olefinic methine.

If we take stock of the remaining pieces from the ^{13}C/DEPT spectra (a ketone carbonyl, two methylene groups, and two quaternary olefinic carbons) and the fact that there is still the unused degree of unsaturation for a ring, we realize that we must draw an unsaturated five member-ring ketone with these pieces. Further evidence allows us to order these pieces. First, the COSY tells us that the two methylene groups (δ 2.30 and 2.45) are vicinally coupled and therefore adjacent. Second, the ^{13}C chemical shift of one of the quaternary olefinic carbons is 170 ppm; such deshielding can only be explained by conjugation with the ketone carbonyl. One obvious structure is shown below. The HMBC (see the correlations to the carbonyl carbon) confirms the ring structure and the lack of an asymmetric carbon atom (there is a plane symmetry in the plane of the page) explains the absence of diastereomeric methylene groups.

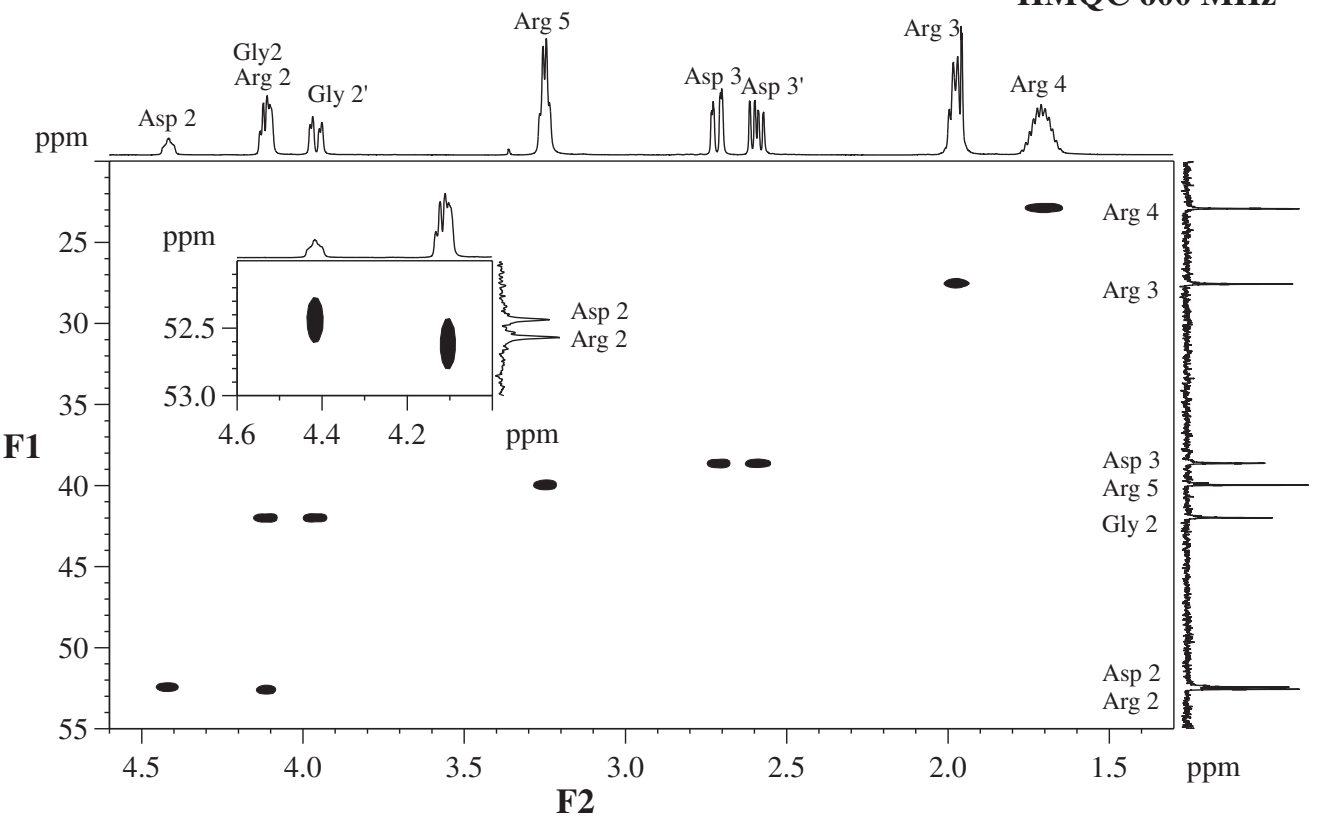


Before we finish, we ask ourselves if there are any constitutional isomers that we need to consider. Yes, if we switch the two substituents (i.e., the 2-pentenyl group and the methyl group), the resulting structure also fits the data so far. Again, the complex HMBC spectrum resolves the issue. For instance, the ring substituted methyl resonance at 17 ppm shows only one correlation to the methylene group at δ 2.45. This methylene group is in the β-position to the carbonyl, thereby confirming the structure given above. Are there other correlations in the HMBC that can confirm the structure? The student can finish the assignments.

