

# Intensity Revival of Weak Symmetric Infrared Band is Possible in Ubiquinone Molecules through the Asymmetric Site-specific Isotope Labeling

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**Abstract:** Modifications of Infrared (IR) spectral bands of ubiquinone molecule ( $UQ_n$ ) upon site-specific  $^{13}C$  labeling at the  $C_5$  or  $C_6$  position are studied in  $CCl_4$  using Gaussian 03. Polarizable continuum model (PCM) has been used to optimize the  $UQ_1$  molecule in solvent. The unlabeled neutral ubiquinone molecule consists of three intense IR bands in the frequency region between  $1700\text{ cm}^{-1}$  to  $1550\text{ cm}^{-1}$ . The symmetric fourth band in this spectral region does not appear in the spectrum because of very weak intensity. However, site-specific  $^{13}C$  labeling at  $C_5$  or  $C_6$  position removes the molecular symmetry and hence there appear four equivalent IR bands in the spectral region thus considered. This observation explains why there appears an extra spectral band in the experimental spectra observed by Brudler, R. et al [1].

**Keywords:** Quinone, ubiquinone, symmetric mode, asymmetric mode, IR spectrum, isotope labeling, vibration, modes of vibration.

## 1. INTRODUCTION:

Infrared spectroscopy is a tool which deals with the molecular vibrations in which corresponding molecule changes its dipole moment [2, 3]. A molecule is chemically influenced in a material from the atoms or group of atoms around it. Its bonding strength depends on the type of bonding, charge on the molecule and electronic environment around the molecule. Studying vibrational frequency and intensity of spectral bands of the infrared spectra digs up state of the molecule and its environment [3]. However, due to the interaction with neighbors the composite spectrum of molecules in vivo is very complex. Isotope labeling is one of the methods of separating contribution of a particular group of vibration in the composite spectrum [1-5]. Frequency of vibration is generally decreases upon isotope labeling of some atom which takes part in the vibration when it is replaced with a heavier isotope. The decrease of frequency upon isotope labeling also changes the extent and type of coupling between two functional groups which are vibrating with nearly equal frequencies. It is of physical importance to study what happens to the infrared weak symmetric band upon asymmetric isotope labeling. Ubiquinone is one of the most studied and useful molecules in this field of research.

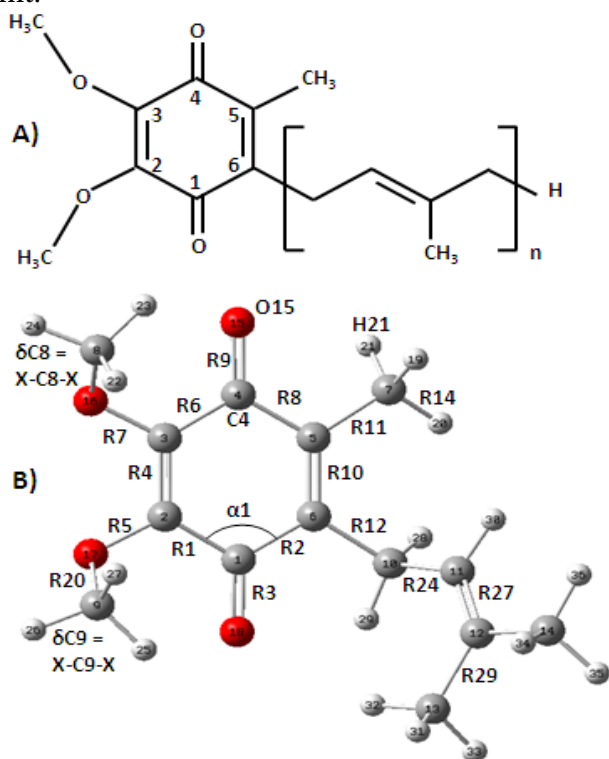
Ubiquinones ( $UQ_n$ : 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone) which play an important role in biological electron and proton transfer processes that occur in both respiration and photosynthesis [6] have intense IR spectral bands in the frequency region between  $1700$  to  $1550\text{ cm}^{-1}$  in the neutral state in solvent and in the purple bacteria reaction centers [1, 4, 5, 7-15]. These IR bands are originated from the stretching vibration of  $C=O$  and  $C=C$  bonds of ubiquinone. Experimental spectra of ubiquinone in the solvent or  $Q_A$  binding site of purple bacteria were taken from ubiquinone having long isoprene tail (10 units) [1, 4]. However, calculated spectra were generated from the ubiquinone with single isoprene unit [10, 16] because of negligible contribution of  $C=C$  groups of the tail and also the computational economy. To explore the environmental effect on the ubiquinone in the purple bacteria reaction center, isotope edited IR double difference spectra were measured in vivo [1, 4]. All of the experiments conform three major neutral ubiquinone IR bands which are down shifted in the purple bacteria reaction center compared to the band positions in the solvents [1, 4, 7]. However, IR spectra of neutral ubiquinones which are site-specific  $^{13}C$  labeled at the  $C_5$  or  $C_6$  positions ( $C_3$  or  $C_2$  positions in author's numbering) contains four distinct bands in  $CCl_4$  [1].

