Importance of the Conformation of Methoxy Groups on the Vibrational and Electrochemical Properties of Ubiquinones

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On the basis of semiempirical calculations, the present study proposes a comprehensive interpretation of the crystallographic, vibrational, and electrochemical data on methoxy-substituted quinones, and in particular for ubiquinones, in terms of the orientation of the methoxy groups relative to the quinone ring plane. "Hindered" and "free" methoxy groups are considered depending on the presence or absence on the quinone ring of a bulky group in ortho position of the considered methoxy group, respectively. The free methoxy groups have their O−CH₃ bond in the quinone ring plane while the hindered methoxy groups cannot adopt this conformation and have their methyl group tilted out of the quinone ring plane. The electron donation of the methoxy is dependent on the orientation of the O−CH₃ bond and is maximum for a free methoxy group. This effect is revealed by the analysis of both electrochemical and IR data. An assignment of the ν(C=O) modes of the quinones bearing such groups is proposed. From electrochemical data in literature, a new coefficient σ_{para}, used in the Hammett equation, is determined for a hindered methoxy group ($\sigma_{\text{para}} = -0.07$ compared to -0.27 for a free methoxy group). In the specific and biologically important case of the bulky group being another methoxy group, such as in ubiquinones (2,3-dimethoxy-substituted 1,4-benzoquinones), two types of conformation have to be considered. In the first type (conformer A), one methoxy adopts the conformation of a free methoxy group and the second that of a hindered methoxy group. In the second type (conformer B), both methoxy groups adopt the conformation of a hindered methoxy group. Both conformers appear equiprobable within the precision of our semiempirical calculations and a low rotational barrier, compared to $k_{\text{B}}T$ at room temperature, is found between them. Only conformers A are encountered in crystals. Using specific ¹³C labeling, IR data show that conformers A are mostly encountered at room temperature in solution while a mixture of both conformers is present at low temperature. On the other hand, electrochemical data on these quinones are best interpreted as the reduction of conformers B. This is explained by the higher electron affinity of conformers B compared to conformers A and by the low rotational barrier between the two conformers. Taking into account IR data of ubiquinone in the bacterial photosynthetic reaction center of *Rhodobacter sphaeroides*, the 70 mV difference found in the redox potential of ubiquinone in the two quinone binding sites can be explained by a difference of orientation of the methoxy groups imposed by the protein. By selecting a different orientation of the methoxy groups in the two sites, the protein would thus tune the redox potential of the quinone present in each site.

Introduction

Quinones are important molecules in biology, where they notably play an essential role in oxido-reduction reactions, such as respiration and photosynthesis.[1] In particular, three classes of *p*-quinones are involved as key electron acceptors in the photosynthetic process: the menaquinones (2-methyl-3-poly-prenyl-1,4-naphthoquinone), the plastoquinones (2,3-dimethyl-5-polyprenyl-1,4-benzoquinone), and the ubiquinones (Q$_n$: 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone); see Figure 1. Compared to menaquinones and plastoquinones, ubiquinones exhibit an unusual behavior concerning their redox properties: while the redox potential of *p*-quinones has been related to the nature of their substituents (Hammett rule), it appears that some *p*-quinones which bear methoxy group(s), and notably the ubiquinones, deviate from this rule.[2−5] They all have in common the presence of a bulky group (*e.g.* methyl, boron, chlorine, methoxy) ortho to the methoxy group. For these quinones it has been proposed that the deviation from the Hammett rule is related to the orientation of the methoxy group relative to the quinone ring plane.[4−6] Crystal data and conformational energy calculations on various methoxy-substituted *p*-benzoquinones have shown that the orientation of a methoxy group, when it is free to rotate, is such that the oxygen to methyl carbon single bond (O−CH₃ bond) lies in the quinone ring plane, the methyl group pointing away from the proximal carbonyl group.[5−7] Due to steric hindrance, such a conformation is no longer possible when a bulky group is ortho to the methoxy group. In the case of *p*-benzoquinones bearing a methyl adjacent to a methoxy group, crystal data and results from calculations show that the O−CH₃ bond is tilted out of the quinone ring plane.[5] In crystal structures of 2,3-dimethoxy-substituted 1,4-benzoquinones, such as Q₀ (2,3-dimethoxy-5-methyl-1,4-benzoquinone) and 2,3-dimethoxy-substituted 1,4-naphthoquinones, one O−CH₃ bond is found to lie in the quinone ring plane while the second is tilted out of this plane.[8−10] In contrast, INDO and CNDO calculations performed

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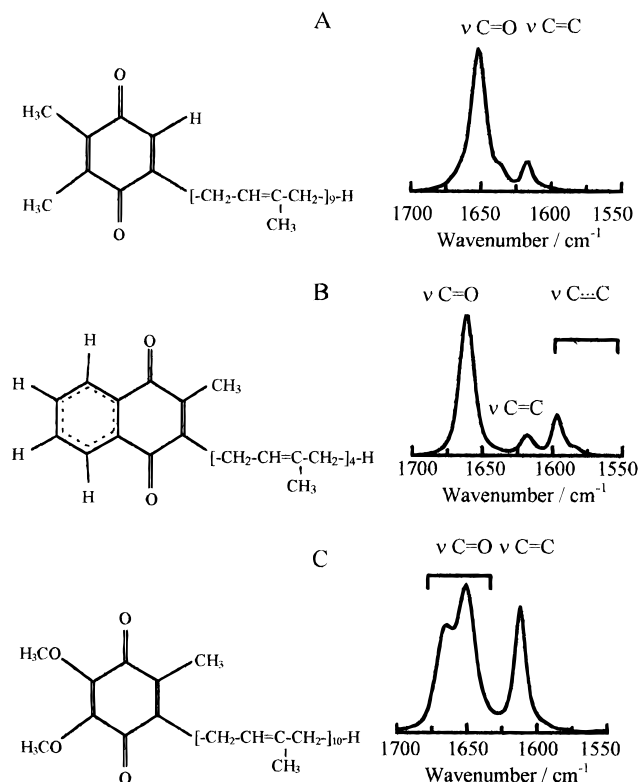⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

Figure 1. Formula and IR spectra in the C=O and C=C stretching region for (A) plastoquinone-9, (B) menaquinone-4, and (C) ubiquinone-10.

on 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone and on Q_1 found the optimal geometry with the two O—CH_3 bonds out of the quinone ring plane.[7] Recently, PM3 semiempirical and density functional calculations showed the possibility of the presence of several nearly isoenergetic conformers depending upon the orientation of the methoxy groups relative to the quinone ring plane.[11,12] This discrepancy between the conformations observed in crystals or derived from calculations might be due to the peculiar environment within crystals while the calculations are performed for quinones in vacuum. This leaves open the question of the methoxy conformation for ubiquinones in solution, which corresponds to the conditions of the electrochemical studies.

Among the spectroscopic techniques that can probe the structural properties of functional groups in small molecules, vibrational spectroscopy has been extensively applied to the study of quinones in solution.[13,14] Notably, the frequency of the C=O stretching modes of quinones has been found to be dependent upon the nature of the substituents.[3,15-17] For naphthoquinones (Figure 1A) and plastoquinones (Figure 1B), the IR spectrum is characterized by one main band in the carbonyl stretching region (around 1650 cm^{-1}), which corresponds to a mode involving out-of-phase stretching of the two carbonyl groups, and band(s) at lower frequency corresponding to C=C stretching mode(s). In contrast, the IR spectrum of ubiquinones (Figure 1C) is characterized by a pair of bands corresponding to C=O stretching modes and a band at lower frequency corresponding to a C=C stretching mode as revealed by the study of isotope effects.[18] The origin of this splitting of the carbonyl bands of ubiquinones is still largely unexplained.[13,15-17]

In view of the key role of ubiquinones in photosynthetic electron and proton transfer reactions, biophysical techniques have been developed to investigate the IR spectra of these quinones within the reaction center protein.[18] There is therefore a need for a better understanding of the effect of the orientation of the methoxy groups on the force constants, vibrational frequencies, and isotopic shifts of isolated ubiquinones. Recently, one of us (M. N.) has reported on conformational analysis and harmonic force fields investigations of 2-methoxy- and 2,3-dimethoxy-1,4-benzoquinone applying density functional methods.[12] Although the results were very satisfactory, these computationally demanding methods will not be applicable to larger quinones of high biological interest. In this respect, there is a need for less expensive calculations allowing for a qualitative understanding of the vibrational properties of the quinones.[11,12]

In the present work, we report on semiempirical PM3 calculations on a large range of methoxy-substituted 1,4-benzoquinones. We demonstrate the usefulness of this approach as a first step toward a quantitative description of the effect of the orientation of the methoxy groups on the vibrational spectra of these quinones. These calculations, together with the IR spectra of ubiquinones selectively ^{13}C-labeled on either one of the two carbonyls, allow the geometrical conformation of their methoxy groups in solution to be determined. Energy calculations on corresponding semiquinones provide a rationale for the peculiar redox properties of ubiquinones both in solution and in bacterial photosynthetic reaction centers.

