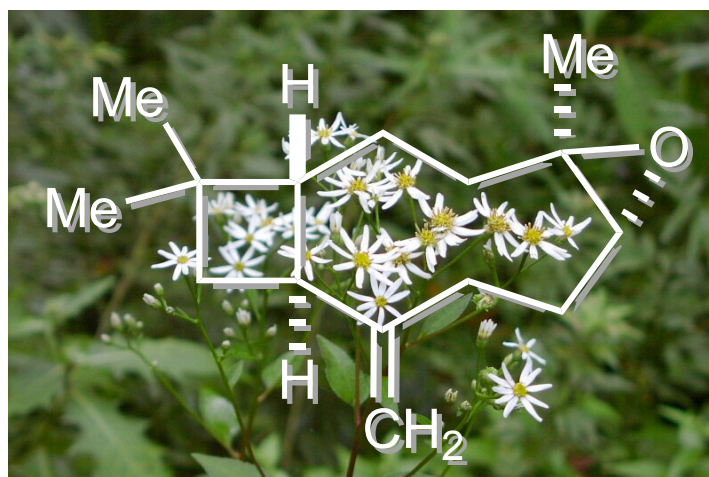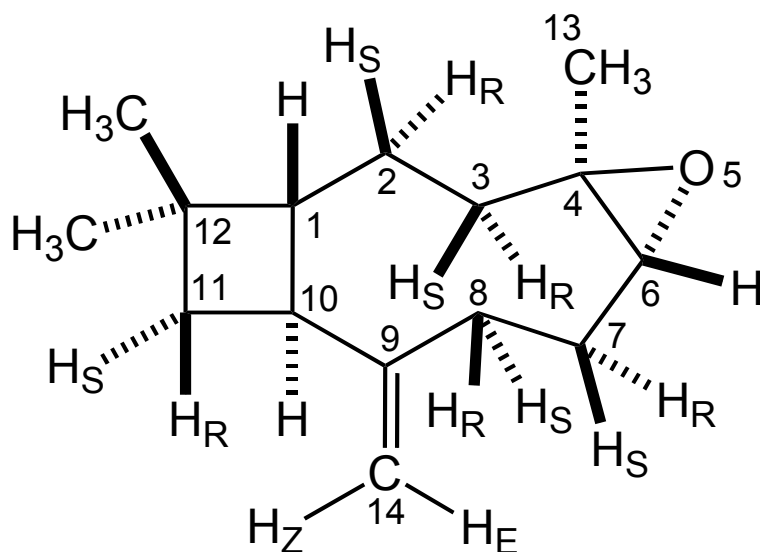# Application of NMR Techniques to the Structural Determination of Caryophyllene Oxide (Unknown #92)

(Photo courtesy of Dr. Kazuo Yamasaki, Hiroshima University)

Christopher J. Morten & Brian A. Sparling
5.46 Spring 2007

Figure 1. Structure of (-)-caryophyllene oxide, or (1*R*,4*R*,6*R*,10*S*)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.0]4,6)]dodecane.

Carbon	Shift (ppm)	^{13}C Integration
C1	50.80	m
C2	27.40	s
C3	39.30	s
C4	60.00	w
C6	63.90	s
C7	30.40	s
C8	29.90	m
C9	152.00	w
C10	48.90	s
C11	39.90	s
C12	34.20	w
C13	17.20	s
C14	113.00	s
C15	30.10	s
C16	21.80	s

Table 1. Carbon signals of caryophyllene oxide.