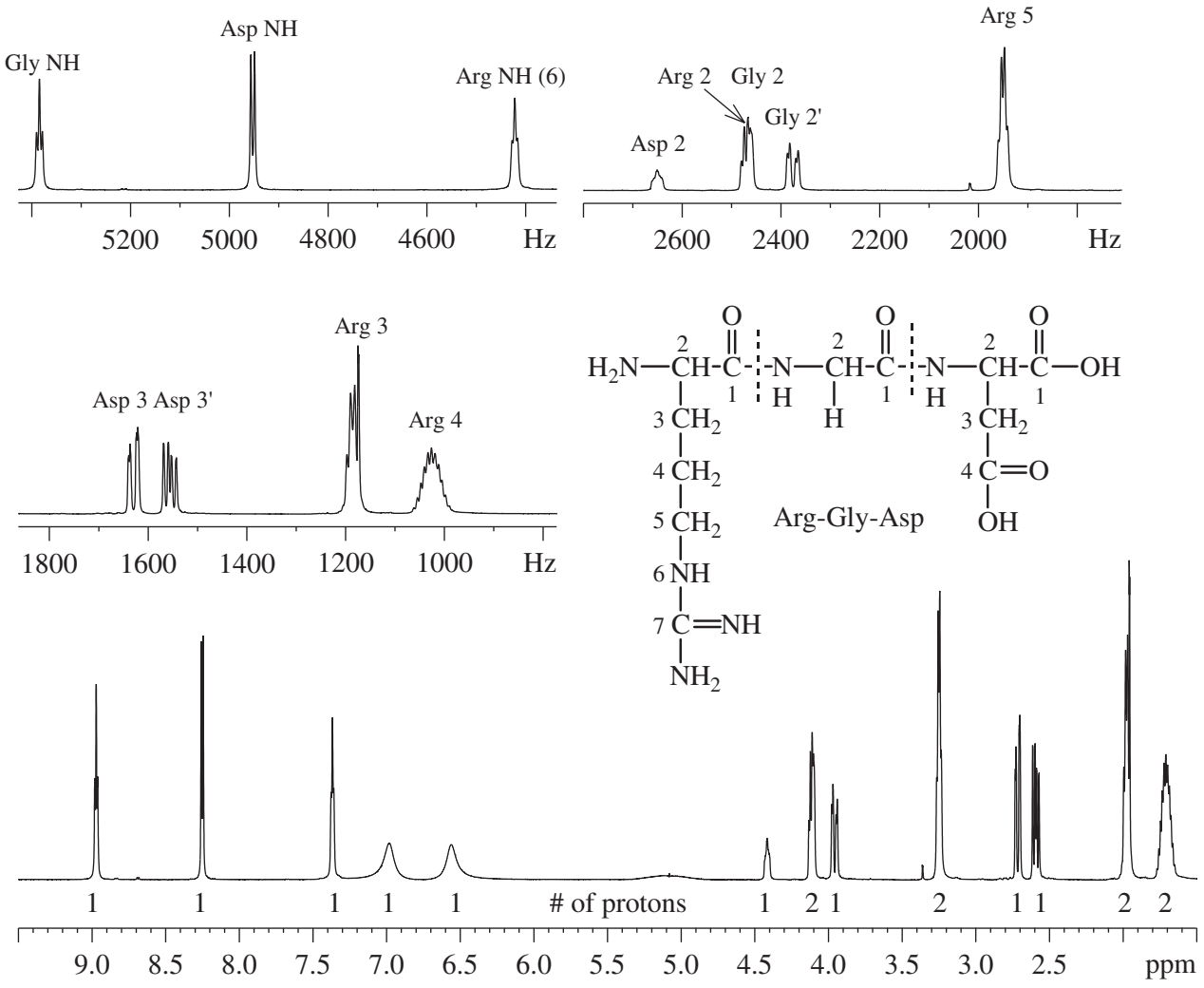
LCMS ES



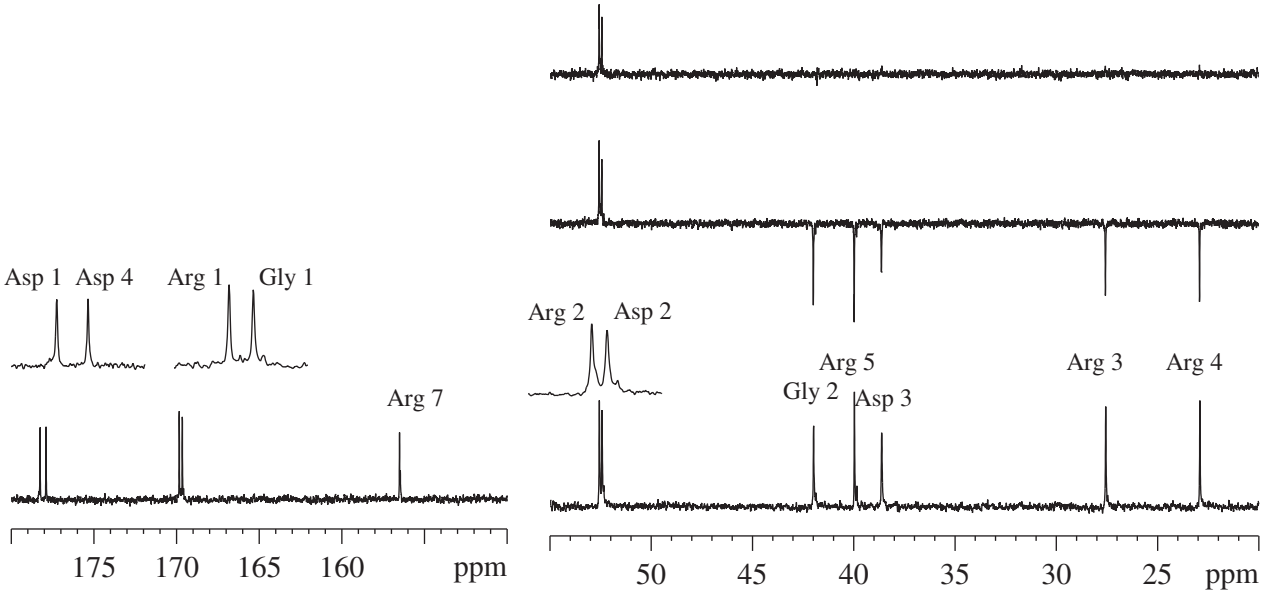
HMQC 600 MHz

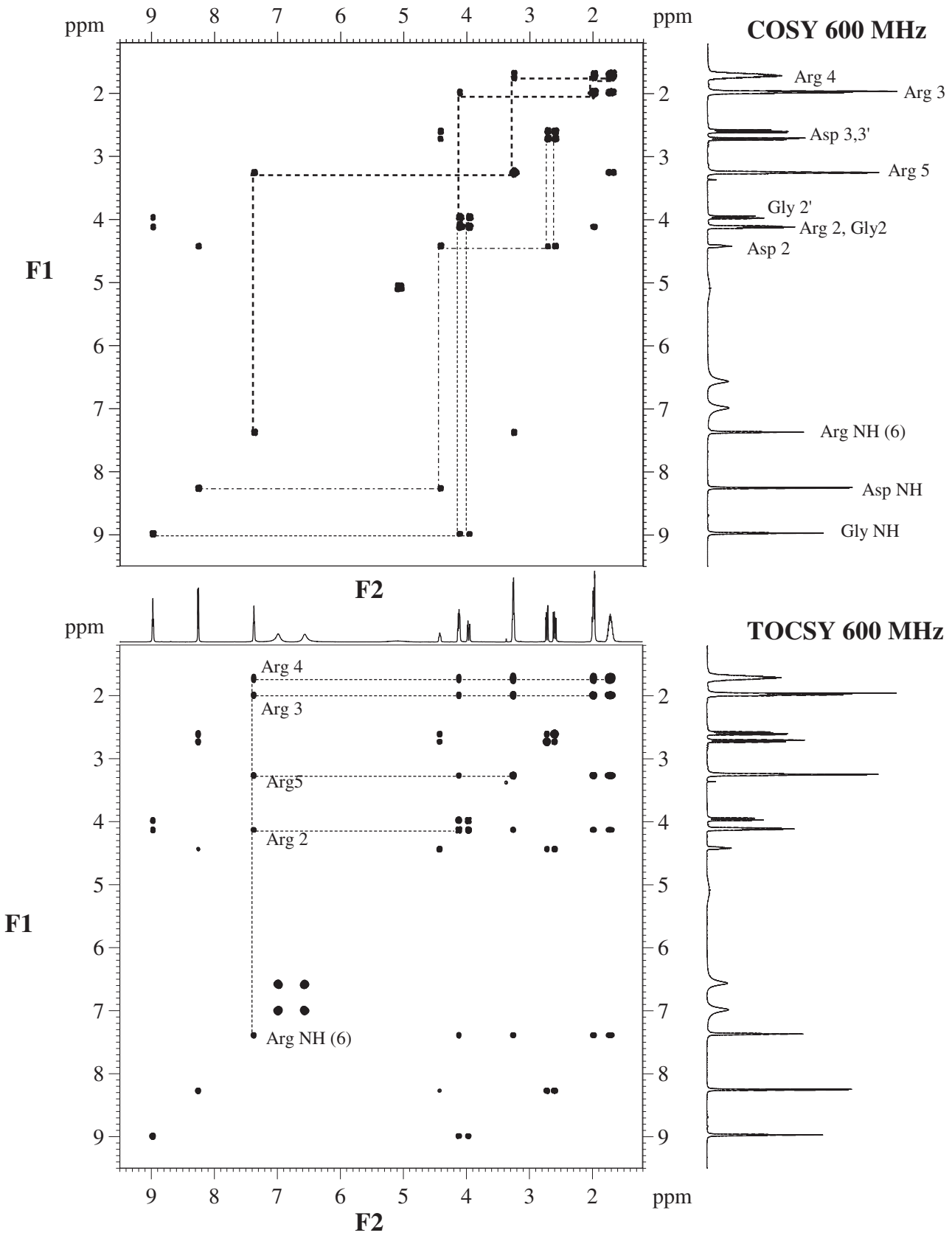


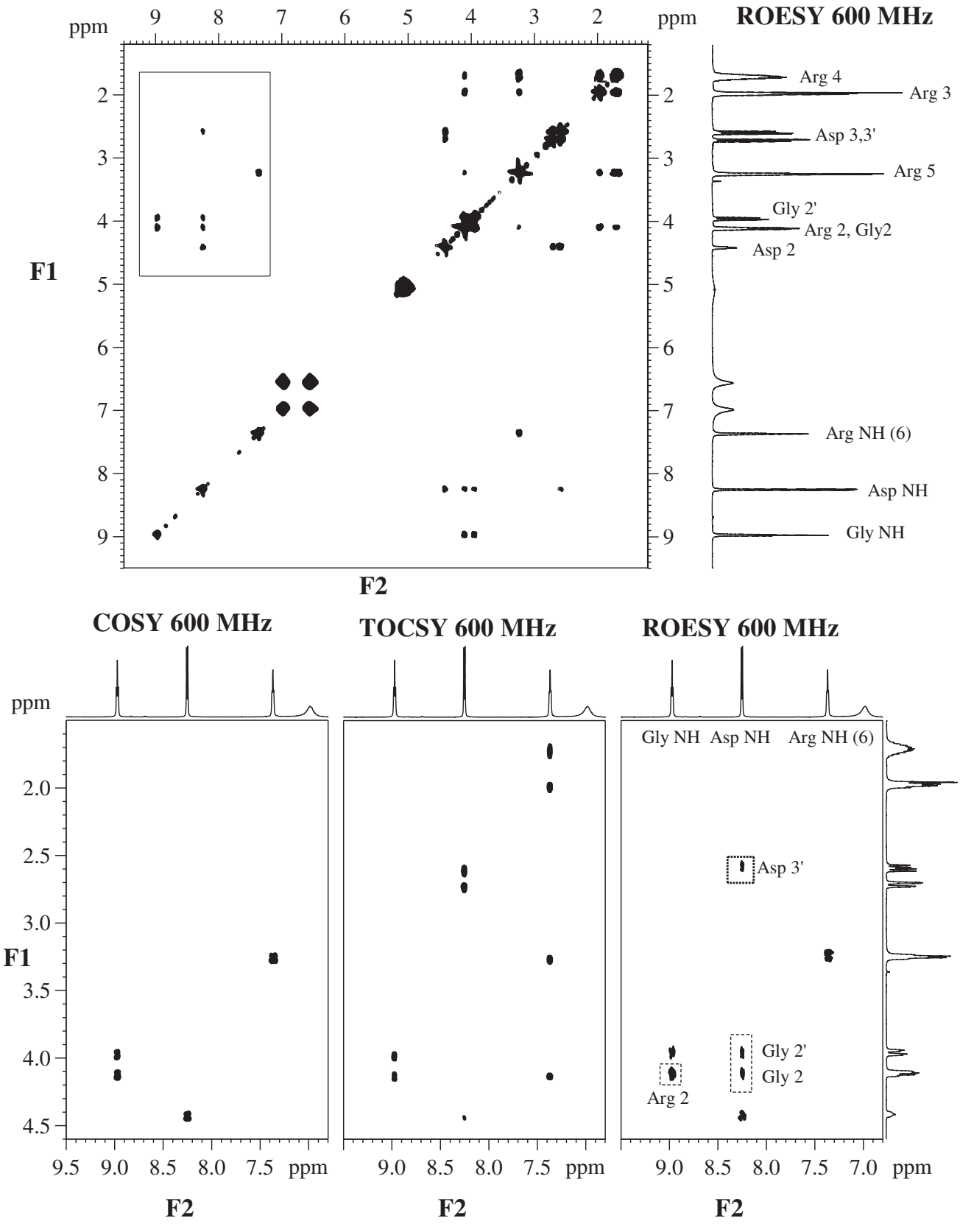
^{1}H NMR 600 MHz

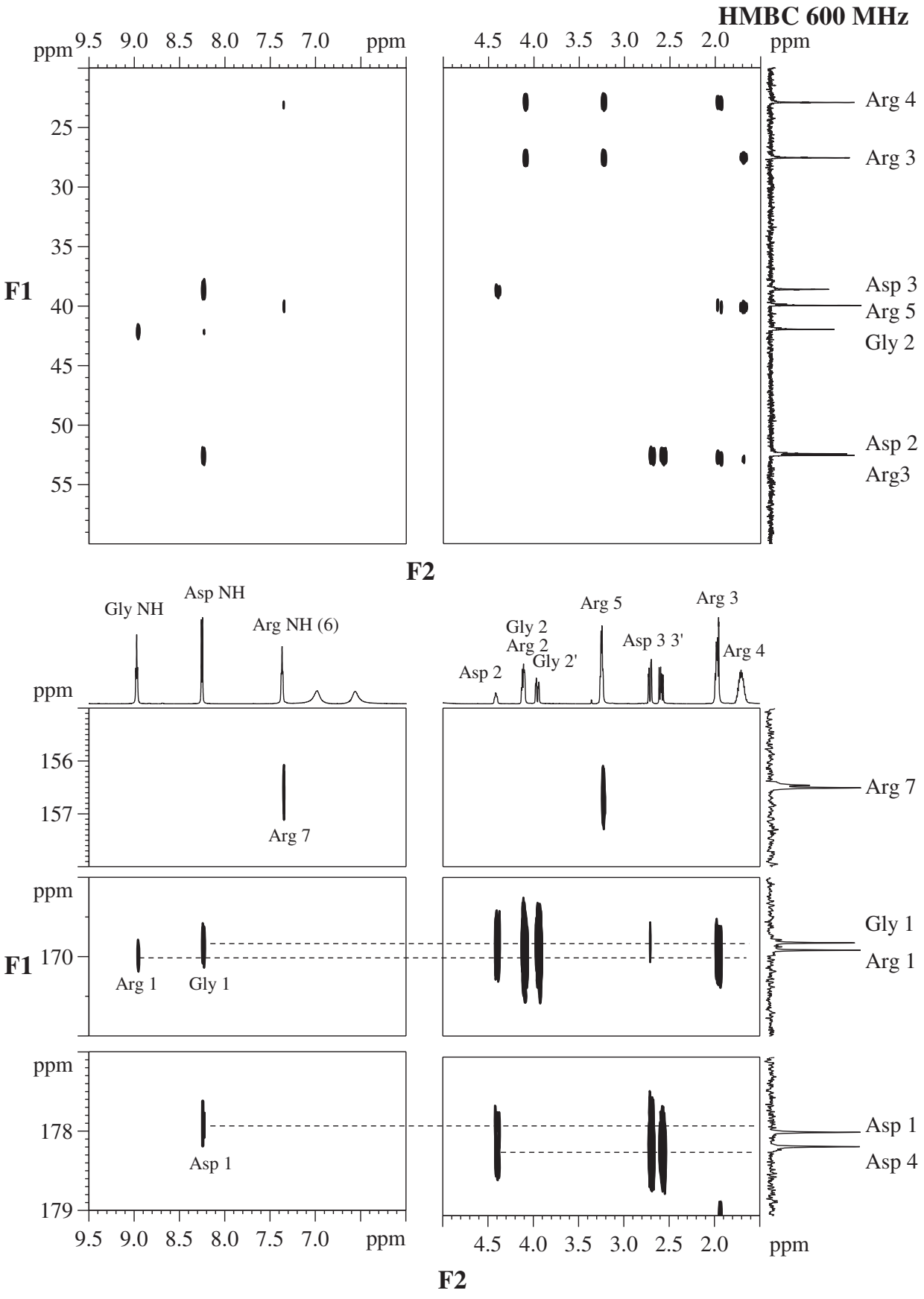


^{13}C/DEPT NMR 150.9 MHz




COSY 600 MHz
ppm
F1
F2
Arg 4
Arg 3