Experimental and Computational Procedures

2-Methoxy-1,4-benzoquinone, 2,3-dimethoxy-1,4-benzoquinone, 2,6-dimethoxy-1,4-benzoquinone, 2,3-dimethoxy-5,6-dimethyl-1,4-benzoquinone (from Apin Chemicals Ltd., U.K.), 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0), ubiquinone-10 (Q_{10}), and menaquinone-4 (from Sigma) were used without further purification. Plastoquinone-9 was extracted from *Synechocystis 6803* as described.[19] $^{13}C_1$ and $^{13}C_4$ ubiquinone-3 (Q_3) were synthesized (Boullais, C., Mioskowski, C., unpublished results) from specific ^{13}C-labeled methylsuccinic anhydrides, by adapting a described procedure for complete synthesis of Q_{10}.[20] Controls by mass spectroscopy have shown a ^{13}C isotopic enrichment larger than 99%. Unlabeled Q_3 was prepared by the same procedure.

IR spectroscopy was carried out on a Bruker IFS88 (at 10 K) and on a Nicolet 60 SX (at room temperature) FTIR spectrometers. The measurements at 10 K were realized in an helium-cooled convection cryostat (TBT). The sample was constituted of a thin film of the studied quinone between two CaF_2 windows. Chainless quinones were dissolved in CCl_4 (spectroscopic grade).

PM3 calculations are made using the MOPAC 6 software on a PC. The choice of the parametrization has been described previously.[11,21] Unless otherwise specified, no symmetry constraints are imposed during the calculations.

For 2-methoxy- and 2-methoxy-3-methyl-1,4-benzoquinones, the dihedral angle θ defining the orientation of the methyl from the methoxy group relative to the C_2=C_3 bond (Figure 2A) is varied from 0° to 180° with 10° steps. At each step, all the other parameters are optimized. Unless specified, the same optimization was performed for all other calculations. For each of these quinones, 19 conformations were studied. For 2,3-dimethoxy-, 2,5-dimethoxy-, 2,6-dimethoxy-, and 2,3-dimethoxy-5-methyl-1,4-benzoquinones, both dihedral angles θ_1 and θ_2 being similarly defined relative to the C=C bonds (Figure 2B–H), θ_1 is varied from 0° to 180° with 10° steps and for each θ_1, θ_2 is varied from 0° to 360° with 10° steps (703 conformations analyzed for each quinone). For 2,3-dimethoxy-5-methyl-6-monoprenyl-1,4-benzoquinone (Q_1), the steps are of 45° and for each couple (θ_1, θ_2), the dihedral angle φ defining the

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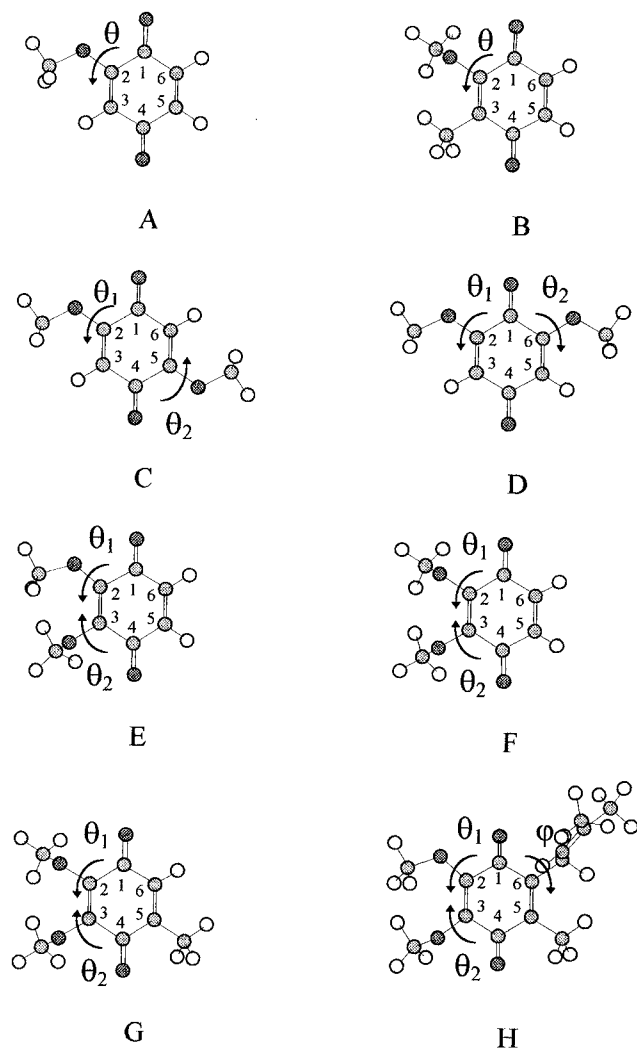

Figure 2. Formula and definition of the dihedral angles θ (and φ) for A) 2-methoxy-1,4-benzoquinone (representation with $\theta = 0°$), (B) 2-methoxy-3-methyl-1,4-benzoquinone ($\theta = 80°$), (C) 2,5-dimethoxy-1,4-benzoquinone ($\theta_1 = \theta_2 = 0°$), (D) 2,6-dimethoxy-1,4-benzoquinone ($\theta_1 = \theta_2 = 0°$), (E) 2,3-dimethoxy-1,4-benzoquinone (conformer A, $\theta_1 = 0°$ $\theta_2 = 100°$), (F) 2,3-dimethoxy-1,4-benzoquinone (conformer B, $\theta_1 = 100°$ $\theta_2 = 100°$), (G) 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0, conformer B, $\theta_1 = 100°$ $\theta_2 = 100°$), and (H) 2,3-dimethoxy-5-methyl-6-monoprenyl-1,4-benzoquinone (Q_1, conformer A_I, $\theta_1 = 0°$ $\theta_2 = 100°$ $\varphi = 90°$). Black-filled circles, oxygen atoms; gray-filled circles, carbon atoms; unfilled circles, hydrogen atoms.

orientation of the monoprenyl chain relative to the $C_5=C_6$ bond (Figure 2H) is varied from 0° to 360° with 45° steps (405 conformations analyzed).

For 2,6-dimethoxy-1,4-benzoquinone, the special case when $\theta_1 = \theta_2 = \theta$ has also been studied using configuration interaction (CI). In these calculations, θ is varied from 0° to 180° with 10° steps. The CI calculations allowed the electrons from the two HOMOs to populate the two LUMOs. Similar CI calculations were realized for 2,3-dimethoxy-1,4-benzoquinone in the special case $\theta_1 = 100°$. θ_2 is then varied from 0° to 360° with 10° steps.

A similar conformational analysis was performed on semiquinones (in this case, UHF calculations were realized) and doubly reduced quinones for 2-methoxy-, 2-methoxy-3-methyl-, 2,3-dimethoxy-, 2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzoquinones.

Using the results of these calculations, the variations of the heat of formation (E) of the molecules versus the rotation angle θ, the couple θ_1,θ_2, or the triplet $\theta_1,\theta_2,\varphi$ are obtained, leading to the curve $E(\theta)$, the surfaces $E(\theta_1,\theta_2)$, or the hypersurface $E(\theta_1,\theta_2,\varphi)$, respectively.

The normal-mode analysis was performed for the conformations corresponding to the minima of $E(\theta)$, $E(\theta_1,\theta_2)$, and $E(\theta_1,\theta_2,\varphi)$. For 2,3-dimethoxy-1,4-benzoquinone, normal-mode calculations with CI were also performed. The quoted frequencies have been scaled with a 0.85 factor, as commonly performed in such calculations.[22,23] This factor has been determined by comparing experimental and PM3 calculated frequencies of a large number of quinones excluding 2,3-dimethoxy-substituted quinones.[11]

Results and Discussion

A. Quinones. In order to evaluate the influence of the orientation of the methoxy group(s) on the C=O stretching modes of *p*-benzoquinones, a normal-mode analysis has to be performed. This in turn requires the determination of the structures of minimum energy. The methoxy-substituted *p*-benzoquinones can be separated in two classes. In the first class, when no bulky group is present in the ortho position, the methoxy group will be designated as "free"; in the second class, the methoxy groups are designated as "hindered".