Proton	Shift (ppm) & Int.	Expected Mult.	Observed Mult.	J Values (Hz)	Environment**
H1	1.76 (1H)	d^3	d^2	10.1, 4.2	CH
H2 *pro-R*	1.42 (1H)	d^4	m	13.6, 4.2, 3.6 1.2	CH_2
H2 *pro-S*	1.65 (1H)	d^4	m	*	CH_2
H3 *pro-R*	2.09 (1H)	d^4	m	3.6*	CH_2
H3 *pro-S*	0.96 (1H)	d^3	ψ-d^3	13.2, 5.0	CH_2
H6	2.88 (1H)	d^2	d^2	10.6, 4.2	CH
H7 *pro-R*	1.32 (1H)	d^4	ψ-d^4	10.6, 4.4, 3.7, 2.1	CH_2
H7 *pro-S*	2.25 (1H)	d^4	ψ-d^4	4.4, 4.2, 3.7, 0.6	CH_2
H8 *pro-R*	2.34 (1H)	d^3	ψ-d^3	4.4, 3.7, 4.5	CH_2
H8 *pro-S*	2.11 (1H)	d^3	m	4.4*	CH_2
H10	2.62 (1H)	d^2	d^2	9.9, 8.5	CH
H11 *pro-R*	1.68 (1H)	d^2	d^2	*	CH_2
H11 *pro-S*	1.61 (1H)	d^2	d^2	*	CH_2
H13	1.20 (3H)	s	s	-	CH_3
H14 *pro-E*	4.86 (1H)	d	d	1.2	CH_2
H14 *pro-Z*	4.97 (1H)	d	d	1.2	CH_2
H15	1.00 (3H)	s	s	-	CH_3
H16	0.98 (3H)	s	s	-	CH_3

Table 2. Proton signals and coupling of caryophyllene oxide.

* Indicates that a full set of coupling constants could not be determined
due to insufficient resolution on the 1D ^{1}H spectrum.

** CH = methine; CH_2 = diastereotopic methylenes (+ *exo*-methylene)

Carbon	Shift (ppm)	Bonded Proton(s)	Environment	C-H Coupling*
a	152.0	-	C	{A, B}, D, {E, G}, {F, N}, I, {J, L}
b	113.0	A, B	CH_2	D, {E, G}
c	63.9	C	CH	{E, G}, {F, N}, {H, R}, O
d	60.0	-	C	C, {F, N}, {H, R}, {K, M}, O
e	50.8	I	CH	{B}, D, {H, R}, {J, L}, {K, M}
f	48.9	D	CH	{A, B}, {E, G}, I, {J, L}, {K, M}, P, Q
g	39.9	J, L	CH_2	{A, B}, D, P, Q
h	39.3	H, R	CH_2	C, I, {K, M}, O
i	34.2	-	C	D (weak), I, {J, L}, P, Q
j	30.4	F, N	CH_2	C, {E, G}
k	30.1	Q	CH_3	I, {J, L}, Q
l	29.9	E, G	CH_2	{A, B}, D, {F, N}
m	27.4	K, M	CH_2	D, {H, R}, I, J
n	21.8	P	CH_3	I, {J, L}, R
o	17.2	O	CH_3	{H, R}

Table 3. HSQC and HMBC coupling data summary table for caryophyllene oxide.

* Braces indicate that either coupling to both diastereotopic protons in a methylene pair is present <u>or</u> coupling to a single proton in one such pair is visible on HMBC.

Methods of Analysis and Assignment:

1D NMR Methods

1. **^{1}H NMR** (Appendix 1)
   a. 24 protons observed
   b. potential entry point: 2 doublets (H_A, 4.97 ppm; H_B, 4.86) with small coupling in alkene region - *exo-methylene*?
   c. potential entry point: 3 methyl singlets (H_O, 1.20 ppm; H_P, 1.00 ppm; H_Q, Q)
   d. H_O and H_P peak strength slightly less than H_Q – some small couplings for these peaks?
   e. peaks between 2 and 3 ppm - somewhat downfield alkyl signals - presence of electronegative heteroatom?
   f. no aromatic protons

2. **^{13}C NMR** (Appendix 2)
   a. 15 carbons
   b. 2 plausible alkene peaks (C_a, 152.0 ppm; C_b, 113.0 ppm), one of which is weak - an internal sp^2 carbon? agrees with *exo*-methylene suggestion (see 1.1.a).
   c. 3 more weak/moderately weak carbon peaks (C_d, 60.0 ppm; C_e, 50.8 ppm, C_i, 34.2 ppm) - 3 quaternary centers?
   d. 2 peaks in region ~60 ppm - possible carbinol carbons? - agrees with suggestion of electronegative atom (see 1.1.d).
   e. no carbonyl peaks
   f. DEPT may be necessary - 3 very close peaks (C_j, 30.4 ppm; C_k, 30.1 ppm, C_l, 29.9 ppm)