Asp 3,3'
Arg 5
Gly 2'
Arg 2, Gly2
Asp 2
Arg NH (6)
Asp NH
Gly NH
TOCSY 600 MHz
Arg 4
Arg 3
Arg5
Arg 2
Arg NH (6)


ROESY 600 MHz
ppm
F1
F2
Arg 4
Arg 3
Asp 3,3'
Arg 5
Gly 2'
Arg 2, Gly2
Asp 2
Arg NH (6)
Asp NH
Gly NH
COSY 600 MHz
TOCSY 600 MHz
ROESY 600 MHz
Gly NH Asp NH Arg NH (6)
Asp 3'
Gly 2'
Gly 2
Arg 2


HMBC 600 MHz
ppm
9.5 9.0 8.5 8.0 7.5 7.0 ppm
4.5 4.0 3.5 3.0 2.5 2.0 ppm
F1
25
30
35
40
45
50
55
Arg 4
Arg 3
Asp 3
Arg 5
Gly 2
Asp 2
Arg3
F2
Gly NH
Asp NH
Arg NH (6)
Asp 2
Gly 2
Arg 2
Gly 2'
Arg 5
Asp 3 3'
Arg 3
Arg 4
ppm
156
157
Arg 7
Arg 7
ppm
F1
170
Arg 1
Gly 1
Gly 1
Arg 1
ppm
178
179
Asp 1
Asp 1
Asp 4
9.5 9.0 8.5 8.0 7.5 7.0 ppm
4.5 4.0 3.5 3.0 2.5 2.0 ppm
F2

PROBLEM 7.6 DISCUSSION

The last of the solved problems in this chapter is quite different from the other problems presented above, and our approach takes a different tack as well. The compound is a tripeptide, and structure elucidation of peptides requires two distinct "solutions." First, the individual amino acids (and their number) are determined and second, the amino acid units are put in order (sequenced). Neither of these exercises is trivial since there are more than 20 common amino acids, and the nature of the peptide bond means that they can be arranged in any order.