(a) Conformational Energy Analysis. p-Benzoquinones with Free Methoxy Groups. For 2-methoxy-, 2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzoquinones, the absolute energy minimum is obtained for $\theta = 0°$, i.e., when the O−CH$_3$ bond(s) is (are) in the quinone ring plane (Figure 3Aa and Table 1). Local energy minima are also observed, at least ≈10 kJ·mol^{-1} higher in energy than the global energy minimum. They correspond to an orientation of one or both methoxy with the O−CH$_3$ bond out of the quinone ring plane with generally $\theta \approx 110°$. The global energy minima, together with the local energy minima and the local energy maxima (defined as the energy of the saddle point between two energy minima), are reported in Table 1. These results are in agreement with crystallographic data and with previous calculations on 2-methoxy-[5,12,24] and 2,6-dimethoxy-1,4-benzoquinones.[6,25] In particular, in the previous work on 2,6-dimethoxy-1,4-benzoquinone, which represents the first *ab initio* study on methoxy-substituted quinones, a local energy minimum was also obtained for $\theta \approx 120°$.[6] These calculations were performed at the Hartree−Fock level imposing a C_2 symmetry which leads to the constraint $\theta_1 = \theta_2$ and using 30° steps. The rotational barrier between the maximum and the absolute minimum can be estimated to ≈37 kJ·mol^{-1} in the *ab initio* work, while in our work, this barrier is 20 kJ·mol^{-1}. This discrepancy is reduced when CI is taken into account in our calculations. This calculation has also been performed in the same conditions and imposing C_2 symmetry to the molecule; however, no differences have been observed in the variation of the heat of formation. As an example, when the electrons of the two HOMOs are allowed to populate the two LUMOs, this barrier is increased to 32 kJ·mol^{-1} and the minima are obtained for almost the same θ values (Table 1). Although this corresponds to a very small CI expansion, it can improve results like the height of torsional barriers or the assignment of normal modes. A recent theoretical study on 2-methoxy-1,4-benzoquinone, using density functional theory (DFT), that accounts for correlation effects, finds a similar barrier.[12]

p-Benzoquinones with Hindered Methoxy Groups. Calculations on 2-methoxy-3-methyl-1,4-benzoquinone show the energy minimum for $\theta \approx 80°$ (Table 1), in agreement with the value of ≈90° previously found.[5] A mean value of 120° is observed in crystal data of other 2-methoxy-3-methyl-substituted 1,4-benzoquinones.[5] The curve $E(\theta)$ (Figure 3Ba) shows a large energy increase when the methyl of the methoxy group starts to clash with the methyl at the 3-position ($\theta \approx 0°$).
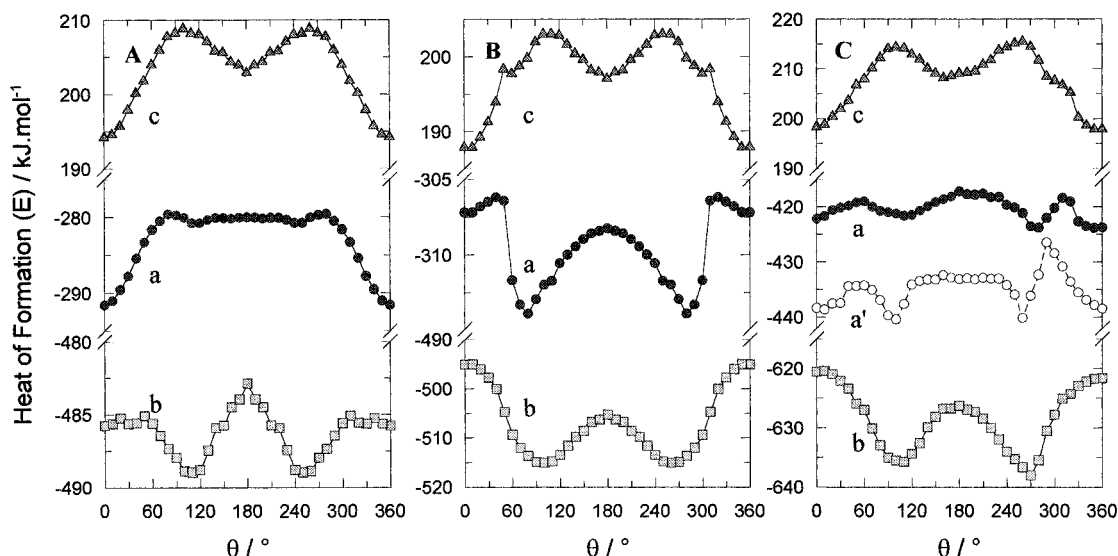
Figure 3. Heat of formation (rotational profiles) for the quinone (a) and semiquinone (b), and electron affinity (c) for (A) 2-methoxy-1,4-benzoquinone, (B) 2-methoxy-3-methyl-1,4-benzoquinone, and (C) 2,3-dimethoxy-1,4-benzoquinone. In the latter case, θ_1 is kept fixed at $100°$ and the curves represent the variations as a function of θ_2 (curve $E(100,\theta)$). The variation of the heat of formation for this quinone obtained using CI during calculations is presented in dotted line (curve a′).

TABLE 1: Heat of Formation (Energy) Minima and Maxima, together with the Corresponding Barriers, for the Different Studied Quinones[a]

		global energy minimum (I)		local energy minimum (II)		local energy maximum (III)		barrier kJ·mol⁻¹	
quinone		kJ·mol⁻¹	deg	kJ·mol⁻¹	deg	kJ·mol⁻¹	deg	III−I	I−II
2-methoxy	θ	−292	0	−281	110	−280	80	12	−11
2-methoxy-3-methyl	θ	−314	80	none		−306	40	8	
2,5-dimethoxy	$\theta_1; \theta_2$	−452	0;0	−441	0;110−170	−439	0;90	12	−10
				−431	170;170	−428	80;80	23	−20
2,6-dimethoxy	$\theta_1; \theta_2$	−449	0;0	−438	0;110−250				
				−430	120;120	−429	90;90	20	−19
no CI	$\theta_1 = \theta_2$	−449	0	−430	110	−429	90	20	−19
CI	$\theta_1 = \theta_2$	−484	0	−457	110	−452	80	32	−27
2,3-dimethoxy	$\theta_1; \theta_2$	−424	360;100						
no CI	$\theta_1 = 100$	−424	360	−424	280	−418	310	5.4	0
	θ_2			−422	110	−419	60	4.8	−2.2
				−422	0				−1.6
		−422	0	−424	100	−418	50	5.4	0
CI		−440	100	−440	260	−432	160	8	−0.2
				−438	360	−434	60	6	−2.0
Q_0	$\theta_1; \theta_2$								
Q_1	$\theta_1; \theta_2$	−486	0;90	−482	135;90	−480	45;90	6	−4
	$\varphi = 90$ or $\varphi = 270$				90;0	−479	90;45	7	−5
				−481	0;225				
					135;315				
				−480	90;90				
					135;0				

[a] For each energy minimum or maximum, the value of θ, the dihedral angle(s) defining the orientation of the methoxy group(s) relative to the quinone ring plane, is (are) given. CI: CI is taken into account during the calculations. Local energy maximum: energy of the saddle point between two energy minima.

In the case of 2,3-dimethoxy-1,4-benzoquinone, the surface $E(\theta_1,\theta_2)$, analogous to that presented in Figure 5B for Q_1, also presents an area of high energy corresponding to very unfavorable conformations when the two methyl groups come almost in contact (θ_1 and θ_2 both close to $0°$).[11] Far from this region, three energy minima are observed. In contrast to the case of quinones with free methoxy group(s), these energy minima are almost isoenergetic (within ≈ 1 kJ·mol⁻¹) (Table 1). They correspond to a conformation where one O−CH$_3$ bond is in the quinone ring plane and the second is out of this plane (Figure 2E, conformer A) or where both O−CH$_3$ bonds are out of the quinone ring plane either on the same side (conformers B) or on opposite sides (conformers B′) of this plane (Figure 2F). Conformer A is observed in the crystal structure while conformer B (B′) is the optimal conformation obtained in a previous

CNDO calculation.[7] The θ value when the O−CH$_3$ bond is not in the quinone ring plane is always $\approx 110°$ (Table 1), as also found in the conformation at the local energy minimum obtained on 2-methoxy-, 2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzoquinones.

The variation of the energy as a function of θ_2 when θ_1 is kept fixed at $100°$ is represented in Figure 3Ca. It appears that the conformer B lies slightly lower in energy than the conformer B′ while conformers A and B are isoenergetic. However, the precision of the calculations is such that this slight difference between the energy of conformers B and B′ might not be significant. The calculated energy barrier between the minima of conformers A and B (B′) is ≈ 5 kJ·mol⁻¹, which is close to $k_B T$ at 293 K (4.9 kJ·mol⁻¹). With CI taken into account, this

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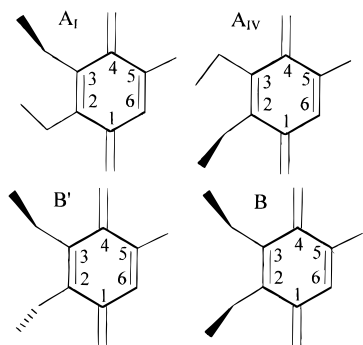
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Figure 4. Formula of conformers A_I, A_{IV}, B, and B′ for 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0).

barrier is increased to 8 kJ·mol^{-1} and all the minima are isoenergetic (Figure 3Ca′).