It is claimed that the extra band at  $1638\text{ cm}^{-1}$  is due to the band shifting from the  $1650\text{ cm}^{-1}$  band on site-specific  $^{13}\text{C}$  labeled at the  $\text{C}_5$  or  $\text{C}_6$  positions. However, origin of the extra band at  $1638\text{ cm}^{-1}$  has not clearly been discussed yet [1]. We have observed that the new band at  $1638\text{ cm}^{-1}$  of site-specific  $^{13}\text{C}$  labeled neutral ubiquinone in  $\text{CCl}_4$  is due to the infrared intensity redistribution among  $\text{C}=\text{C}$  and  $\text{C}=\text{O}$  bands up on asymmetric isotope labeling of the molecule. This finding may also be valuable to explore the origin of various bands of ubiquinone difference spectrum in the purple bacteria reaction centers [1, 4].

## 2. MATERIALS AND METHODS

### UQ structure and numbering

Figure 1 shows the structure and numbering scheme for  $\text{UQ}_n$ . The subscript,  $n$ , refers to the number of isoprene units in the chain at position 6. The ubiquinone molecule has two carbonyl groups at  $\text{C}_1$  and  $\text{C}_4$  positions, two methoxy groups at positions at  $\text{C}_2$  and  $\text{C}_3$ , a methyl group at  $\text{C}_5$  position and an isoprene unit at  $\text{C}_6$  position. In this work we have removed the part of the tail after the first isoprene unit.



**Figure 1** (A) Structure and numbering scheme for  $\text{UQ}_n$ . The subscript  $n$  refers to the number of isoprene units associated with the chain attached at position 6. (B) Energy minimized  $\text{UQ}_1$  model used in calculations. Atom numbering scheme and brief representations of internal coordinates are also shown. R,  $\alpha$  and  $\delta$  represent for bond stretching, angle bending and combination of angle bending at a vertex atom respectively.

### 3. MOLECULAR OPTIMIZATION

Molecular geometry optimizations and harmonic vibrational frequency calculations of ubiquinone molecules were performed using hybrid DFT methods, employing the B3LYP functional and the 6-31+G(d) method within Gaussian 03 [17]. Minimum energy conformations of ubiquinone molecules were explored by calculating single point energy for  $10^6$  steps of 2- and 3- methoxy dihedral angles. The constrained minimum energy conformers were further optimized in dichloromethane using PCM method [18] without any constrained.

### 4. SPECTRAL CALCULATIONS OF UNLABELED AND SITE SPECIFIC ISOTOPE LABELED UBIQUINONE MOLECULE

Vibrational frequency calculations of optimized ubiquinone molecule were performed at DFT/B3LYP/6-31+G(d) level for both unlabeled molecule and  $^{13}\text{C}$  labeled molecule at  $\text{C}_5$  or  $\text{C}_6$  carbon position of ubiquinone molecule. Infrared spectra were generated from the Gaussian output files of frequency calculations using Gauss View 5.0 at  $4\text{ cm}^{-1}$  resolution. Potential energy distribution is calculated using gar2ped program [19].

