Dynamic Structure of the Potent Uncoupler SF 6847 (3,5-Di-*t*-butyl-4-hydroxybenzylidenemalononitrile) and Its Derivatives

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The steric and electronic structures of the most potent uncoupler of oxidative phosphorylation known, SF 6847, and its derivatives were studied. ^{1}H and ^{13}C NMR measurements showed that the malononitrile moiety is not planar with the benzene ring and that its intramolecular motional freedom is reduced on acid dissociation of the phenolic proton. The activation energies of the tumbling motion of the malononitrile moiety in the anionic forms of SF 6847 and its derivatives were also determined from their ^{1}H NMR measured at various temperatures. The CNDO/2 and *ab initio* MO calculations of the model compounds of SF 6847 showed that the planar form is quite unstable and that the malononitrile moiety is twisted considerably relative to the benzene ring. The effect of the dynamic structure on the acidity of SF 6847 was discussed based on the above results.

It is widely known that phenols exhibit a wide variety of biological activities, such as fungicidal, bacteriocidal and acaricidal effects, probably due to uncoupling of the phosphorylation reaction from the oxidoreduction reaction in microorganisms and organelles.[1-4] Among phenols, 3,5-di-*t*-butyl-4-hydroxybenzylidenemalononitrile (SF 6847) was found to show the most potent uncoupling activity in mitochondria.[4] This compound is characterized by being hydrophobic and having a strong electron-withdrawing group (malononitrile group) and an acid dissociable proton (phenolic hydroxyl group). These features are common to other potent uncouplers of oxidative phosphorylation, such as carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), 4,5,6,7-tetrachloro-2-trifluoromethoxybenzimidazole (TTFB) and 5-chloro-3-*t*-butyl-2'-chloro-4'-nitrosalicylanilide (S-13).[5-7] However, the structural requirements of uncouplers required for their activities are not yet fully clarified, although considerable attention has been paid to this problem.[8-12]

The neutral molecular form of SF 6847 has an absorption band at about 365 nm and the anionic form has one at about 456 nm.[13,14] On binding of SF 6847 to mitochondria and liposomes with various phospholipid compositions, its absorption spectrum changes greatly.[13,14] Changes in spectral properties are also observed when this compound is dissolved in organic solvents or when it forms a dimer.[13-17] These results suggest that the structural properties of SF 6847 change greatly in different circumstances. This paper deals with the dynamic structure of SF 6847 and its derivatives examined by NMR spectroscopic and molecular orbital (MO) studies.

Experimental

All the 3,5-dialkylbenzylidenemalononitriles were prepared by the procedure of Horiuchi *et al.*[18] and purified by chromatography and repeated recrystallization.

Fourier-transformed ^{1}H and ^{13}C NMR spectra were obtained at 90 MHz and 22.5 MHz, respectively, with a JEOL FX-90Q NMR spectrometer.

Molecular orbital (MO) calculations were performed according to the CNDO/2 method.[19] Since the energy of the dissociated forms (anions) of SF 6847 derivatives did not converge in the usual CNDO/2 program, we adopted a modified program using the density matrix method proposed by McWeeny.[20] *Ab initio* (STO 3G) MO calculations were also carried out using the GAUSSIAN 70 program.[21] The standard bond angles and bond lengths were used in the calculation referring to the crystal geometry of SF 6847 determined by X-ray diffractometry.[22]

Results and Discussion

^{1}H and ^{13}C NMR. Figure 1A shows the 90 MHz ^{1}H NMR spectrum of the neutral (undissociated) form of SF 6847 in $CDCl_3$ at room temperature. From the signal intensity in the figure, all the signals are easily assigned; δ 1.46 (*t*-butyl), 6.04 (-OH), 7.65 (benzylidene), and 7.80 (aromatic). The chemical shift

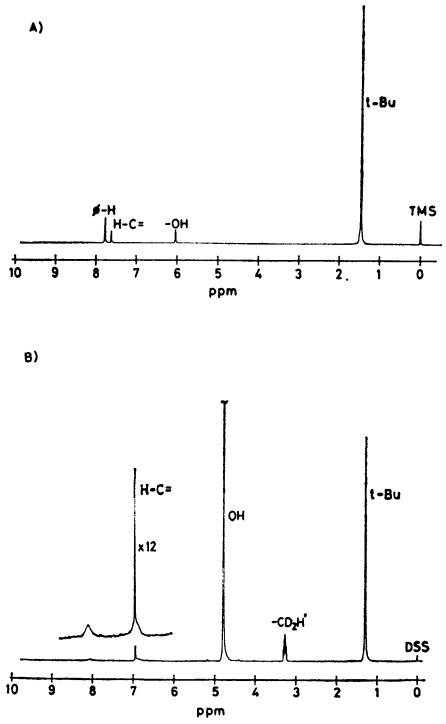


Fig. 1. 90 MHz ^{1}H NMR spectrum of SF 6847 in $CDCl_3$ (A) and in methanol-d_4 containing 0.1 mol dm^{-3} sodium hydroxide (B).