Table 1 also shows the results of the calculations performed on Q_0 and Q_1. Two energy minima corresponding to the two types of conformer A and B are obtained. It should be noted that for these quinones, two subconformers A_I and A_{IV} have to be considered whether the O−CH$_3$ bond in the quinone ring plane is at the 2-position (A_I) or the 3-position (A_{IV}) (Figure 4). They are found isoenergetic within the accuracy of the calculations. For Q_0, the influence of a methyl group at position 5 on the geometry and relative energy of the different conformers is small. For Q_1, the influence of the orientation of the chain relative to the quinone ring plane was studied by rotating the φ angle with 45° steps for a given orientation of the two methoxy groups: two almost isoenergetic minima were found at $\varphi = 90°$ and $\varphi = 270°$, independent of the orientation of the methoxy groups (see Figure 5A). These results are in agreement with previous INDO and CNDO calculations on the optimal orientation of the monoprenyl chain in Q_1.[7] This influence is similar to the one of a methyl group.

According to literature, the orientation of the methoxy groups relative to the quinone ring plane is governed by three effects: (1) the delocalization of two electrons from the methoxy oxygen onto the electron-poor quinone ring, (2) the Coulombic repulsion between the charge density of the methoxy oxygen lone pairs and the charge density of the carbonyl oxygen lone pairs that lie in the plane of the quinone ring, and (3) a steric interaction between the methoxy methyl group and the carbonyl oxygen.[6] Effect 1 (orbital delocalization) will be maximized at 0° and 180° and will be negligible at 90°. Effect 2 (Coulombic repulsion) will monotonically decrease over the range from 0° to 180° whereas effect 3 (steric repulsion) will monotonically increase over the same range. The orientation of free methoxy group(s) in *p*-benzoquinones is thus mostly governed by effect 1.[6] When a bulky group (methyl, chlorine, boron, methoxy) is present, ortho to the methoxy group, new steric and Coulombic repulsions exist which result in hindered methoxy groups, with the O−CH$_3$ bond out of the quinone ring plane. When the methyl of the methoxy group is not in the quinone ring plane, the oxygen atom has a sp^3 hybridization, while when it is in the quinone ring plane, the delocalization of two electrons from the methoxy oxygen onto the electron-poor quinone ring is possible through a sp^2 hybridization of the oxygen atom.[8] This is seen in the study of the molecular orbitals obtained with the calculations presented here.[11] For a free methoxy at position 2, the semiempirical calculations indicate that the molecular orbital resulting from the combination of the atomic orbitals from the oxygen atom and the pz orbitals from the C$_2$ and C$_3$ atoms is bonding for the C$_2$−O, C$_1$=O, and C$_2$=C$_3$ bonds and nonbonding for the C$_2$−C$_1$ bond.

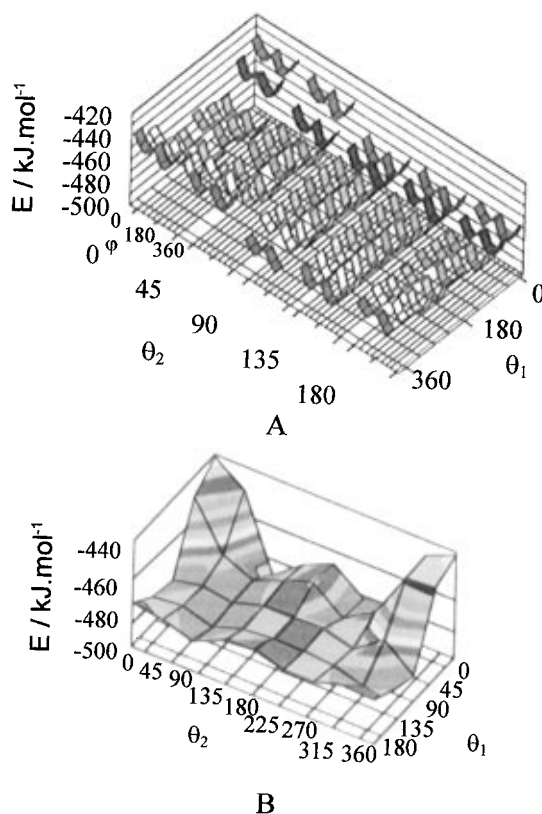In contrast to the cases of 2-methoxy-, 2-methoxy-3-methyl-,

Figure 5. (A) Representation of $E(\theta_1,\theta_2,\varphi)$ for Q_1. For a given couple (θ_1,θ_2), the curve $E_{\theta_1,\theta_2}(\varphi)$ is represented. It appears that in each of these curves, two minima are present at 90° and 270°. Taking the minimum of each of these curves, it is possible to construct the surface $E(\theta_1,\theta_2,)$ presented in (B). For a stable conformation corresponding to a minimum of the surface $E(\theta_1,\theta_2,)$, there exist two subconformers of minimal energy depending on whether $\varphi = 90$ or 270°.

2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzoquinones, where the conformation of the methoxy group(s) seems to be known without ambiguity, the closeness of the energy of conformers A and B for 2,3-dimethoxy-substituted 1,4-benzoquinones and the small magnitude compared to k_BT at room temperature of the energy barrier between these two energy minima (allowing the interconversion between the two conformers at room temperature) raise the question of the presence of only one or both conformers in solution. This question can be addressed by vibrational spectroscopy.

(b) Vibrational Spectra Analysis. The stretching motion of the two carbonyl groups in *p*-benzoquinones normally couples in two modes, a mode where their carbonyls stretch in phase (predominantly Raman active) and a second one where they stretch out of phase (predominantly infrared active). This results in one main band in the IR spectra of these quinones,[16,17] as observed in Figure 1, A and B, for menaquinone and plastoquinone. The normal-mode analysis realized on these quinones, at the same level of calculation as used for the conformational energy analysis, is presented for the C=O stretching modes in Table 2.[11,21] The two C=O stretches are coupled giving rise to in-phase and out-of-phase modes. For each mode, the composition of the potential energy distribution (PED) is of 29% and 25% of each C=O group. The calculated IR intensity of the band is found 28 and 7 times higher for the asymmetric mode than for the symmetric mode for menaquinones and plastoquinones, respectively.[11] These results are thus in good agreement with the presence of a main C=O stretching band in the IR spectra of these quinones, despite the approximations inherent to the method.[11,21]

In contrast, ubiquinones present a pair of bands in the region

TABLE 2: Normal Mode Decomposition for Quinones in the C=O Stretching Region

quinone	intensity (km.mol^{-1})		potential energy distribution (%)		
			C$_1$=O	C$_4$=O	C−C
2,3-dimethyl-1,4-	17	sym	29	25	36
naphthoquinone	467	asym	25	29	36
2-methyl-3-isoprenyl-1,4-	87	sym	29	24	46
naphthoquinone	408	asym	24	30	45
duroquinone	62	sym	28	26	46
	438	asym	26	28	45
plastoquinone-1	59	sym	29	24	45
	449	asym	25	29	45
2-methoxy-1,4-	87		50	4	42
benzoquinone	403		4	50	42
2-methoxy-3-methyl-1,4-	81		38	16	32
benzoquinone	440		16	38	32
2,5-dimethoxy-1,4-	0.3	sym	28	26	45
benzoquinone	494	asym	26	28	45
2,6-dimethoxy-1,4-	104		53	1	44
benzoquinone	390		1	52	45

around 1650 cm^{-1} (Figure 1C) which, according to their sensitivity to ^{18}O substitution, can be assigned to modes involving strong C=O stretching contributions.[18] It is important to note that when the spectra of ubiquinone and menaquinone with similar chains of comparable length are normalized on the ν(C−H) (3100−2700 cm^{-1}) and δ(C−H) (1500−1350 cm^{-1}) bands of the chain, the integrated intensity of the single band of the menaquinone is comparable to the sum of the integrated intensity of the two bands of the ubiquinones (data not shown), which reinforces the attribution of these two bands in the ubiquinones spectra to the ν(C=O) stretching modes. As discussed above, the methoxy groups can adopt two distinct orientations relative to the quinone ring plane in *p*-benzoquinones, with the O−CH$_3$ bond in or out of the quinone ring plane. We will examine below the influence of the presence and of the orientation of the methoxy groups on the C=O stretching modes of *p*-benzoquinones.

Monomethoxy-Substituted p-Benzoquinones. The normal-mode analysis indicates that the presence of a methoxy group at the 2-position always increases the bond strength of the C$_1$=O group. The amplitude of this effect depends on the orientation of the O−CH$_3$ bond relative to the quinone ring plane and is maximum when the O−CH$_3$ bond is in this plane (data not shown), as also found in DFT calculations on 2-methoxy-1,4-benzoquinone.[12] This in turn induces a variable uncoupling of the two C=O stretches compared to what is observed in menaquinone and plastoquinone, as reflected by the normal-mode composition shown in Table 2. For 2-methoxy-1,4-benzoquinone, where the O−CH$_3$ bond is in the quinone ring plane, the coupling is almost lost (50% and 4% in the PED of each mode), while for 2-methoxy-3-methyl-1,4-benzoquinone, where the O−CH$_3$ bond is tilted at $\theta = 80°$, a coupling of 38% and 16% is calculated compared to the 29% and 25% found in menaquinone and plastoquinone (Table 2).