### 5. RESULTS

Ubiquinone has two flexible methoxy groups and a long isoprene tail. Length and orientation of isoprene units does not affect on the vibrational frequency provided that the molecule contains at least one isoprene [10, 15]. However, orientations of methoxy groups have vital roles on the vibrational frequency, modes of vibration, and hence the shape of spectrum. Thus, to extract realistic absorption spectra from possible structures at room temperature, all possible minimum energy conformers of neutral ubiquinone molecules should be considered. The minimum

energy conformers of neutral ubiquinone molecules in  $\text{CCl}_4$  have been explored by calculating single point energies at the step of  $10^\circ$  dihedral angles of the methoxy groups with respect to the ubiquinone head. Eight minimum energy conformers labeled A, B, C, D, E, F, G and H have been observed [10]. The Boltzmann weight factors [20] for conformers in the composite spectrum were calculated from the calculated energy difference of the neutral ubiquinone molecules at room temperature.

The calculated ground state energy, energy difference, Boltzmann factors and dihedral angles of methoxy groups of the conformers at room temperature are shown in Table 1. Table 1 shows that conformers A, B, C and D have relatively lesser energies than that of the remaining conformers E, F, G and H. Among conformers four low energy conformers C has the lowest energy at room temperature. Thus, the energy differences were calculated with respect to the energy of conformer C. Boltzmann factors were calculated at room temperature 298 K.

**Table 1:** Calculated optimized ground state energies, dihedral angles of methoxy groups and corresponding Boltzmann factors of 8 neutral  $\text{UQ}_1$  conformers in  $\text{CCl}_4$ . At 298 K,  $k_B T \sim 0.592$  kcal/mol.

Ubiquinone Conformers	2-methoxy dihedralangle	2-methoxy dihedralangle	Energy (kcal/ Mole)	$\Delta E$ (kcal/ Mole)	Boltzmann Factors
A	-9.3	120.9	-530364.8585	0.0788	0.2082
B	10.7	-121.7	-530364.8912	0.0461	0.2201
C	-122.5	10.4	-530364.9373	0	0.2379
D	117.2	-5.2	-530364.8081	0.1292	0.1912
E	118.4	123.3	-530363.887	1.0502	0.0404
F	-144.5	141.9	-530363.7736	1.1637	0.0333
G	-123.8	-122.7	-530364.0127	0.9245	0.0499
H	139.4	-142.3	-530363.4424	1.4949	0.0190

Calculated vibrational frequencies, infrared intensities, and potential energy distribution (ped) of the  $\text{C}=\text{O}$  and  $\text{C}=\text{C}$  stretching modes of vibration of unlabeled,  $^{13}\text{C}_5$  and  $^{13}\text{C}_6$  isotope labeled neutral ubiquinone one ( $\text{UQ}_1$ ) conformers in  $\text{CCl}_4$  are given in Table 2. The tail  $\text{C}=\text{C}$  stretching mode is not considered here because this band has very weak IR intensity and also it is not coupled with other modes of vibrations. Corresponding composite spectra generated from weighing spectra of eight conformers with Boltzmann factors of unlabeled,  $^{13}\text{C}_5$  and  $^{13}\text{C}_6$  isotope labeled neutral  $\text{UQ}_1$  in  $\text{CCl}_4$  are shown in Figure 2. Figure 2 shows that the unlabeled neutral  $\text{UQ}_1$  has three intense IR bands at 1676, 1650 and 1604  $\text{cm}^{-1}$ . The 1676  $\text{cm}^{-1}$  band is mainly ( $\sim 80\%$

