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^{1}H NMR, ^{13}C NMR, and Computational DFT Studies of the Structure of 2-Acylcyclohexane-1,3-diones and Their Alkali Metal Salts in Solution

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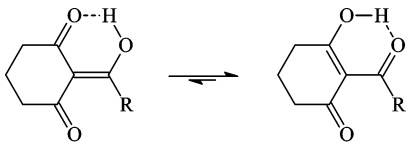

exo-enol *endo*-enol

^{1}H and ^{13}C NMR spectra of 2-acyl-substituted cyclohexane-1,3-diones (acyl = formyl, **1**; 2-nitrobenzoyl, **2**; 2-nitro-4-trifluoromethylbenzoyl, **3**) and lithium sodium and potassium salts of **1** have been measured. The compound **3**, known as NTBC, is a life-saving medicine applied in tyrosinemia type I. The optimum molecular structures of the investigated objects in solutions have been found using the DFT method with B3LYP functional and 6-31G** and/or 6-311G(2d,p) basis set. The theoretical values of the NMR parameters of the investigated compounds have been calculated using GIAO DFT B3LYP/6-311G(2d,p) method. The theoretical data obtained for compounds **1**−**3** have been exploited to interpret their experimental NMR spectra in terms of the equilibrium between different tautomers. It has been found that for these triketones an *endo*-tautomer prevails. The differences in NMR spectra of the salts of **1** can be rationalized taking into account the size of the cation and the degree of salt dissociation. It seems that in DMSO solution the lithium salt exists mainly as an ion pair stabilized by the chelation of a lithium cation with two oxygen atoms. The activation free energy the of formyl group rotation for this salt has been estimated to be 51.5 kJ/mol. The obtained results suggest that in all the investigated objects, including the free enolate ions, all atoms directly bonded to the carbonyl carbons lie near the same plane. Some observations concerning the chemical shift changes could indicate strong solvation of the anion of **1** by water molecules. Implications of the results obtained in this work for the inhibition mechanism of (4-hydroxyphenyl) pyruvate dioxygenase by NTBC are commented upon.

Introduction

Several very efficient herbicides such as mesotrione[1] or sulcotrione[2] being presently in use to control a wide range of broad-leaved and grass weeds in maize belong to the 2-acylcyclohexane-1,3-dione family. The discovery of these active substances was connected with the observation that the California bottlebrush plant excreted a herbicidal compound, causing the bleaching of surrounding plants and preventing their growth. This active substance was found to be leptospermone (2-isovaleryl-4,4,6,6-tetramethylcyclohexane-1,3,5-trione). Later studies evidenced that the mode of action of those herbicides was the inhibition of (4-hydroxyphenyl)pyruvate dioxygenase (HPPD), the enzyme that catalyzes the conversion of (4-hydroxyphenyl)pyruvate to homogentisate. In plants, homogentisate is essential for the synthesis of plastoquinone and α-tocopherol. Lack of those substances causes plant bleaching. In animals, the transformation of (4-hydroxyphenyl)pyruvate to homogentisate, catalyzed by HPPD, is a step of the tyrosine catabolism path, the final products of which are fumarate and acetoacetate, which are excreted in urine. One of the severe human hereditary diseases connected with the tyrosine catabolism is tyrosinemia type I.[3] It is caused by fumaryl acetoacetate hydrolase deficiency, leading to an accumulation of fumaryl and maleyl acetoacetate and their succinyl analogues, resulting

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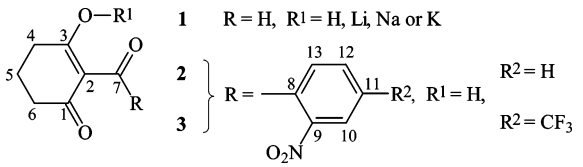
in liver and kidney damage. For a long time, the only effective treatment of this disease was liver transplantation. Progress in the biochemical investigations on benzoylcyclohexadiones led to the discovery that one of them, 2-[2-nitro-4-(trifluoromethyl)-phenyl]cyclohexane-1,3-dione (NTBC), can be successfully applied to treat tyrosinemia type I.[4] Blocking effectively HPPD, it prevents the production of homogentisate and, in consequence, also the hepatotoxic compounds mentioned above.