According to these calculations, the following effects are thus expected on the IR spectra of 2-methoxy-1,4-benzoquinones bearing methyl(s) at different positions: when no methyl group is present at the 3-position, two bands corresponding to the C$_1$=O (high-frequency band) and C$_4$=O stretching modes are expected, while when a methyl group is present at the 3-position one main band is expected. The experimental IR frequencies of such monomethoxy-substituted quinones in CCl$_4$ are reported in Table 3A. The IR spectrum of all these quinones is characterized by a band at 1658−1645 cm^{-1}. Moreover, all the quinones that do not bear a methyl group in position 3 present a high-frequency band (1683−1675 cm^{-1}). Thus the

predictions of the calculations are fully supported by the experimental observations. This leads us to assign in the spectra of the 2-methoxy-substituted 1,4-benzoquinones having no substituent at the 3-position (i.e., having the O−CH$_3$ bond in the quinone ring plane according to the calculations) the high-frequency band to the C$_1$=O stretching mode and the low-frequency band to the C$_4$=O stretching mode. In 2-methoxy-3-methyl-substituted 1,4-benzoquinones the band at 1658−1650 cm^{-1} corresponds to the asymmetrically coupled C=O stretching mode.

Dimethoxy-Substituted p-Benzoquinones. The experimental IR frequencies of some dimethoxy-substituted *p*-benzoquinones in the C=O stretching region are reported (Table 3B). Among the listed quinones, only the 2,5-dimethoxy- and 2,6-dimethoxy-1,4-benzoquinones are expected to have both O−CH$_3$ bonds in the quinone ring plane (Table 1 and literature [6]). The normal-mode analysis on these quinones (Table 2) indicates different behaviors. For 2,5-dimethoxy-1,4-benzoquinone, the two carbonyl groups are strongly coupled while for 2,6-dimethoxy-1,4-benzoquinone the two carbonyl groups vibrate almost independently. For the latter quinone, the weak coupling between these two modes is found to be even smaller than in the 2-methoxy-1,4-benzoquinone. The upshift of the C$_1$=O stretching frequency is thus expected to be higher in this quinone than the one observed in 2-methoxy-1,4-benzoquinone. Indeed, two bands are observed in the 2,6-dimethoxy-1,4-benzoquinone spectrum at 1697 and 1647 cm^{-1} compared to 1682 and 1650 cm^{-1} in the 2-methoxy-1,4-benzoquinone spectrum. For 2,5-dimethoxy-1,4-benzoquinone, only one main band is expected in the IR spectrum, which is indeed observed experimentally at 1658 cm^{-1}. This is further supported by the fact that in 1,4-cyclohexanedione, where the two C=O groups cannot get into resonance due to the absence of C=C bonds, the C=O stretching modes are found at ≈1710 cm^{-1}, compared to ≈1666 cm^{-1} in 1,4-benzoquinone.[13,26] These observations reinforce the proposed model of the influence of the conformation of the methoxy group on the carbonyl stretching modes and warrant this approach to investigate the geometry of the methoxy groups in 2,3-dimethoxy-substituted quinones by analyzing their IR spectra.

For 2,3-dimethoxy-1,4-benzoquinone, three conformers A, B, and B′ (Figure 3Ca,a′) are possible according to our calculations (Table 1). The normal-mode analysis shows the behavior of the C=O stretching modes to be dependent on the type of conformation adopted by the methoxy groups (Table 4). In conformer A, these modes are almost uncoupled (Table 4). In conformers B and B′, the two C=O stretches are coupled (in-phase and out-of-phase modes). All calculations on B and B′ (normal modes, isotope effects) give similar results such that no discrimination can be made between these conformers using our experimental data. Therefore, we will thereafter no longer consider conformer B′. Normal-mode calculations performed on Q$_0$ and Q$_1$ show that the influence of a methyl or a chain on the degree of coupling of the C=O modes for each conformer is small.[11] The presence of two bands corresponding to C=O stretching modes in the IR spectra of these quinones (Figures 1C and 4, Table 3) seems to indicate that conformer A is favored in solution.

Study of isotope effects is a key step in vibrational mode analysis. The difference of the methoxy conformation must appear as different behaviors of the C=O stretching modes upon labeling in conformers A and B. This difference can be used to better ascertain the conformation of ubiquinone in solution. Among the different possible labeling, specific ^{13}C labeling at the 1- or 4-position is very important as they break the symmetry

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TABLE 3: Experimental IR Frequencies and Assignments in the C=O and C=C Stretching Region for (A) Methyl-Substituted 2-Methoxy-1,4-benzoquinones and (B) Dimethoxy-Substituted 1,4-Benzoquinones

A

substituents according to the position					frequencies (cm^{-1}) and modes			
2	3	5	6		C$_1$=O	C$_4$=O	C=C	C=C
OMe[f]	H	H	H	a	1681	1656	1623	1595
				c	1682	1655–1650	1625	1596
OMe	H	Me[g]	H	a	1681	1653		1608
OMe	H	H	Me	a	1683	1653	1637	1605
OMe	H	Me	Me	a	1675	1645		1610
					sym C=O[d]	as C=O[e]	C=C	C=C
OMe	Me	H	H	a	1669 sh	1653		1597
OMe	Me	Me	H	a		1653		1610
OMe	Me	H	Me	a		1658		1613
OMe	Me	Me	Me	a		1650	1621	

B

					sym C=O	as C=O	sym C=C	as C=C
OMe	H	OMe	H	b		1658		1595
					C$_1$=O	C$_4$=O	C=C	C=C
OMe	H	H	OMe	c	1697	1647	1625	1599
				b	1695	1645	1623	1595
					C=O[h]	C=O[i]	C=C	C=C
OMe	OMe	H	H	c	1674	1661	1639	1593
OMe	OMe	Me	H	c	1672	1659		1605
OMe	OMe	Me	Me	c	1666	1651		1614
OMe	OMe	Me	triprenyl	c	1664	1650		1611

[a] Quinones in CCl$_4$; Flaig, W.; Salfeld, J. C. *Justus Liebigs Ann. Chem.* **1959**, *626*, 215. [b] Quinones in KBr pellet; Flaig, W.; Salfeld, J. C. *Justus Liebigs Ann. Chem.* **1959**, *626*, 215. [c] Quinones in CCl$_4$; this work. [d] sym (C=O or C=C): symmetric mode. [e] as (C=O or C=C): asymmetric mode. [f] OMe: methoxy. [g] Me: methyl. [h] With the ortho OMe free. [i] With the ortho OMe hindered.

of the C=O with respect to the methoxy groups. Such labeling is available experimentally on Q$_3$. They will be compared to calculations performed on Q$_1$. The effects of specific ^{13}C labeling of one carbonyl on Q$_1$ have been calculated for the different conformers (Figure 4): conformer A$_I$, conformer A$_{IV}$, and conformer B. The calculated effect of the labeling on the normal modes is found to be dependent upon the conformation of the methoxy groups (Table 4):

(i) In unlabeled conformer A$_I$, the two bands calculated at 1663 cm^{-1} and 1653 cm^{-1}, respectively, have both substantial IR intensity. This also applies to the unlabeled conformer A$_{IV}$ which, depending on the conformation of the chain, gives rise to the two subconformers A$_{IV-1}$ and A$_{IV-2}$ (Figure 5). As the two C=O stretches are almost uncoupled in the ^{12}C quinone (45%, 9%), specific ^{13}C labeling of one C=O group will affect only the frequency of this group and less the relative IR intensities. For conformer A$_I$, the calculated 1663 cm^{-1} band is shifted to 1621 cm^{-1} upon ^{13}C$_1$ labeling and unshifted upon ^{13}C$_4$ labeling while the calculated 1653 cm^{-1} band is shifted to 1616 cm^{-1} upon ^{13}C$_4$ labeling and unshifted upon ^{13}C$_1$ labeling. For the two subconformers A$_{IV-1}$ (A$_{IV-2}$), an opposite effect is found with the downshift of the calculated 1664 (1666) cm^{-1} band upon ^{13}C$_4$ labeling to 1621 (1624) cm^{-1} and of the calculated 1651 (1656) cm^{-1} band upon ^{13}C$_1$ labeling to 1612 (1618) cm^{-1}.

(ii) In unlabeled conformer B, the two bands calculated at 1662 and 1653 cm^{-1} correspond to the symmetric and antisymmetric coupling of the C=O stretch, respectively. Both have similar C$_1$=O and C$_4$=O stretch (33%, 21%) while only the band calculated at 1653 cm^{-1} has a significant IR intensity. The selective ^{13}C labeling leads to the uncoupling of the two C=O modes. This results for the IR spectrum of the labeled quinone in two bands calculated at 1657 and 1619 cm^{-1} of comparable IR intensity. The high-frequency band corresponds to the almost pure ^{12}C=O stretch while the low-frequency band corresponds to the ^{13}C=O stretch. The effect of labeling at C$_1$ or C$_4$ is identical.