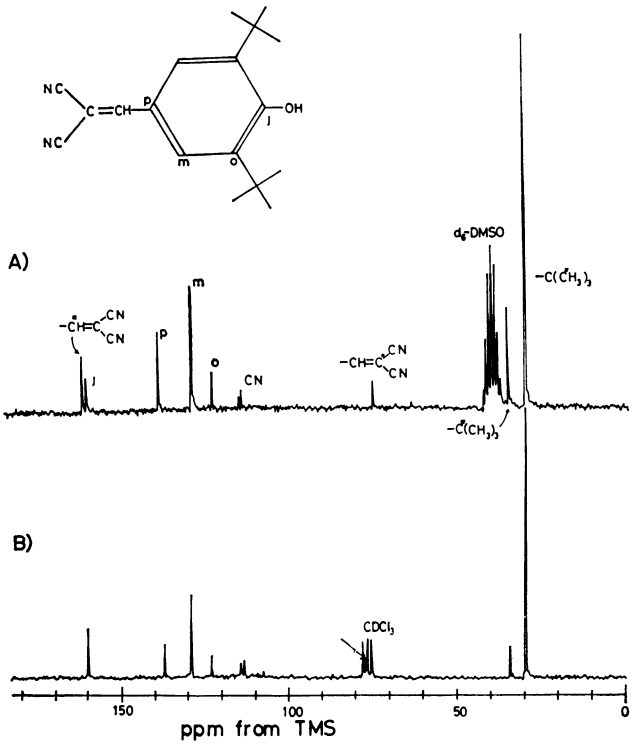


Fig. 2. 22.5 MHz ^{13}C NMR spectrum of SF 6847 in dimethyl-d_6 sulfoxide (A) and in $CDCl_3$ (B).

of the two aromatic protons (meta to the hydroxyl group) is the same in Fig. 1A, and this still exhibited a single resonance when the temperature was lowered to −90 °C in $CDCl_3$ (spectrum not shown). Figure 2 shows the ^{13}C NMR of the neutral form of SF 6847 in $CDCl_3$ (A) and in DMSO-d_6 (dimethyl-d_6 sulfoxide) (B). The carbon signals were assigned as shown in Fig. 2, with reference to the splitting pattern in the proton-coupled spectra (spectrum not shown). It is noted in the figure that the 2- and 3-carbons are equivalent to the 6- and 5-carbons, respectively. The ^1H and ^{13}C NMR spectra indicate that the malononitrile moiety of the neutral form of SF 6847 does not remain fixed in the same plane as the benzene ring.

On the other hand, the ^1H NMR spectrum of the anionic form of SF 6847 in alkaline methanol solution in Fig. 1B shows broad signals of the aromatic protons at 6.9 and 8.1 ppm from DSS. Figure 3 shows the temperature dependence of the ^1H NMR spectrum of the aromatic protons of SF 6847 in the anionic form. As can be seen, the two separate proton signals become narrower when the temperature is decreased to 0 °C from room temperature (23 °C), while they become broad (at 28 °C) and then coalescence into a single resonance (at 58 °C) when the temperature is raised from 23 °C. These results suggest that the malononitrile moiety of the anionic form is less mobile than that in the neutral form. Figure 3 also shows that the signal of the benzylidene proton shifts to a higher field when the temperature is raised. This is attributable to the change of the inter- or intramolecular anisotropic shielding effect of the benzene ring in SF 6847 molecules.