Many questions concerning the mechanism of inhibition of HPPD by NTBC have already been answered, but many others are still under investigation.[5,6] Undertaking this work, we aimed at checking if NMR measurements, supported by theoretical calculations, could provide new information on the solution structure of NTBC and its analogues.

Results and Discussion

The chemical structures and atom numbering of investigated objects are shown below:



2-Formylcyclohexane-1,3-dione, 1. The size of the NTBC molecule does not impose any limitation on spectroscopic investigations. On the other hand, the level of theory applied in the calculation is dictated by the size of the investigated molecule. Therefore, to obtain reliable results it is reasonable to perform some of the calculations for carefully selected model compounds rather than for NTBC. The investigated object should be as small as possible to enable the use of a high level of theory but still possessing all the important structural features as the object of primary interest. 2-Formylcyclohexane-1,3-dione, **1**, fulfils this demand. It was also expected that the spin−spin interaction of the formyl proton with carbon atoms could give valuable information on the molecule conformation.

In the proton NMR spectra of **1** in $CDCl_3$ or CD_2Cl_2 solution at room temperature two sharp pseudotriplets are observed for H4 and H6 protons, indicating that the rotation around the C2−C7 bond is stopped in the NMR time scale. The same conclusion comes out from the observation that in the ^{13}C NMR spectra of **1** there are separate signals for C1 and C3 as well as for C4 and C6 carbon atoms. This situation is not affected by the concentration changes of **1**. The hydroxyl proton signal in the ^1H NMR spectra is broad, and its half-height-width depends on the concentration: in about 0.5 M solution, $w_{1/2} = 130$ Hz and $\delta = 15.16$ ppm, whereas in the solution diluted 1500 times, $w_{1/2} = 9$ Hz and $\delta = 15.87$ ppm. At the same time, no other changes are observed in the spectra except small downfield shifts of the remaining signals. These observations evidence the intermolecular hydroxyl proton exchange occurring despite the existence of the strong intramolecular hydrogen bond. Expecting that the spin−spin interaction between hydroxyl proton and H7 could give important information on the structure of the investigated compound, we measured the low-temperature spectra of **1** in CD_2Cl_2 solution. Indeed, at −90 °C, when the hydroxyl proton exchange was slowed, the signals of the mentioned protons split into doublets. The line-shape analysis of this AX spin system gave the coupling constant $J = 2.0$ Hz. Even at the lowest temperature applied, the spectra gave no evidence for the restriction of the cyclohexane ring inversion.

TABLE 1. Experimental and Calculated ^{13}C Chemical Shifts (ppm) for 1 in $CDCl_3$ Solution

carbon number	δ_{exp}	δ_{calc}[a]	σ_{endo}[b]	σ_{exo}[b]
1	195.1	194.4	−17.7	−18.3
2	113.8	112.1	66.2	65.0
3	195.6	196.8	−18.9	−29.7
4	31.2	30.8	149.6	142.8
5	19.3	20.5	159.3	159.9
6	36.5	36.5	143.2	142.0
7	191.3	191.8	−16.9	−1.7

[a] Chemical shifts calculated from eq 1. [b] Theoretical absolute values of carbon atom shielding constants [GIAO DFT B3LYP/6-311G(2d,p) PCM].

To achieve a deeper interpretation of the obtained NMR data, the optimum structure of **1** has been calculated using the DFT B3LYP/6-311G(2d,p) method. The influence of solvent has been described by the polarizable continuum model (PCM).[7] The calculations show that the enolic proton exists in the potential with two minima, which correspond to exo and endo forms of the investigated enol. The calculated energy of the *exo*-enol is higher by 2.8 kJ/mol than that of *endo*-enol, which suggests that the endo form of **1** should prevail in solution. This is in agreement with the literature data concerning cyclic α-formyl ketones.[8] To get further evidence that could verify the above conclusion, the NMR parameters have been calculated for both tautomers of **1**. We have found the values of the coupling constants between OH and H7 protons in both tautomers to be: $J_{exo} = 12.3$ Hz and $J_{endo} = 0.4$ Hz. From these values and the experimental value $J_{exp} = 2.0$ Hz, the population of the *endo*-enol could be estimated to be about 0.87. Moreover, we have found that the experimental values of the carbon chemical shifts of **1** can be accurately reproduced using the calculated absolute values of the shielding constants for both enols (Table 1) by means of the following equation:

$$\delta_{exp} = a[x\sigma_{endo} + (1 - x)\sigma_{exo}] + b \qquad (1)$$

The values of the adjustable parameters, $a = -0.9817$, $b = 176.9$ ppm, and $x = 0.88$ (std dev = 0.97, $R = 0.9999$), were found using the least-squares method. The population of e*ndo*-enol, *x*, calculated in this manner, is in full agreement with the former estimation. It is noteworthy that all the above results indicate that the population of the tricarbonyl tautomer of **1** in solution to be negligible, if any.

Alkali Metal Salts of 1. Tricarbonyl compounds of the type discussed here are acidic ($pK_a \sim 3-4$).[9] Thus, one may expect that in living organisms they exist, exclusively or predominantly,

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TABLE 2. Dissociation Effect Caused by Changes of Counterion and Measurement Conditions Monitored by ^{13}C Chemical Shifts for the Salts of 1 in DMSO-d_6

	carbon atom				
$\Delta\delta$	1,3	7	2	4,6	5
δ_{Li}	197.7	188.5	117.2	39.1	21.4
$\delta_{Li} - \delta_{Na}$	0.7	0.1	0.0	−0.6	−0.3
$\delta_{Li} - \delta_{K}$[a]	1.6	1.1	0.0	−0.8	−0.4
$\delta_{Li} - \delta_{K}$[b]	2.0	1.4	0.1	−0.8	−0.4
$\delta_{Li} - \delta_{K}$[c]	2.56	1.98	0.26	−1.0	−0.5
$\delta_{Li} - \delta_{anion}$[d]	3.9	4.2	0.9	−0.9	−0.5

[a] Concentration = 0.15 M. [b] Concentration = 0.03 M. [c] Concentration = 0.03 M + kryptofix 222. [d] Theoretical values calculated using GIAO DFT B3LYP/6-311G(2d,p) PCM basis set.

in anionic form. Therefore, an investigation of the structure of their salts may be useful in understanding their mode of action toward enzymes. For this reason, we prepared lithium, sodium, and potassium salts of **1** and recorded their NMR spectra in a DMSO solution with a concentration of about 0.15 M. Additionally, the spectra of the diluted solution of the potassium salt (about 0.03 M) and of the same solution with the addition of the potassium cation complexing agent, kryptofix 222, were measured. Theoretical calculations of molecular geometries and carbon chemical shifts for the lithium salt and for the free anion of **1** were also performed. The results of these measurements and calculations are collected in Table 2.

The following conclusions can be drawn from the obtained data:

1. The observed carbon chemical shifts represent the averaged values of the chemical shifts of the associated salt and free anion, dependent on the populations of these two species. They change monotonically going from lithium salt to diluted potassium salt containing the potassium cation complexing agent, kryptofix 222. These changes may reflect the degree of dissociation, which is small in the former case and probably complete in the latter, though the influence of the cation size on the carbon chemical shifts of the associated form should also be considered.

2. In the free anion, sp^2 carbon atoms are deshielded, whereas sp^3 carbon atoms are shielded when compared with those of the lithium salt. This observation is in agreement with theoretical calculations. The size of the chemical shift difference between lithium salt and free anion, however, is only roughly reproduced.

3. The calculated molecular geometries of the main parts of the free anion, the lithium ion pair and **1** itself are very similar. All oxygen and carbon atoms in these molecules, with the exception of C5, are located on the same plane. It would suggest that the stabilizing effect of the π electron delocalization is stronger than the destabilizing effect caused by the repulsion of the negatively charged oxygen atoms. In the case of lithium salt, the chelating effect of the lithium cation should also be taken into consideration. The importance of this effect is reflected in the barriers to the formyl group rotation in the lithium salt, when compared with that for the free anion (see below).

4. The hydrogen bond between the aldehyde proton and the ring carbonyl group may participate in the stabilization of the planar conformation of the anion. Calculated bond lengths suggest that this interaction is stronger in the free anion and in the lithium salt than in **1** (see Supporting Information, Table S2).