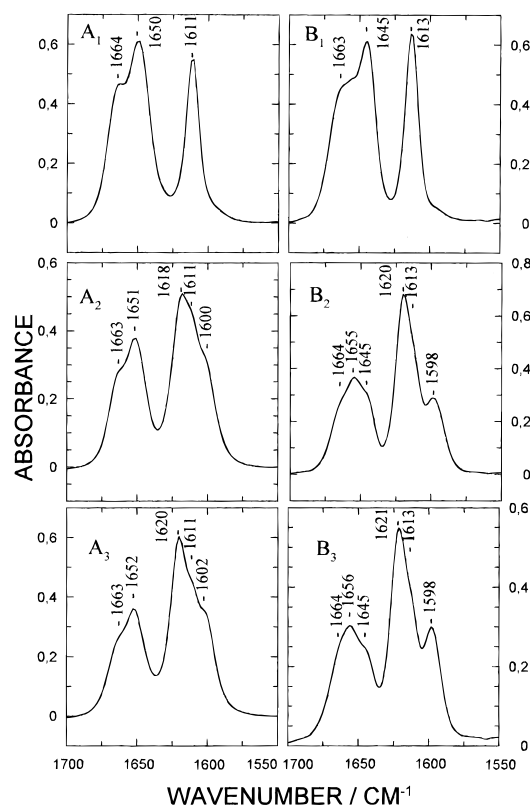Experimentally, specific ^{13}C labeling at the 1- or 4-position

Figure 6. Specific ^{13}C labeling effects on the IR spectrum of Q$_3$: (A) effects at room temperature; (B) effects at 10 K. (1) Unlabeled Q$_3$; (2) ^{13}C$_1$-labeled Q$_3$; (3) ^{13}C$_4$-labeled Q$_3$. The spectra at a given temperature have been normalized on the absorption of the CH$_2$ and CH$_3$ bending modes in the 1400–1500 cm^{-1} region. The same samples have been used for the measurements at the two temperatures.

on Q$_3$ leads in both cases to a decrease of the intensity of both bands at 1663 and 1650 cm^{-1}, while bands appear at 1618 or 1620 cm^{-1} (Figure 6A). The presence of two remaining bands at ≈1663 and ≈1650 cm^{-1} and the absence of a median band

TABLE 4: Calculated Effect of Labeling on the IR Frequency and Normal-Mode Decomposition of Q_I for the C=O Stretching Modes for Two Different Conformations (Conformer A or B)

	ν	intensity (km·mol^{-1})	potential energy distribution (%)		
conformer A_I			C$_1$=O	C$_4$=O	C−C
^{12}C	1663	131	9.2	45.3	37.4
	1653	334	45	9.3	37.3
^{13}C$_1$	1655	165	**53.7**	0.6	44.6
	1621	268	**0.7**	55.4	42.9
^{13}C$_4$	1662	220	0.3	**54.2**	44.9
	1616	232	55.4	**0.4**	42.8
conformer A_{IV-1}			C$_1$=O	C$_4$=O	C−C
^{12}C	1664	133	49.5	5.0	41
	1651	335	5.1	49.2	40.8
^{13}C$_1$	1652	218	**0.8**	53.5	44.4
	1621	246	**55.1**	0.9	42.8
^{13}C$_4$	1663	195	54.2	**0.3**	45
	1612	255	0.5	**55.4**	42.6
conformer A_{IV-2}			C$_1$=O	C$_4$=O	C−C
^{12}C	1666	115	45.3	9.1	37.6
	1656	353	9.2	45.0	37.6
^{13}C$_1$	1658	224	**0.7**	53.6	44.8
	1624	240	**55.3**	0.8	42.9
^{13}C$_4$	1665	191	54.1	**0.3**	45
	1618	263	0.4	**55.4**	43
conformer B					
^{12}C	1662	66	33.2	21.1	27.7
	1653	409	21.2	33.2	27.7
^{13}C$_1$	1657	189	**0.4**	54	28
	1619	285	**55**	0.4	28
^{13}C$_4$	1657	214	54	**0.4**	28
	1619	261	0.4	**55**	28

TABLE 5: Value of the Dihedral Angle(s) θ in the Optimal Conformation for Some Methoxy-Substituted 1,4-Benzoquinones in Different Oxidation States

substituent	Q	Q$^{•-}$	Q^{2-}
2-methoxy	0°	110°	150°
	110° lm[a]		
2,3-dimethoxy	0°/90°	90°/90°	150°/210°
	90°/90° and 90°/270°	90°/270°	150°/150°
2,5-dimethoxy	0°/0°	110°/110°	150°/150°
	110°/110° lm	110°/250°	150°/210°
2,6-dimethoxy	0°/0°	110°/110°	150°/150°
	110°/110° lm	110°/250°	150°/210°

[a] lm: local minimum.

centered at ≈1656 cm^{-1} exclude the dominant contribution of conformer B in solution. On the other hand, the experimental data are fully compatible with an equivalent mixture of conformer A_I and A_{IV}, leading to a similar decrease of both the 1663 and 1650 cm^{-1} bands, without frequency shifts, upon either ^{13}C$_1$ or ^{13}C$_4$ labeling.

Indeed, if conformers A_I and A_{IV} are equiprobable, the 1663 cm^{-1} band in the IR spectrum of Q_3 arises for one half from the C$_1$=O stretch of conformers A_I and for the other half from the C$_4$=O stretch of conformers A_{IV}. Similarly, the 1650 cm^{-1} band arises for one half from the C$_1$=O stretch of conformers A_{IV} and for the other half from the C$_4$=O stretch of conformers A_I. The specific ^{13}C$_1$ labeling of Q_3 thus induces a shift of the C$_1$=O stretching mode of conformers A_I at 1663 cm^{-1}, resulting in a decrease by ≈50% of the intensity of the 1663 cm^{-1} band (the remaining intensity corresponding to the C$_4$=O stretch of conformers A_{IV}) as well as a shift of the C$_1$=O stretching mode of conformers A_{IV} at 1650 cm^{-1}, resulting in a decrease by ≈50% of the intensity of the 1650 cm^{-1} band (the remaining intensity corresponding to the C$_4$=O stretch of conformers A_I).

A ^{13}C$_4$ specific labeling would have a very similar effect. For both ^{13}C$_1$ and ^{13}C$_4$ labels, new bands are expected around 1620 cm^{-1}, corresponding to the ^{13}C=O stretch, and are indeed observed.

Correlation effects have been shown to be essential in a normal-mode analysis performed on benzoquinone and naphthoquinone with ab initio calculations.[27−30] When CI is taken into account in our normal-mode calculations, coupling appears between the C=O and C=C stretches (data not shown). The presence of these couplings is in better agreement with the observed labeling effects. Indeed, experimentally, ^{18}O, ^{13}C$_1$, and ^{13}C$_4$ labeling of the carbonyl groups also induce small shifts of the band at 1611 cm^{-1} attributed to a C=C stretching mode.[18,31,32] Similarly, ^{13}C specific labeling at the 5- or 6-position induces small shifts of the ν(C=O) bands at 1663 and 1650 cm^{-1}.[32] Despite the approximations of the method used in the present work, important conclusions can be obtained on the influence of the conformation of the methoxy groups on the IR spectra of ubiquinones. However, in order to study the details of the isotope effects, it is necessary to supplement the normal-mode analysis presented here with a more quantitative approach. In this respect, DFT calculations have already provided detailed information on the vibrational properties of similar quinones.[12,27,28] The full understanding of the IR spectra of these quinones requires, however, more experimental results on isotope labeling complemented by calculations. This work is in progress.[33]

(c) Conformation of the Methoxy Groups of Ubiquinones in Solution Derived from IR Data. While energy calculations have indicated that the methoxy groups of ubiquinones can adopt different geometries, the vibrational data allow the preferred geometry of ubiquinone in solution to be determined. The analysis of IR spectra of several methoxy-substituted quinones leads to the following:

(i) The frequency of the C=O stretching mode is upshifted by the presence of a methoxy group in ortho position and this upshift is maximum when the O−CH$_3$ bond is in the quinone ring plane.

(ii) The presence of two C=O bands in ubiquinones and the effects of specific ^{13}C labeling (at the 1- or 4-position) lead us to conclude that in solution at room temperature the main conformation of ubiquinones is with one O−CH$_3$ bond in and the second out of the quinone ring plane. The highest frequency band in the spectra of these quinones is attributed to the C=O stretch of the carbonyl group proximal to the methoxy group with the O−CH$_3$ bond in the quinone ring plane.

However, even if the effect of specific labeling unambiguously demonstrates that conformer A is favored in solution, it cannot be excluded that a small proportion of conformers B is also present due to the low rotational barrier between conformers A and B obtained in our calculations (Table 1). This population would thus correspond to transient species. An indication of the existence of this population is obtained in the IR spectra of Q_3 recorded at low temperature. Indeed, when the spectra are recorded at 10 K, the effect of specific ^{13}C labeling is slightly different from that observed at room temperature. In this case (10 K), a third band appears between the two bands observed in the spectra of the unlabeled quinone upon ^{13}C specific labeling (Figure 6). This can be interpreted by the fact that at this temperature the rotational barrier is higher than k_BT (0.2 kJ·mol^{-1}). In this case, the state at higher energy becomes long-lived and three populations have to be considered: the two populations corresponding to the conformers A_I and A_{IV} and the third where both O−CH$_3$ bonds are out of the quinone ring plane (conformer B). While at higher temperature the high-

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energy state (conformer B) becomes more populated, kinetics effects, related to the fact that k_BT is higher than the rotational barrier, make the lifetime of this state very short. According to the uncertainty principle, this manifests in a line broadening which limits observation of this state in the IR spectra at room temperature. A quantitative description of the line shape versus temperature, which would require statistical density matrix formulation, is beyond the scope of the present work.