Before discussing the actual data, some discussion of sample handling is worthwhile. The mass spectrum of the tripeptide was obtained using an electrospray LCMS (see Chapter 1). Electrospray (ES) is a "soft" method of ionization (a type of chemical ionization), which suppresses or limits fragmentation and enhances pseudomolecular ions (depends on the number of charges on the ion, z). The NMR experiments were obtained in 95% H_2O and 5% D_2O at 0°C. The reasoning for using these solvents and the details are given in Section 5.11.

The LCMS ES gives an M + 1 of m/z 347, which corresponds to a molecular formula of $C_{12}H_{22}N_6O_6$. Derivation of this formula is not considered in detail since Appendix A in Chapter 1 only goes up to 250 amu. The small peak at m/z 369 (M + 23) is due to the presence of sodium ions, which are ubiquitous in aqueous solutions. Although there is only limited fragmentation, the fragments that do appear can be quite useful to an experienced interpreter, and some of the cleavages are shown in the Problem 7.6A.

To ascertain the three amino acids, we use information from the ^{1}H NMR spectrum, the ^{13}C/DEPT spectra, the COSY spectrum, the TOCSY spectrum, and the HMQC spectrum. Chemical shifts for protons of amino acids are given in Appendix I of Chapter 3. Peptides are chiral molecules, and all methylene groups are diastereotopic, even those of glycine. (The methylene group of free glycine is enantiotopic.) A starting point for peptides (and other compounds made of distinct units such as oligo- and polysaccharides) is the TOCSY. The 2-D TOCSY shows correlation among all spins in a spin system, but no correlations to spins outside the system. For a tripeptide, there are three distinct spin systems, and they are easy to find in the TOCSY spectrum (Problem 7.6C). The N—H resonance at 8.95 ppm reveals one spin system showing correlations to two resonances at 3.96 and 4.13 ppm. If we feed this information into the HMQC spectrum, we find that these two proton-resonances correlate to the same carbon atom at 41.9 ppm (i.e., a diastereotopic methylene group). This amino acid is identified as a glycine residue, and since there is correlation to an amide N—H group, we conclude that the glycine is not the N-terminus. (This point will be confirmed using other methods.)

Another spin system is evident with a convenient starting point with the N—H resonance at 8.26 ppm. There are three correlations to this resonance for a total of four moieties in this spin system. If we again take this information directly to the HMQC spectrum, we can find the corresponding carbon resonances. Of course, there is no correlation to the N—H resonance. The proton resonance at 4.41 ppm correlates to a carbon resonance at 52.4 ppm, and the ^{13}C/DEPT spectra confirm that this is a methine group. The remaining spins in the system are proton resonances at 2.58 and 2.72 ppm, which correlate to a single carbon resonance at 38.6 ppm. The ^{13}C/DEPT confirms that this is a methylene group. This residue is identified as aspartic acid, and again we conclude that it is not the N-terminus.

A starting point for the final spin system is the N—H resonance at 7.35 ppm. There are four other proton resonances in this spin system; the HMQC spectrum and the ^{13}C/DEPT spectra indicate that these resonances represent one methine group and three methylene groups. Thus, this amino acid residue is arginine.

The COSY spectrum could be used to confirm our analysis thus far but it has not been necessary. However, the COSY and TOCSY are not redundant, and, in fact, they are complementary in at least one important aspect. While the TOCSY shows all of the spins in a spin system, it does not reveal which spins are actually coupled to one another. For instance, the N—H proton at 8.26 ppm (from aspartic acid) shows only a correlation to a methine group in the COSY; we can safely conclude that this N—H group is involved in a peptide linkage and that the methine group is the α- or asymmetric carbon of the amino acid. (Glycine is a trivial case and not considered here.) The N—H proton at 7.35 ppm, which we assigned to an aspartic acid residue, correlates with all of the spins in the TOCSY, but only shows coupling to a methylene group at 2.24 ppm in the COSY. This information allows us to draw two conclusions that were not available from the TOCSY. First, the N—H resonance is not coupled to the α-carbon of arginine and therefore must represent the N—H from the guanadino group and the not α-amino group. Second, the arginine residue must be the N-terminal residue because there is no correlation from the methine proton at 4.13 ppm and an N—H proton in either the COSY or the TOCSY. In the discussion of the sequence of the amino acids, we will confirm this second point.