3. **DEPT** (Appendix 3)
   a. resolves confusion in region ~30 ppm - C_j and C_l are likely methyls (strong signals, positive phasing); C_k is methylene (negative phasing)

4. **Tentative Conclusions from 1D**
   a. partial molecule formula - $C_{15}H_{24}$
   b. even number of H's would require even number of N's, or any number of O's
   c. if oxygens present, IHD = 15 - 12 + 1 = 4; one alkene implied by *exo*-methylene; no carbonyls or other alkenes present suggests that 3 rings may exist

2D NMR Methods:

1. **HSQC** (Appendix 4) – see spectra for specific assignments of H_A-H_R and C_a-C_o
   a. 3 methyls, 6 methylenes, 3 methines, and 3 quaternary centers total
   b. further confirmation of *exo*-methylene – H_A and H_B on same carbon (C_b)
   c. H_C, H_D, and H_I confirmed as methine H's, while several isolated peaks with

integration of 1(for H_E, H_F, H_M, and H_N) are in fact diastereotopic methylene H's

d. carbons ~ 30 ppm can be cleared up completely – definitely 2 methylenes and a methyl

e. H_R, an apparent multiplet partially buried under H_Q at 0.96 ppm, is definitely not an impurity but rather a methylene H on C_h, along with H_H – shifts are very well separated (0.96 and 2.09 ppm), implying some interesting electronic effects at work

f. assumed quaternary C's (C_d, C_e, and C_i) confirmed as such – no crosspeaks

2. **HMBC** (Appendix 5) – *please note:* (#) after each entry below corresponds to a structure in Figure 1 on next page, showing gradual solving of connectivity.

a. The *exo*-methylene carbon (C_b) is coupled to protons on two separate carbons, C_f and C_l → indicates protons *alpha* to the alkene functionality (**1**).

b. C_l, aside from protons on C_b and C_f, is also coupled to protons C_j, which are not coupled to the C_f nucleus (**2**).

c. C_j is only coupled to one other proton than those previously identified: H_C on C_c (**3**).

d. Other protons coupled to C_c include those found on C_h and C_o. Additionally, C_d, a quaternary center, is coupled to protons on C_j, C_c, C_h, and C_o along with protons on another carbon, C_m. Since the connectivity of the C_j-C_c fragments has been established, the position of C_d can be established (**4**).

e. With this information, C_o can be connected to either C_c (**5a**) or C_d (**5b**). However, the fact that C_o is only coupling to protons on C_h, which couple to C_d but not C_c eliminates the possibility of **5a**. This also alludes to C_h-C_d connectivity (**6**).

f. C_h is additionally coupled to protons on C_e and C_m. However, the fact that C_e is coupled to all the same protons as C_m but some additional protons, *including the protons on C_f and C_b*, not only leads to the establishment of C_e and C_m connectivity, but evidences the closure of a **nine-membered ring**. This is supported by the coupling of alkenyl C_a, which couples to the proton of C_e (**7**).

g. The four "unconnected" carbons remaining (C_g, C_i, C_k, and C_n) can only adopt one structural motif. Because C_k and C_n both contain methyl singlets in the ^{1}H NMR, and C_i is a quaternary carbon, **8** is the only possible fragment. All protons on these carbons couple to one another, supporting this hypothesis.

h. The only question that remains is how fragment **8** is attached to fragment **7**: either **9a** or **9b**. Either way, a **four-membered ring** is necessary. The fact that C_g couples to protons on C_f but not C_e, suggests **9b** over **9a**.