B. Semiquinones. The addition of substituents to the quinone ring has an influence on the value of the half-wave reduction potential. According to the nature of these substituents, this potential is increased or decreased relative to the potential of the unsubstituted quinone. This effect has been quantified through the Hammett equation:[2]

$$\Delta E = \rho \sum \sigma_{para} + \text{constant} \qquad (1)$$

In this equation, ΔE represents the difference between the redox potential of the substituted and unsubstituted quinone, ρ is a factor depending on the nature of the quinone (benzoquinone, naphthoquinone) and on the nature of the solvent. $\sum \sigma_{para}$ is the sum, over all the substituents present on the quinone ring, of the coefficient σ_{para}, each of which characterizes the electron donating or electron-withdrawing effect of the substituent. It has been shown that the appropriate σ_{para} for quinones are those for para-substituents in benzene derivatives.[34,35] However, the redox potential of several methoxy-substituted quinones fails to be predicted by this rule.[2-5] These quinones all have methoxy group(s) hindered by the presence of a bulky group (methyl, chlorine, boron, methoxy) in ortho position.

(a) Electrochemistry of Methoxy-Substituted Quinones. It has been previously stressed that the effect of a methoxy group on the redox properties of quinones is related to the conformation of this methoxy.[4] However, the σ_{para} value available in the literature for a methoxy group (-0.27) corresponds to the effect of a free methoxy group, with the $O-CH_3$ bond in the quinone ring plane. This negative value reflects the donation effect of the methoxy group in the quinone ring. When the $O-CH_3$ bond of the methoxy group is not in the quinone ring plane, as is the case with hindered methoxy groups, electron donation to the quinone ring is less than that observed for free methoxy groups. This explains why the Hammett equation is only applicable for methoxy-substituted *p*-benzoquinones with free methoxy group(s). It is thus important to derive a σ_{para} for a hindered methoxy group.

From the electrochemical data in the literature on methoxy-substituted quinones with methyl and chlorine groups adjacent to the methoxy group(s) and using the Hammett equation, we have deduced a σ_{para} for a hindered methoxy group by subtracting the σ_{para} of the bulky group alone from that of the couple methoxy plus bulky group. The same value $\sigma_{para} = -0.07$ is obtained whether the bulky group is a methyl or a chlorine. This value of -0.07, compared to the value of -0.27 for a free methoxy group, does reveal that the electron donation is less from a hindered compared to a free methoxy group.

For quinones bearing adjacent methoxy groups, a $\sigma_{para} = -0.10$ has been previously derived in dimethylformamide for the effect of the two adjacent methoxy groups.[4] Using a larger set of quinones and solvents,[3-5] we have calculated from electrochemical data a $\sigma_{para} = -0.16$. It is surprising to find that this value is inconsistent with the preferred quinone conformation in solution suggested from IR data (conformer A) for which a $\sigma_{para} = -0.34$ is calculated. It is also inconsistent with the conformer with two free methoxy groups ($\sigma_{para} = -0.54$). On the other hand, it is in good agreement with the

value ($\sigma_{para} = -0.14$) calculated for a quinone with two hindered methoxy groups (conformer B).

While IR data show that the main conformation of adjacent methoxy groups in *p*-quinones is with one methoxy group bearing its $O-CH_3$ bond in the quinone ring plane and the second bearing its $O-CH_3$ bond out of the quinone ring plane (conformer A), the electrochemical potential measured for these quinones corresponds to that of a quinone with both methoxy groups bearing their $O-CH_3$ bond out of the quinone ring plane (conformer B). This can be explained as follows: (i) From IR data at room temperature, it can be deduced that most of these quinones in solution are conformers A. However, IR data at 10 K are consistent with the presence of a population of conformer B. Although this population increases at higher temperature, the lifetime of this conformer becomes so short that line-broadening effects prevent its observation in the IR spectra. Furthermore, the low rotational barrier calculated to convert conformer A in conformer B compared to k_BT at room temperature suggests that an interconversion between the two conformers exists in solution. (ii) Due to the smaller effect of electron donation for hindered methoxy groups compared to free methoxy groups, the redox potential of a conformer B is higher than the redox potential of a conformer A ($\sigma_{para} = -0.14$ and -0.34, respectively). (iii) From points (i) and (ii), it can be proposed that when the potential of the quinone solution is lowered, conformers B will be reduced first. Owing to the possible interconversion between both conformers, in fact all the quinones will be reduced, *via* conformer B. It can be predicted that the determination of the redox potential of these quinones at low temperature (i.e., when the interconversion between conformers A and B is no longer possible) should present two waves, one corresponding to the reduction of the population of conformers B and the second corresponding to the reduction of the population of conformers A, with a difference determined by the following rule:

$$\Delta E_2 - \Delta E_1 = \rho(-0.27 + 0.07) = -0.2\rho \qquad (2)$$

(b) Analysis of Semiquinone and Doubly Reduced States. The variation of the heat of formation of 2-methoxy-1,4-benzosemiquinone is presented in Figure 3Ab. It indicates that the optimal orientation of the $O-CH_3$ bond is no longer in the quinone ring plane, as in the neutral quinone, but with a dihedral angle of 110°, which is close to the angle corresponding to the local minimum in the case of 2-methoxy-1,4-benzoquinone. For 2,3-dimethoxy-, 2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzosemiquinones, the calculated potential energy surfaces indicate that the optimal conformations are with both $O-CH_3$ bonds out of the quinone ring plane. This result is in good agreement with the results from *ab initio* calculations on 2,6-dimethoxy-1,4-benzosemiquinone and semiempirical calculations on 2,3-dimethoxy-1,4-benzoquinone.[6] ENDOR data have been used in order to determine the orientation of the methoxy groups in the semiquinones.[36-40] Although the practical determination is very difficult due to the use of the protons of the methyl, which are far from the quinone ring and bear low electron density, a value of ≈60° out of the quinone ring plane has been determined, which is in good agreement with the results of our calculations (70°). As previously observed in the *ab initio* calculations on 2,6-dimethoxy-1,4-benzosemiquinone, the additional electron in the semiquinone is not localized at the carbonyl oxygen atoms, which results in distortions of bond lengths and angles evenly distributed throughout the ring system.[6] The calculations performed on the doubly reduced states of these quinones indicate that the $O-CH_3$ bond is always out of the quinone ring plane, with an angle of ≈150° and that

distortions, larger than in the case of the semiquinones, are evenly distributed throughout the ring system.

The optimal values of the dihedral angles obtained for semiquinones and doubly reduced quinones result from a difference in the balance between the three effects proposed to drive this orientation.[6] In particular, the presence of the additional electron(s) in the normally electron-poor quinone ring reduces the influence of effect 1. In contrast, effect 2 (Coulombic repulsion) will be increased and its role is strengthened in the determination of the orientation of the methoxy groups.

(c) Effect of the Conformation of Semiquinones on the Electrochemical Behavior of Methoxy-Substituted Quinones. Quinone reduction is performed without geometry reorientation.[6] For 2-methoxy-, 2,5-dimethoxy-, and 2,6-dimethoxy-1,4-benzoquinones, the $O-CH_3$ bond of their methoxy group(s) has different orientations relative to the quinone ring plane in the quinone and semiquinone states. The reduction of these quinones in solution thus proceeds in two steps: first an electron is transferred to the quinone which, then, is followed by a geometry relaxation leading to a reorientation of the methoxy group(s) relative to the quinone ring plane. On the other hand, for 2,3-dimethoxy-1,4-benzoquinone, while the reduction of conformer A will proceed in two steps (electron transfer followed by the reorientation of the methoxy group with the $O-CH_3$ bond in the quinone ring plane), the reduction of conformer B does not require a tilt of the methoxy groups after the electron transfer. Furthermore, when the conformation of the methoxy groups is fixed in a given geometry (by specific interactions as in crystals or proteins), the electron affinity is then related to the difference between the curves $E(\theta)$ in the quinone and semiquinone states or $E(\theta_1, \theta_2)$ surfaces for dimethoxy-substituted quinones. It appears in Figure 3, Ac, Bc, and Cc, that the electron affinity does change according to the orientation of the methoxy in the quinone state. It is thus possible to tune the electrochemical properties of ubiquinones by imposing a specific conformation of the methoxy groups.[6]

Quinones are essential electron acceptors in the electron transfer process in the photosynthetic reaction centers of purple bacteria where the quinones Q_A and Q_B play the role of one-electron and two-electron gates, respectively. In several purple bacteria, both sites are occupied by the same ubiquinone (Q_{10}). However, a difference of ≈ 70 mV exists between the redox potentials of the same quinone in the Q_A or Q_B sites.[41] The protein thus tunes differently the midpoint redox potential of the quinone in each site. A possible explanation that has been frequently propounded would be a difference in the orientation of the methoxy groups of the ubiquinones in the two sites.[4-6] Could the results from the present study help in the verification of this hypothesis? In reaction centers of *Rhodobacter sphaeroides*, specific $^{13}C_1$ and $^{13}C_4$ labeling of Q_3 in the Q_A and Q_B sites have revealed that the IR absorption of the ubiquinone bound to the two sites are quite different. In Q_A, both carbonyl groups stretch independently.[31] On the other hand, in the Q_B site, both carbonyl groups stretch at the same frequency.[42] The simplest explanation of these data would be that the quinone in the Q_A site is likely to be a conformer A, while the quinone in the Q_B site is a conformer B. The difference of the redox potential of the ubiquinone in the two binding sites would then come, at least in part, from the different geometries induced by the protein on the methoxy groups of the ubiquinones. The difference of ≈ 70 mV observed for the quinones *in situ* is relatively close to the calculated difference, using eq 2, between the redox potential of the two conformers of Q_1 in aqueous solution (126 mV).[3] This observation suggests that the orientation of the methoxy groups in the protein might be the predominant effect which tunes the electrochemical properties of the ubiquinones in the reaction center.