The combined information from the ^{13}C/DEPT, COSY, TOCSY, and HMQC enables us to assign all protons in the ^{1}H spectrum except those that are exchanging rapidly (i.e., the carboxyl and free amino protons) and all of the non-quaternary carbon resonances in the ^{13}C spectrum. There is no need to assign the rapidly exchanging protons, and the non-quaternary carbons will be assigned during the sequencing discussion.

The second main objective is to "sequence" the peptide or place the amino acids in their proper order. Two powerful tools from our NMR experimental repertoire are HMBC and ROESY (or NOESY). Recall that the HMBC shows long range $^{1}H—^{13}C$ coupling (generally 2-bond, $^{2}J_{CH}$, and 3-bond, $^{3}J_{CH}$ couplings). For sequencing purposes, this experiment shows correlation between adjacent amino acids, as it were, "seeing through" the amide (peptide) carbonyl to the amide N—H. This exercise will also enable us to assign the carbonyls.

The ROESY experiment facilitates sequencing utilizing the inevitable through space correlations between adjoining amino acids. We expect to find through space connectivities from one amino acid's N-H to the adjoining amino acid's α- or C-2 proton(s). The data from either experiment should be sufficient; together they provide strong confirmatory evidence.

The full ROESY spectrum is shown in Problem 7.6D (top part). ROESY cross peaks show both COSY correlations and NOE correlations. For easy comparison, therefore, the area of interest for sequencing (the boxed area) is shown along with the corresponding COSY and TOCSY (bottom part of Problem 7.6D). The glycine N—H, which correlates with the adjacent methylene group in both the COSY and TOCSY spectra, gives an additional correlation in the ROESY spectrum to H-2 of arginine. This correlation shows a linkage between glycine and arginine. Some might consider this correlation inconclusive because of the overlap between H-2 of arginine and one of the H-2's of glycine. In this case, confirmation is desirable (see below).

The other connectivity can be established by way of the NOE interaction between the aspartic acid N—H and the two glycine H-2's. There is no ambiguity or overlap in this correlation thus proving the sequence given in Problem 7.6A. An interesting aside worth noting is the NOE correlation between the aspartic acid N—H and only one of the two diastereotopic methylene protons of aspartic acid (H-3). This selective interaction suggests restricted rotation and allows steric differentiation and assignment between the diastereotopic protons.

Confirmation of this sequence and assignment of the quaternary carbons is accomplished with the HMBC, which is shown in Problem 7.6E. The bottom part of this page is pertinent. Before confirming the sequence, a simple assignment of a quaternary carbon is made. The assignment of the C-7 carbon of arginine can be made by noting the correlation between the arginine N—H (H-6) and the quaternary carbon at 156.5 ppm.

The analysis of the HMBC in the region of the carbonyl carbons is hampered by the lack of digital resolution along the F1 axis. Recall that the HMBC experiment is proton detected giving good resolution in the proton or F2 dimension. The only way to improve resolution along the F1 axis is to increase the number of FIDs in the experiment, which has serious practical limitations. The lines drawn in the insets help clarify the correlations.

The glycine N—H correlates with the arginine carbonyl (C-1) confirming the arginine–glycine linkage. The assignment of the arginine carbonyl is accomplished by noting the correlation of the carbonyl resonance at about 170 ppm with the arginine methine H-2. The other linkage is established by the correlation of the aspartic acid N—H and the glycine carbonyl (C-1); the glycine carbonyl is pinpointed from its correlations with the glycine diastereotopic methylene protons. The sequence of the tripeptide is confirmed by two independent methods.

STUDENT EXERCISES

The following exercises are given for the student to "solve." The structures and spectra for two compounds as shown on the next page. The student should "prove" the structure from the spectra and assign all protons and carbons.