i. The current [7.2.0]bicycloundecane core accounts for all the carbons but accounts for only 3 degrees of hydrogen deficiency – not the 4 suggested by our tentative molecular formula. Additionally, all carbons currently adhere to the octet rule except C_d and C_c. This information, combined with the fact that C_c and C_d along with their bonded hydrogens are considerably

deshielded leads to the establishment of an **epoxide** functionality across this C-C bond, establishing the full connectivity of the unknown (**10**).
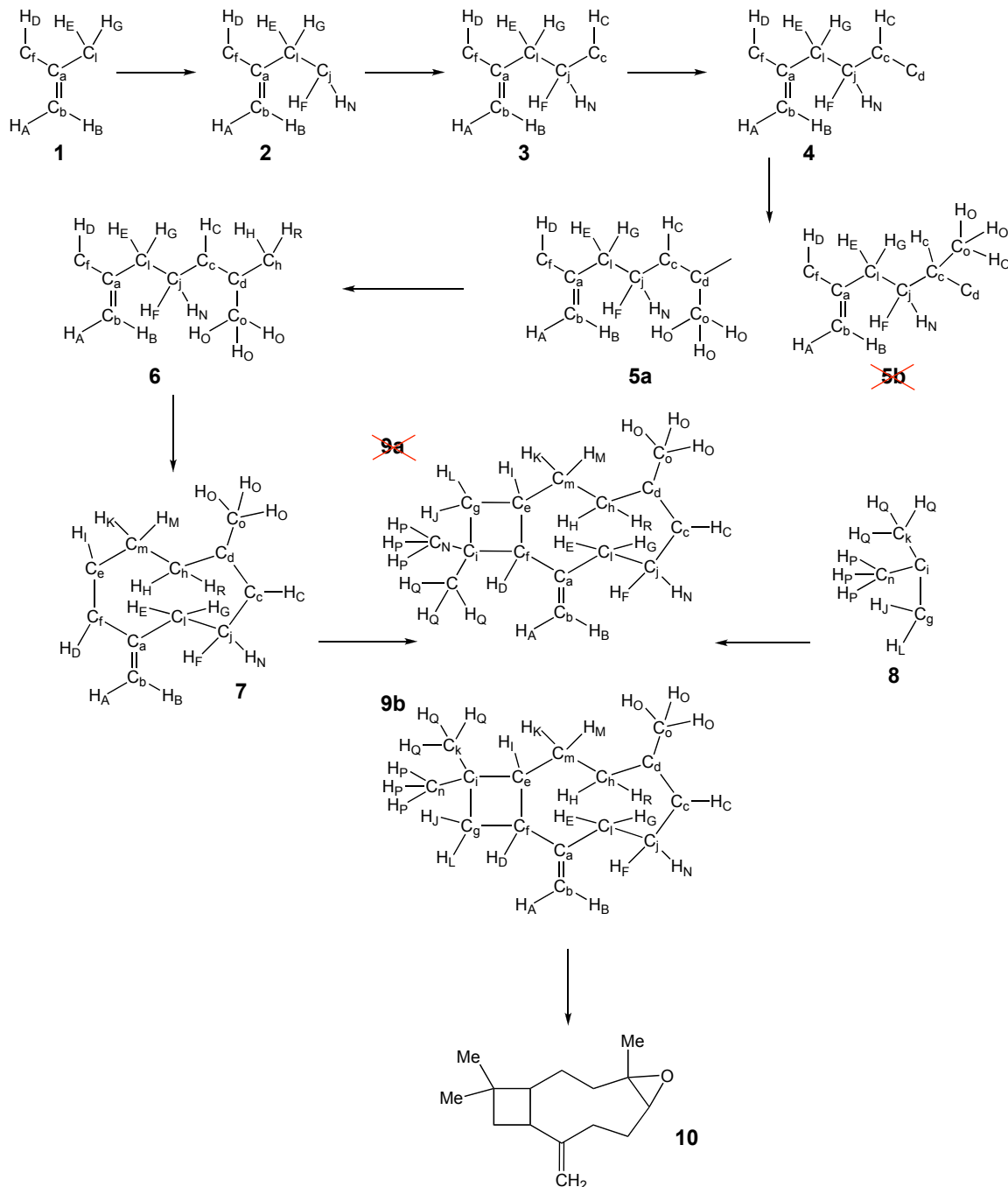
**Figure 1.** Logical assembly of structure based on HMBC data.