From the temperature dependence of the signal

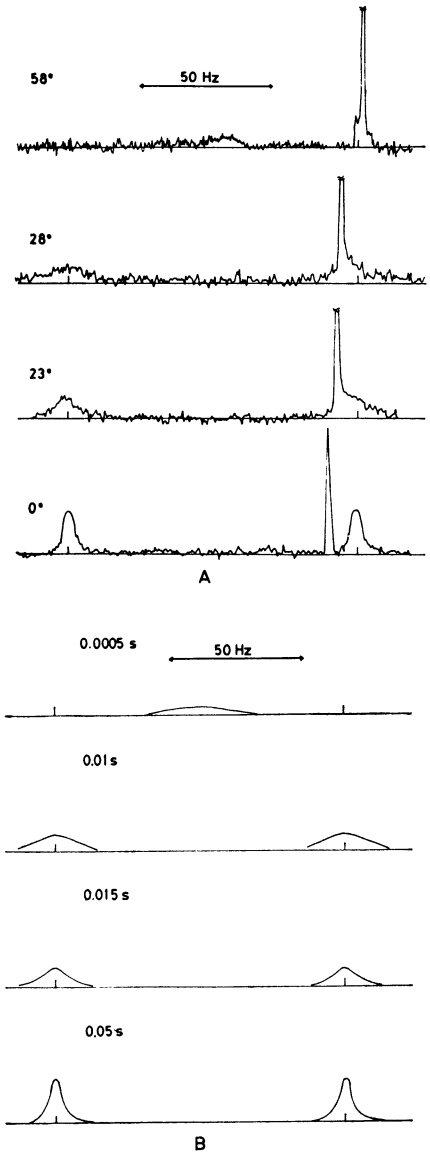
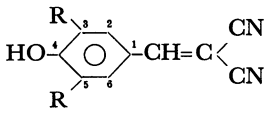


Fig. 3. (A) Temperature dependent ^1H NMR spectra of the benzylic protons of SF 6847 in methanol-d_4 containing 0.1 mol dm^{-3} sodium hydroxide. (B) Calculated spectra for the mean lifetime (τ) indicated. The sharp signal in (A) is due to the benzylic proton.

pattern of the aromatic protons, it is possible to calculate the activation energies of the rotational or tumbling motion around the C–C bond between the benzene ring and the malononitrile moiety. Table 1 gives the calculated activation energies based on a simple line-shaped analysis[23] for SF 6847 and its dialkyl derivatives (**1**—**5**), together with the differences of the chemical shifts between the two aromatic protons at low temperatures and the coalescence temperatures. Table 1



R = H	**1**
Methyl	**2**
Ethyl	**3**
Isopropyl	**4**
t-Butyl	**5**

indicates that the motional barrier tends to increase with increase in the bulkiness of the 3,5-dialkyl groups, and that the activation energy (E_a) of the di-*t*-butyl

Table 1. Activation energies (E_a) in the anionic forms of SF 6847 (**5**) and its derivatives

Compound	R	$\Delta\nu$[a] Hz	T_c[b] K	E_a K J mol^{-1}	ΔE_a[c] K J mol^{-1}
1	H	90	213	42±2	—
2	Methyl	90	233	46±2	4
3	Ethyl	93	251	50±2	4
4	Isopropyl	97	261	54±2	4
5	*t*-Butyl	109	327	64±3	10

a) Difference between the chemical shifts of the two aromatic protons at low temperatures. b) Coalescence temperature. c) Difference between E_a values due to introduction of an additional carbon atom.

derivative **5** (SF 6847) is higher than those of derivatives substituted with smaller alkyl groups (**1**—**4**). It is of interest to note that the energy difference of E_a between **4** and **5** is apparently greater than that between **1** and **2**, or **2** and **3**, or **3** and **4**, indicating that the "steric" effect of the *t*-butyl group is significant.

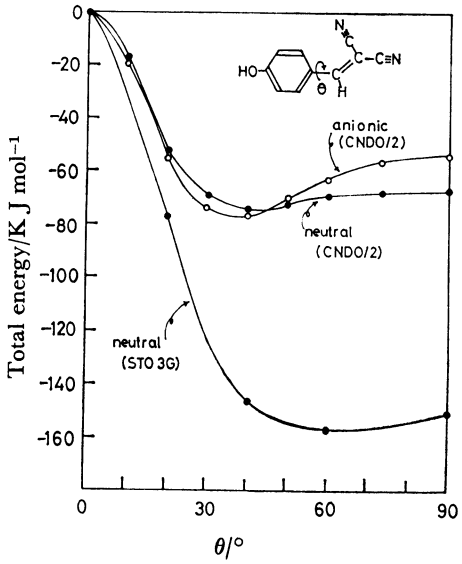


Fig. 4. Changes of the total energy with the angle θ between the benzene ring and the malononitrile moiety in the anionic and neutral forms of **1** calculated using CNDO/2 and *ab initio* (STO 3G) MO calculations. The total energy of the planar form (θ=0°) is standardized to zero.