ped) due to  $\text{C}_1=\text{O}$  stretching vibration in conformers A and B and  $\text{C}_4=\text{O}$  stretching vibration in conformers C and D. The 1650  $\text{cm}^{-1}$  band is due to remaining  $\text{C}=\text{O}$  stretching modes of vibrations coupled with  $\text{C}=\text{C}$  stretching modes of vibration. The third band at 1604  $\text{cm}^{-1}$  is due to the asymmetric stretching modes of vibration coupled with  $\text{C}=\text{O}$  modes which give rise to 1650  $\text{cm}^{-1}$  band. The symmetric  $\text{C}=\text{C}$  stretching modes nearly at 1653  $\text{cm}^{-1}$  have very weak IR intensities and is not seen in the spectrum. Because of higher energies and lesser Boltzmann factors, conformers E, F, G and H have very small contribution to the composite spectrum and hence they are not considered in the discussions here.

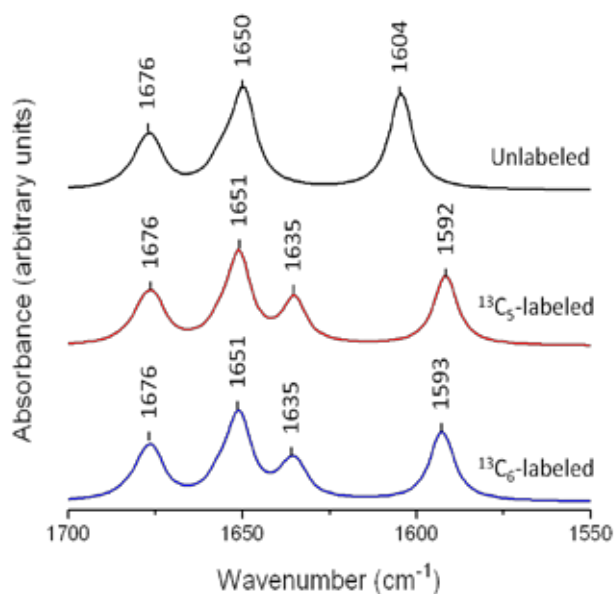
**Table 2.** Calculated vibrational frequencies (cm<sup>-1</sup>), infrared intensities (km/mole) in parenthesis and potential energy distribution (%) of unlabeled, <sup>13</sup>C<sub>5</sub> and <sup>13</sup>C<sub>6</sub> isotope labeled neutral ubiquinone one in CCl<sub>4</sub>.