With connectivity established it becomes convenient to abandon the H_A-H_R and C_a-C_o naming system and switch to numbering carbons on our structure:
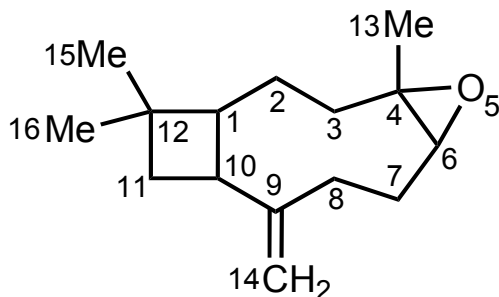
**Figure 2.** Structure showing connectivity of unknown compound.

3. **gCOSY** (Appendix 6)
   a. Confirms connectivity established by HMBC. Of particular note is that there are two nearly isolated spin systems apparent on the gCOSY – one can "chain-walk" from strong coupling to strong coupling from the 2 H_{11}'s through H_{10}, to H_1, to both H_2's, and so on down to the 3 methyl protons on C_{13}, and similarly one sees strong couplings among H_6-H_8, but there is only weak coupling between the 2 H_8's and one of the 2 H_{14}'s, and there is similarly weak coupling between H_6 and H_{13}, which suggests that these are not ^3J but rather longer-range couplings and corroborates our structure.
   b. can be used to determine relative stereochemistry. We assume arbitrarily that H_1 is up and that C_1's stereochemical assignment is then R:
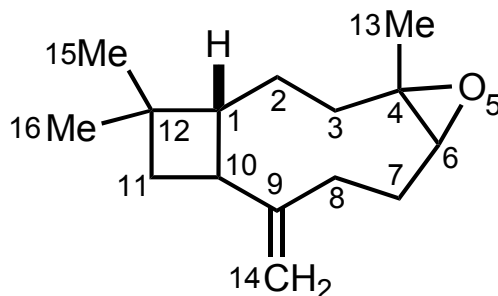

   c. assuming for now that all methylenes around 9-membered ring will rotate into a roughly staggered conformation to minimize energy, we then guess that H_1 will couple more strongly to H_{2R}, which will be *anti* to H_1 whereas H_{2S} is likely *syn*. gCOSY shows H_1 coupling more strongly to peak at 1.42 than to peak at 1.65 ppm, implying that 1.42 is H_{2R}:

1.65 1.42
S R 13Me
H

15Me

16Me 12 1 2 3 4 O5
11 10 9 8 7 6
14CH₂
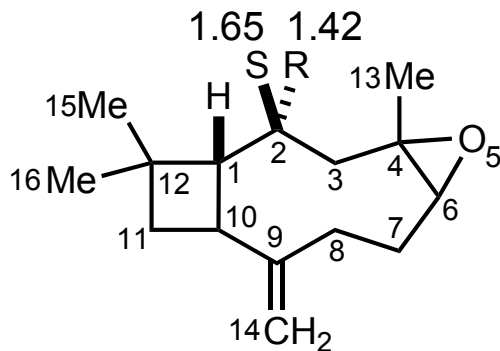
d. H_{2R} couples more strongly to one H_3 (2.09 ppm) than to the other (0.96); again, stronger coupling probably to anti, so 2.09 is likely H_{3R} and 0.96 is H_{3S}.

e. H_{3R} is far downfield compared to H_{3S} – the effect of interaction with the epoxide O? We guess that deshielding is through-space effect and O is up, closer to H_{3R} than H_{3S}, which puts C_{13} down and makes C_4 *R*:

13Me
H
15Me

16Me 12 1 2 3 4 O5
11 10 9 8 7 6
14CH₂
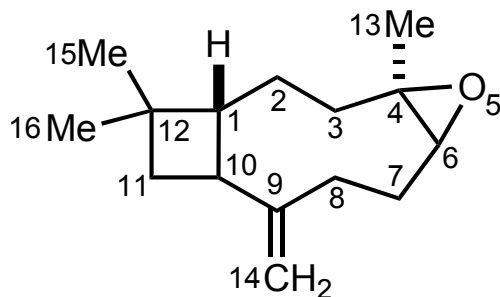
f. H_6 couples with H_{13}. This is a ^4J and therefore probably a W-coupling, which puts H_6 up and makes C_6 *R*:

13Me
H
15Me

16Me 12 1 2 3 4 O5
11 10 9 8 7 6
14CH₂
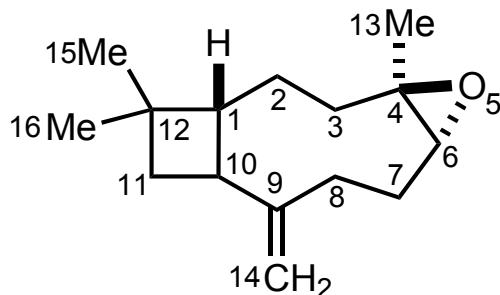
g. H_6 couples about equally with both H_7's (see volume integrals). However, coupling constants observed on 1D ^1H are clearly very different, with one J value ~11 Hz and the other ~4 Hz. 4 Hz is more consistent with a dihedral angle of 60°, while 11 Hz is more consistent with ~180°. Visual inspection of the 1D ^1H shows wider splitting only in H_7 at 1.32 ppm, implying that this proton is *anti* and theforefore H_{7R}, while the other H_7 at 2.25 ppm has nearly constant coupling constants (~4 Hz) and is therefore more likely *syn* and H_{7S}.

h. H_{7S} couples more strongly with H_8 at 2.11, so 2.11 anti, so H_{8S}, making 2.34 H_{8R}.

i. H_{10} has strong coupling with H_1, implying trans ring junction and making C_{10}

S:
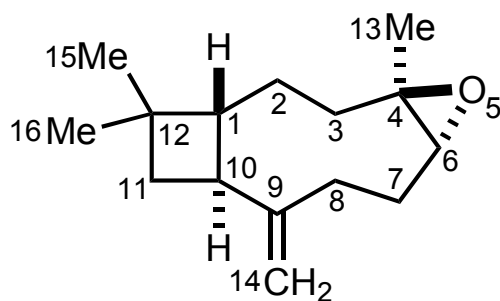
j. H_{10} couples more strongly to H_{11} at 1.68, so H_{11R} (anti), making 1.61 H_{11S}.

k. H_{11S} has crosspeak with H_{15}, implying W-coupling. H_{11S} is down, suggesting that C_{15} is up. C_{16} is likely down:
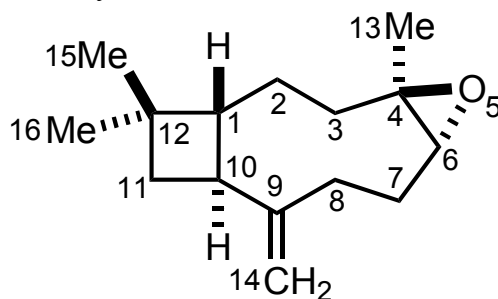
Confirmation of Structure, Including Stereochemistry
- Molecular modeling in Spartan and Chem3D of proposed relative stereochemistry shows bond angles reasonably consistent with staggered assumption made in COSY analysis. Also, lone pair on epoxide O is appropriately positioned for interaction with H_{3R}.
- (-)-caryophyllene oxide, a natural product derived from chamchwi, a flowering plant, happens to have the same absolute stereochemistry as the structure we propose. It is commercially available from Aldrich. The enantiomer does not appear to be commercially available.
- Comparison by brief inspection with 1D ^1H spectra from Aldrich shows a good match.
- Thus we propose that our unknown is, indeed, (-)-caryophyllene oxide.