Conclusions

Quinones bearing methoxy groups have revealed specific characteristics in their IR spectra and electrochemical behavior. The present study relates these properties to the effect of the conformation of the methoxy group(s) relative to the quinone ring plane. The influence of this orientation on the $\nu(C=O)$ modes in the IR spectra is determined. In the particular case where two adjacent methoxy groups are present, as in the biologically important class of ubiquinones, the IR data are interpreted in terms of a preferred conformation of the quinones with one methoxy group (free) oriented in the quinone ring plane and the other one (hindered) tilted out of this plane (conformer A). From electrochemical data, a new $\sigma_{para} = -0.07$ has been deduced for a hindered methoxy group (compared to $\sigma_{para} = -0.27$ for a free methoxy group). The electrochemical data on ubiquinones suggest that these quinones are reduced from a conformation where both methoxy groups are hindered (conformer B). This is possible due to the low rotational barrier calculated to convert a conformer A into a conformer B.

The present work shows that it is possible to determine the conformation of the methoxy groups in ubiquinones from their IR spectra and to quantitatively relate their electrochemical properties to the conformation of the methoxy groups. This is essential in the interpretation of protein−quinone interactions. It has been proposed that proteins might tune the redox properties of ubiquinones *in situ* by affecting the orientation of the methoxy groups.[6] The conclusions drawn from the redox and IR properties of ubiquinones in solution, together with the IR data on these quinones in the Q_A and Q_B sites,[18,31,42] strengthen the importance of this effect in the bacterial photosynthetic reaction centers.

References and Notes

(1) Trumpower, B. L. *Function of Quinones in Energy Conserving Systems*; Academic Press: New York, 1982.

(2) Johnson, C. D. *The Hammett Equation*; Cambridge University Press: London, 1980.

(3) Flaig, W.; Beutelspacher, H.; Riemer, H.; Kälke, E. *Liebigs Ann. Chem.* **1968**, *719*, 96.

(4) Prince, R. C.; Dutton, P. L.; Bruce, J. M. *FEBS Lett.* **1983**, *160*, 273.

(5) Prince, R. C.; Halbert, T. R.; Upton, T. H. In *Advances in Membrane Biochemistry and Bioenergetics*; Kim, C. H., Tedeshi, H., Diwan, J. J., Salerno, J. C., Eds.;Plenum Press: New York, 1988; p 469.

(6) Robinson, H. H.; Khan S. D. *J. Am. Chem. Soc.* **1990**, *112*, 4728.

(7) Breen, D. L. *J. Theor. Biol.* **1975**, *53*, 101.

(8) Silverman, J.; Stam-Thole, I.; Stam, C. H. *Acta Crystallogr.* **1971**, *B27*, 1846.

(9) Schmalle, H.; Jarchow, O.; Hausen, B.; Schulz, K. H. *Acta Crystallogr.* **1984**, *C40*, 1090.

(10) Cannon, J. R.; Matsuki, I.; Patrick, V. A.; White, A. H. *Aust. J. Chem.* **1987**, *40*, 1191.

(11) Burie J.-R. Ph.D. Thesis, Université de Paris VI, 1994.

(12) Nonella, M.; Brändli, C. *J. Phys. Chem.* **1996**, *100*, 14549.

(13) Becker, E. D.; Ziffer, H.; Charney, E. *Spectrochim. Acta* **1963**, *19*, 1871.

(14) Castresana, J.; Alonso, A.; Arrondo J.-L. R.; Goñi, F. M.; Casal, H. *Eur. J. Biochem.* **1992**, *204*, 1125.

(15) Meyerson, M. L. *Spectrochim. Acta* **1985**, *41A*, 1263.

(16) Bellamy L. J. *The Infrared Spectra of Complex Molecules*, 2nd ed.; Chapman and Hall: London, 1980; Vol. 2.

(17) Flaig, W.; Salfeld, J. C. *Justus Liebigs Ann. Chem.* **1959**, *626*, 215.

(18) Breton, J.; Burie, J.-R.; Berthomieu, C.; Berger, G.; Nabedryk, E. *Biochemistry* **1994**, *33*, 4953.

(19) Berger, G.; Kléo, J.; Breton, J.; Gilles, N.; Lirsac, P. N. *J. Liq. Chromatogr.* **1994**, *17*, 4531.

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(20) Rüttimann, A.; Lorenz, P. *Helv. Chim. Acta* **1990**, *73*, 790.

(21) Burie J.-R.; Nonella, M.; Nabedryk, E.; Tavan, P.; Breton, J. In *Fifth International Conference on the Spectroscopy of Biological Molecules*; Theophanides T., Anastassopoulou J., Fotopoulos, N., Eds.; Kluwer Academic Publishers: Dordrecht, 1993; p 27.

(22) Pulay, P.; Fogarasi, G.; Pongor, G.; Boggs, J. E.; Vargha, A. *J. Am. Chem. Soc.* **1983**, *105*, 7037.

(23) Pople, J. A.; Schlegel, H. B.; Krishnan, R.; Defrees, D. J.; Binkley, J. S.; Frisch, M.; Whiteside, R. A.; Hout, R. F.; Hehre, W. J. *Int. J. Quantum Chem.* **1981**, *S15,* 557.

(24) Keegstra, E. M. D.; Spek, A. L.; Zwikker J. W.; Jenneskens, L. W. *J. Chem. Soc., Chem. Commun.* **1994**, 1633.

(25) Schmalle, H. H.; Jarchow, O. *Naturwissenschaften* **1977**, *64*, 534.

(26) *Aldrich Library of Infrared Spectra*, 3rd ed.; Aldrich Chemical Co: Milwaukee, WI, 1981; p 266H.

(27) Nonella, M.; Tavan, P. *Chem. Phys.* **1995**, *199*, 19.

(28) Nonella, M. *J. Mol. Struct. (THEOCHEM)* **1996**, *362*, 7.

(29) Liu, R.; Zhou, X.; Pulay, P. *J. Phys. Chem.* **1992**, *96*, 4255.

(30) Yamakita, Y.; Tasumi, M. *J. Phys. Chem.* **1995**, *99*, 8524.

(31) Breton, J; Boullais, C.; Burie, J.-R.; Nabedryk, E.; Mioskowski, C. *Biochemistry* **1994** *33*, 14378.

(32) van Liemt, W. B. S.; Steggerda W. F.; Esmeijer, R.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 153.

(33) Nonella, M.; Burie, J.-R.; Boullais, C.; Mioskowski, C.; Nabedryk, E.; Breton, J., manuscript in preparation.

(34) Zuman, P. *Substituents Effect in Organic Polarography*; Plenum: New York, 1967.

(35) Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979.

(36) Spoyalov, A. P.; Samoilova, R. I.; Tyryshkin, A. M.; Dikanov, S. A.; Liu, B.-L.; Hoff, A. J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1519.

(37) MacMillan F.; Lendzian, F.; Lubitz, W. *Magn. Reson. Chem.* **1995**, *33*, S81.

(38) Samoilova, R. I.; van Liemt, W.; Steggerda, W. F.; Lugtenburg, J.; Hoff, A. J.; Spoyalov, A. P.; Tyryshkin, A. M.; Gritzan, N. P.; Tsvetlov, Y. D. *J. Chem. Soc., Perkin Trans. 2* **1994**, 609.

(39) Holton, D. M.; Murphy, D. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 2173.

(40) Sullivan, P. D.; Bolton, J. R.; Geiger Jr., W. F. *J. Am. Chem. Soc.* **1970**, *92*, 4176.

(41) Kleinfeld,D.; Okamura, M. Y.; Feher, G. *Biochim. Biophys. Acta* **1984**, *766*, 126.

(42) Breton, J.; Boullais, C.; Berger, G.; Mioskowski, C.; Nabedryk, E. *Biochemistry* **1995**, *34*, 11606.