Calculated Ubiquinone Conformers and IR Bands in CCl <sub>4</sub>				
Conf.	I	II	III	IV
Unlabeled Ubiquinone				
A	1678(224) C <sub>1</sub> =O (83%)	1650(414) C <sub>4</sub> =O (69%), C <sub>5</sub> =C <sub>6</sub> (7%)	1653(31) C <sub>2</sub> =C <sub>3</sub> (22%), C <sub>5</sub> =C <sub>6</sub> (42%)	1604(379) C <sub>2</sub> =C <sub>3</sub> (41%), - C <sub>5</sub> =C <sub>6</sub> (19%), C <sub>4</sub> =O (7%)
B	1679(220) C <sub>1</sub> =O (80%)	1650(418) C <sub>4</sub> =O (68%), C <sub>5</sub> =C <sub>6</sub> (8%)	1653(34) C <sub>2</sub> =C <sub>3</sub> (22%), C <sub>5</sub> =C <sub>6</sub> (41%)	1604(377) C <sub>2</sub> =C <sub>3</sub> (41%), - C <sub>5</sub> =C <sub>6</sub> (19%), C <sub>4</sub> =O (6%)
C	1676(308) C <sub>4</sub> =O (80%)	1649(316) C <sub>1</sub> =O (51%), C <sub>5</sub> =C <sub>6</sub> (20%)	1654(69) C <sub>2</sub> =C <sub>3</sub> (21%), C <sub>5</sub> =C <sub>6</sub> (29%), - C <sub>1</sub> =O (24%)	1604(392) C <sub>2</sub> =C <sub>3</sub> (42%), - C <sub>5</sub> =C <sub>6</sub> (18%)
D	1675(309) C <sub>4</sub> =O (80%)	1648(309) C <sub>1</sub> =O (48%), C <sub>5</sub> =C <sub>6</sub> (21%)	1654(74) C <sub>2</sub> =C <sub>3</sub> (22%), C <sub>5</sub> =C <sub>6</sub> (27%), - C <sub>1</sub> =O (26%)	1605(400) C <sub>2</sub> =C <sub>3</sub> (41%), - C <sub>5</sub> =C <sub>6</sub> (19%)
E	1664(7) C <sub>1</sub> =O (47%), C <sub>4</sub> =O (31%)	1657(653) C <sub>1</sub> =O (35%), - C <sub>4</sub> =O (43%)	1651(65) C <sub>2</sub> =C <sub>3</sub> (28%), C <sub>5</sub> =C <sub>6</sub> (36%)	1607(211) C <sub>2</sub> =C <sub>3</sub> (40%), - C <sub>5</sub> =C <sub>6</sub> (29%)
F	1661(14) C <sub>1</sub> =O (36%), C <sub>4</sub> =O (23%), C <sub>5</sub> =C <sub>6</sub> (12%)	1650(639) C <sub>1</sub> =O (18%), - C <sub>4</sub> =O (58%)	1653(111) C <sub>2</sub> =C <sub>3</sub> (16%), C <sub>5</sub> =C <sub>6</sub> (33%), - C <sub>1</sub> =O (28%)	1607(251) C <sub>2</sub> =C <sub>3</sub> (46%), - C <sub>5</sub> =C <sub>6</sub> (20%)
G	1664(7) C <sub>1</sub> =O (46%), C <sub>4</sub> =O (29%)	1656(654) C <sub>1</sub> =O (35%), - C <sub>4</sub> =O (44%)	1651(51) C <sub>2</sub> =C <sub>3</sub> (26%), C <sub>5</sub> =C <sub>6</sub> (38%), - C <sub>4</sub> =O (8%)	1606(216) C <sub>2</sub> =C <sub>3</sub> (41%), - C <sub>5</sub> =C <sub>6</sub> (27%)
H	1660(7) C <sub>1</sub> =O (28%), C <sub>4</sub> =O (34%), C <sub>5</sub> =C <sub>6</sub> (10%)	1649(573) -C <sub>1</sub> =O (20%), C <sub>4</sub> =O (46%), - C <sub>5</sub> =C <sub>6</sub> (10%)	1652(177) C <sub>2</sub> =C <sub>3</sub> (15%), C <sub>5</sub> =C <sub>6</sub> (27%), - C <sub>1</sub> =O (28%)	1602(252) C <sub>2</sub> =C <sub>3</sub> (45%), - C <sub>5</sub> =C <sub>6</sub> (22%)
Calc.	1676	1650	-	1604
Expt.	1666	1650	-	1611
<sup>13</sup> C labeled at C <sub>5</sub> position				
A	1678(226) C <sub>1</sub> =O (81%)	1650(357) C <sub>4</sub> =O (71%), -C <sub>2</sub> =C <sub>3</sub> (11%)	1635(200) C <sub>2</sub> =C <sub>3</sub> (28%), C <sub>5</sub> =C <sub>6</sub> (24%), C <sub>4</sub> =O (11%)	1592(266) C <sub>2</sub> =C <sub>3</sub> (24%), - C <sub>5</sub> =C <sub>6</sub> (41%)
B	1679(221) C <sub>1</sub> =O (80%)	1651(365) C <sub>4</sub> =O (71%), -C <sub>2</sub> =C <sub>3</sub> (11%)	1635(197) C <sub>2</sub> =C <sub>3</sub> (29%), C <sub>5</sub> =C <sub>6</sub> (24%), C <sub>4</sub> =O (10%)	1592(266) C <sub>2</sub> =C <sub>3</sub> (24%), - C <sub>5</sub> =C <sub>6</sub> (41%)
C	1675(315) C <sub>4</sub> =O (82%)	1651(315) C <sub>1</sub> =O (74%), -C <sub>2</sub> =C <sub>3</sub> (8%)	1635(163) C <sub>2</sub> =C <sub>3</sub> (30%), C <sub>5</sub> =C <sub>6</sub> (25%)	1591(294) C <sub>2</sub> =C <sub>3</sub> (24%), - C <sub>5</sub> =C <sub>6</sub> (40%)