MO Calculation. In order to gain further insight into the dynamic structure of the SF 6847 molecule, MO calculations were performed for **1**. The changes of the total energy with the angle θ between the benzene ring and the malononitrile moiety in the anionic and neutral forms of compound **1** are shown in Fig. 4. The CNDO/2 calculation shows that the "planar form" (θ=0°) is quite unstable and the minimum energy is found at about θ=40° for both molecular forms. As shown in Fig. 4, essentially the same results were obtained in the *ab initio* calculations, *i.e.*, the planar form is the most unstable conformation. However, the position at the minimum energy determined by the *ab initio* calculation is slightly shifted giving a greater angle θ than that in the CNDO/2 MO calculation. These MO results show clearly that the energy barrier at θ=90° is very small in the neutral form of **1** and a little greater in the anionic form. Similar angular dependence of the total energy based on the MO calculation has also been observed with compound **2**.[17] In view of these results, all the compounds in the series of 3,5-dialkyl derivatives of SF 6847 (compounds **3**, **4**, and **5**) can be regarded as having a similar profile of energy change with θ.

Dynamic Structure. From the above results obtained by NMR and MO calculations, the following conclusions can be drawn concerning the dynamic structure of SF 6847 (**5**) and its derivatives (**1**—**4**.)

(1) The planar conformation (θ=0°) is quite unstable.

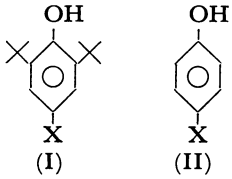
(2) In the most stable conformation of these molecules the benzene ring and the malononitrile moiety are twisted.

(3) The energy barrier at θ=90° is rather small, and thus the malononitrile group can tumble beyond the barrier at θ=90°.

(4) The activation energy of the intramolecular tumbling motion of the malononitrile moiety is greater in the anionic form than in the neutral form.

Here it should be stressed that the degree of conjugation between the benzene ring and the malononitrile moiety in SF 6847 and its derivatives is quite small in solutions. A recent X-ray diffraction study on SF 6847 (**5**)[22] showed that **5** is almost planar in a single crystal. This may be due to the stressed packing of **5** in the crystal, because of the rather strong intermolecular interaction between the benzene ring and the malononitrile moieties of SF 6847 molecules.[22] Difference in the conformation between in crystalline and in solution was also reported for the case of biphenyl.[24]

On Acidity of SF 6847. Next with regard to the acidity of SF 6847 and its derivatives, it is well known[25,26] that introduction of *t*-butyl groups into a molecule near an acidic or basic site causes a large change in acidity or basicity of the molecule. The acid ionization constants (pK_a) of series of 4-substituted 2,6-di-*t*-butylphenols (I) and their unhindered analogues (II) in water and methanol have been reported by some workers.[25,26] It was reported that the hindered



phenol (I) is a weaker acid than its unhindered analogue (II).[27,28] However, in the case of X=–CH=C(CN)$_2$, the acidity of the hindered phenol (R=*t*-butyl, SF 6847) is stronger than that of the unhindered one (R=H, the compound **1**), *i.e.*, the pK_a for SF 6847 is 6.83 and for its unhindered derivatives is 7.25.[27,28] This is explained as follows. As discussed above, in the dissociated (anionic) form the activation energy of the rotational motion around the C–C bond between the benzene ring and the malononitrile moiety of the hindered phenol, SF 6847, is higher than that of the corresponding

unhindered phenol (*cf.* Table 1). Thus, the nonplanarity of the π conjugated system, *i.e.*, the angle between the benzene ring and the malononitrile moiety, is possibly reduced in the hindered form, accompanied with the increase in the activation energy at $\theta=90°$. This implies that the conjugation between the benzene ring and the malononitrile moiety is greater when di-*t*-butyl groups are introduced onto the 3,5-carbons of the benzene ring. Consequently the electron-withdrawing power of the malononitrile moiety is stronger in SF 6847 than in the unhindered analogue. Thus, the *t*-butyl groups electronically enhance the acidity of SF 6847, and this overcomes the steric effect of the bulky *t*-butyl groups which weaken the acidity. Therefore, SF 6847 is more acidic than the unhindered derivative.

It is sometimes pointed out that for exhibiting uncoupling activity, the anionic form of an uncoupler should be present in the hydrophobic biological membrane, and thus that a bulky hydrophobic group adjacent to the acid dissociable proton and the electron-withdrawing group is essential for a potent uncoupler.[7,12,29,30] The unique effect of the hydrophobic *t*-butyl group on the acid dissociation of SF 6847 will explain why SF 6847 is such a very potent uncoupler of oxidative phosphorylation.

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