D	1675(315) C <sub>4</sub> =O (82%)	1651(296) C <sub>1</sub> =O (72%), -C <sub>2</sub> =C <sub>3</sub> (10%)	1635(187) C <sub>2</sub> =C <sub>3</sub> (29%), C <sub>5</sub> =C <sub>6</sub> (24%), C <sub>1</sub> =O (9%)	1591(295) C <sub>2</sub> =C <sub>3</sub> (23%), - C <sub>5</sub> =C <sub>6</sub> (41%)
E	1664(12) C <sub>1</sub> =O (54%), C <sub>4</sub> =O (29%)	1657(694) C <sub>1</sub> =O (29%), - C <sub>4</sub> =O (54%)	1639(74) C <sub>2</sub> =C <sub>3</sub> (49%), C <sub>5</sub> =C <sub>6</sub> (18%)	1590(157) C <sub>2</sub> =C <sub>3</sub> (19%), - C <sub>5</sub> =C <sub>6</sub> (50%)
F	1660(25) C <sub>1</sub> =O (55%), C <sub>4</sub> =O (26%)	1650(726) C <sub>1</sub> =O (27%), - C <sub>4</sub> =O (56%)	1638(66) C <sub>2</sub> =C <sub>3</sub> (39%), C <sub>5</sub> =C <sub>6</sub> (26%)	1591(201) C <sub>2</sub> =C <sub>3</sub> (27%), - C <sub>5</sub> =C <sub>6</sub> (41%)
G	1663(13) C <sub>1</sub> =O (54%), C <sub>4</sub> =O (28%)	1655(684) C <sub>1</sub> =O (29%), - C <sub>4</sub> =O (55%)	1638(70) C <sub>2</sub> =C <sub>3</sub> (47%), C <sub>5</sub> =C <sub>6</sub> (19%)	1590(163) C <sub>2</sub> =C <sub>3</sub> (21%), - C <sub>5</sub> =C <sub>6</sub> (48%)
H	1659(4) C <sub>1</sub> =O (43%), C <sub>4</sub> =O (39%)	1650(740) C <sub>1</sub> =O (39%), - C <sub>4</sub> =O (44%)	1636(67) C <sub>2</sub> =C <sub>3</sub> (41%), C <sub>5</sub> =C <sub>6</sub> (25%)	1589(199) C <sub>2</sub> =C <sub>3</sub> (26%), - C <sub>5</sub> =C <sub>6</sub> (44%)
Calc.	1676	1651	1635	1592
Expt.	1666	1650	1638	1596
<sup>13</sup> C labeled at C <sub>6</sub> position				
A	1678(221) C <sub>1</sub> =O (81%)	1650(394) C <sub>4</sub> =O (75%), -C <sub>2</sub> =C <sub>3</sub> (7%)	1635(138) C <sub>2</sub> =C <sub>3</sub> (31%), C <sub>5</sub> =C <sub>6</sub> (26%)	1592(290) C <sub>2</sub> =C <sub>3</sub> (25%), - C <sub>5</sub> =C <sub>6</sub> (39%)
B	1679(215) C <sub>1</sub> =O (80%)	1650(402) C <sub>4</sub> =O (75%), -C <sub>2</sub> =C <sub>3</sub> (6%)	1637(135) C <sub>2</sub> =C <sub>3</sub> (31%), C <sub>5</sub> =C <sub>6</sub> (26%)	1592(289) C <sub>2</sub> =C <sub>3</sub> (25%), - C <sub>5</sub> =C <sub>6</sub> (39%)
C	1675(315) C <sub>4</sub> =O (81%)	1652(274) C <sub>1</sub> =O (70%), -C <sub>2</sub> =C <sub>3</sub> (11%)	1634(211) C <sub>2</sub> =C <sub>3</sub> (26%), C <sub>5</sub> =C <sub>6</sub> (24%), C <sub>1</sub> =O (12%)	1594(284) C <sub>2</sub> =C <sub>3</sub> (26%), - C <sub>5</sub> =C <sub>6</sub> (39%)
D	1675(316) C <sub>4</sub> =O (81%)	1652(255) C <sub>1</sub> =O (67%), -C <sub>2</sub> =C <sub>3</sub> (13%)	1634(237) C <sub>2</sub> =C <sub>3</sub> (26%), C <sub>5</sub> =C <sub>6</sub> (23%), C <sub>1</sub> =O (15%)	1594(284) C <sub>2</sub> =C <sub>3</sub> (25%), - C <sub>5</sub> =C <sub>6</sub> (40%)
E	1663(9) C <sub>1</sub> =O (51%), C <sub>4</sub> =O (32%)	1657(681) C <sub>1</sub> =O (32%), - C <sub>4</sub> =O (49%)	1639(88) C <sub>2</sub> =C <sub>3</sub> (47%), C <sub>5</sub> =C <sub>6</sub> (18%)	1591(158) C <sub>2</sub> =C <sub>3</sub> (20%), - C <sub>5</sub> =C <sub>6</sub> (49%)
F	1660(19) C <sub>1</sub> =O (51%), C <sub>4</sub> =O (30%)	1651(708) C <sub>1</sub> =O (31%), - C <sub>4</sub> =O (48%)	1638(85) C <sub>2</sub> =C <sub>3</sub> (38%), C <sub>5</sub> =C <sub>6</sub> (26%)	1591(204) C <sub>2</sub> =C <sub>3</sub> (28%), - C <sub>5</sub> =C <sub>6</sub> (39%)
G	1663(8) C <sub>1</sub> =O (51%), C <sub>4</sub> =O (32%)	1656(676) C <sub>1</sub> =O (32%), - C <sub>4</sub> =O (49%)	1638(79) C <sub>2</sub> =C <sub>3</sub> (46%), C <sub>5</sub> =C <sub>6</sub> (20%)	1591(165) C <sub>2</sub> =C <sub>3</sub> (22%), - C <sub>5</sub> =C <sub>6</sub> (47%)
H	1659(9) C <sub>1</sub> =O (38%), C <sub>4</sub> =O (43%)	1650(704) C <sub>1</sub> =O (44%), - C <sub>4</sub> =O (36%)	1637(95) C <sub>2</sub> =C <sub>3</sub> (39%), C <sub>5</sub> =C <sub>6</sub> (24%)	1590(201) C <sub>2</sub> =C <sub>3</sub> (27%), - C <sub>5</sub> =C <sub>6</sub> (41%)

Calc.	1676	1651	1635	1592
Expt.	1666	1650	1638	1596

Note: The calculated frequencies are scaled by 0.973. Experimental values were taken from reference [1]. The minus sign means the out of phase vibration. (Calc. = Calculated, Expt. = Experimental)



**Figure 2.** Calculated IR spectra of unlabeled,  $^{13}\text{C}_5$  and  $^{13}\text{C}_6$  isotope labeled neutral ubiquinone one in  $\text{CCl}_4$ . Each spectrum is the weighted average spectra of eight conformers (A, B, C, D, E, F, G and H) using Boltzmann factors 0.2082, 0.2201, 0.2379, 0.1912, 0.0404, 0.0333, 0.0499 and 0.0190 respectively.

Upon site specific asymmetric  $^{13}\text{C}_5$  or  $^{13}\text{C}_6$  labeling both C=C symmetric and asymmetric modes downshifted by  $\sim 18$  and  $14 \text{ cm}^{-1}$  respectively. The C=C symmetric mode also gained much IR intensity upon asymmetric  $^{13}\text{C}$  labeling at  $\text{C}_5$  or  $\text{C}_6$  positions and as a result a new band appeared at  $1635 \text{ cm}^{-1}$  in the corresponding IR spectrum. However, the C=C asymmetric band loses some intensity. On the other hand, the C=C coupled C=O mode at  $1650 \text{ cm}^{-1}$  is up-shifted by  $\sim 1 \text{ cm}^{-1}$  and also lost some IR intensity.

## 6. DISCUSSIONS

Spectral modification of  $\text{UQ}_1$  in  $\text{CCl}_4$  upon site specific  $^{13}\text{C}$  labeling can be compared with the experiment carried out by R. Brudler et al. [1] on  $\text{UQ}_{10}$  in  $\text{CCl}_4$ . Their numbering is different from ours in such a way that the atom numbers  $\text{C}_2$  and  $\text{C}_3$  are same as our atom numbers  $\text{C}_6$  and  $\text{C}_5$ . They also observed three intense IR bands of unlabeled

neutral  $\text{UQ}_{10}$  in the spectral region between  $1700$  and  $1600 \text{ cm}^{-1}$  in  $\text{CCl}_4$  at  $1666$ ,  $1650$  and  $1611 \text{ cm}^{-1}$ . Upon site specific asymmetric  $^{13}\text{C}$  labeling at  $\text{C}_2$  or  $\text{C}_3$  positions (author's naming) they also observed four intense IR bands at  $1666$ ,  $1650$ ,  $1638$  and  $1596 \text{ cm}^{-1}$ . The unchanged values of the frequency and intensity of the highest frequency C=O stretching mode in the experiment carried by R. Brudler et al. is exactly revived in our calculations too. We have also observed decrease in IR intensity of  $1650 \text{ cm}^{-1}$  band upon  $^{13}\text{C}$  labeling at  $\text{C}_5$  or  $\text{C}_6$  positions (our naming) as the experimenters observed. Our observed result of  $\sim 12 \text{ cm}^{-1}$  wavenumber downshift of C=C asymmetric band is comparable with the  $15 \text{ cm}^{-1}$  downshift of C=C asymmetric band in the experiment of  $\text{UQ}_{10}$  in  $\text{CCl}_4$ .

The origin of new band at  $1638 \text{ cm}^{-1}$  upon asymmetric  $^{13}\text{C}$  labeling at  $\text{C}_2$  or  $\text{C}_3$  positions (their naming) was not clearly explained by the experimenters. However, they implied that this new band might be  $12 \text{ cm}^{-1}$  wavenumber shifted from  $1650 \text{ cm}^{-1}$  band. On the basis of the experimental data, they claimed that whatever the origin of the new band the symmetry of IR spectra of  $^{13}\text{C}$  labeled at  $\text{C}_2$  or  $\text{C}_3$  positions of  $\text{UQ}_{10}$  showed that these two positions were chemically equivalent. We have clearly seen that the slight change in the IR intensity and frequency upon asymmetric  $^{13}\text{C}$  labeling is due to the modification of the scheme of coupling between lower frequency C=O stretching vibration and C=C stretching vibrations. The frequency downshift and intensity change of the asymmetric C=C stretching vibrations are also isotope mass increase and some changes in the coupling scheme between low frequency C=O mode and C=C stretching modes of vibrations. Moreover, the asymmetric  $^{13}\text{C}$  labeling at  $\text{C}_2$  or  $\text{C}_3$  positions of neutral ubiquinone not only down-shifts the symmetric C=C stretching mode but also increases its IR intensity. The spectral modification observed in the calculations of vibrational frequencies of neutral

unlabeled and asymmetric  $^{13}\text{C}$  labeled ubiquinone molecule may also be useful to explain experimental spectral modifications observed in the ubiquinone spectra in the  $\text{Q}_\text{A}$  binding site of the purple bacteria reaction centers.

## 7. CONCLUSIONS

Infrared band intensity of very weak symmetric mode of vibration of symmetric molecules can be enhanced by asymmetric isotope labeling of participating atoms. This technique will be useful to explore positions of silent symmetric IR modes of the relevant molecules.

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