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TABLE 3. Comparison of Calculated and Experimental Effect of Water Solvation on the Carbon Chemical Shifts of the Free Anion of 1

	C1,C3	C7	C2	C4,C6	C5
δ_{D_2O}[a]	205.2	191.8	119.1	39.9	22.4
δ_{DMSO}[b]	195.2	186.5	116.9	40.0	21.9
$\delta_{D_2O} - \delta_{DMSO}$	10.0	5.2	2.2	−0.1	0.4
$\sigma_{DMSO} - \sigma_{H_2O}$[c]	4.7	2.8	0.2	−2.8	−0.6

[a] Measured for the potassium salt of **1**. [b] Measured for 0.03 M potassium salt of **1** with kryptofix 222. [c] Absolute values of shielding constants calculated for the anion of **1** in which each oxygen atom is solvated by one molecule of water.

Triketones, used as biologically active agents, are to work in water media. Thus, it seemed interesting to perform the appropriate measurements for water solutions and to compare their results with those described above. Therefore, the ^{13}C NMR spectrum of the potassium salt of **1** dissolved in D_2O has been measured. It may be expected that in this solvent the salt is completely dissociated. Strangely enough, the observed chemical shifts, especially those for oxygen-bonded carbon atoms, were remarkably different from those obtained for potassium salt with kryptofix in DMSO. This discrepancy might have an origin in the specific solvation of the carbonyl oxygen atoms with water molecules. To verify this hypothesis, we performed the geometry optimization of a solvate of the **1** anion, in which each of its carbonyl oxygen atoms was hydrogen-bonded with one water molecule, and then we calculated magnetic shielding constants for the carbon nuclei in such a species (Table 3).

One may find that the calculated changes of the chemical shifts caused by the solvation mimic experimental effects observed for potassium salt solutions in DMSO and D_2O. The importance of the water solvation for the carbonyl carbon chemical shift can also be illustrated by the data for acetone (see Supporting Information, Table S3). The carbonyl carbon signal of acetone is strongly downfield-shifted in D_2O solution when compared with its position in DMSO. All the above observations indicate that in water solutions carbonyl oxygen atoms strongly interact with solvent molecules.

Barrier to Formyl Group Rotation in the Lithium Salt of 1. NMR spectra of all investigated salts of **1** in DMSO recorded at ambient temperature indicate fast rotation about the C2−C7 bond in the NMR time scale. It was, however, observed that for lithium salt at 27 °C, the common signal of C1 and C3 is broadened. Therefore, a series of low-temperature spectra of this salt in DMF-d_7 has been measured. At −50 °C, the signal mentioned above splits into two relatively sharp singlets ($\Delta\delta$ = 2.0 ppm). In the coalescence region, the s/n ratio achievable in a reasonable measurement time precluded the recording of any reliable line shapes. Nevertheless, neglecting the temperature dependence of the chemical shift difference between the C1 signal and the C3 signal, the rotation rates could be estimated on the basis of the signal broadenings measured at four temperatures above the coalescence point. Then, the free energy of activation of the rotation about the C2−C7 bond could be calculated using the Eyring equation. The determined mean value of $\Delta G^{\ddagger}$ at about 10 °C is 51.5 kJ/mol. It might be surprising that slow rotation does not affect the signals of C4 and C6 and the protons bonded to them; at −50 °C they are still singlet and triplet, respectively. These observations may be rationalized taking into account calculated chemical shift differences between appropriate carbon signals. They are 3.00 ppm for $\delta_{C1} - \delta_{C3}$ (in rough agreement with the experiment) and only 0.17 ppm for $\delta_{C4} - \delta_{C6}$.

The theoretical molecular geometry optimization predicts a planar conformation of the free anion of **1** to be the most stable. It was interesting to check whether it would be possible to confirm this prediction by NMR. For this reason, the low temperature ^{13}C NMR spectra of potassium salt solution in DMF-d_7, with the addition of kryptofix 222, were measured. Splitting of the C1,C3 signal was not observed down to −50 °C, the lowest temperature available for measurements, but at about −40 °C the signal apparently started to broaden. Probably in this case the coalescence temperature is lower despite the fact that the calculated chemical shift difference $\delta_{C1} - \delta_{C3} =$ 5.6 ppm is higher than that for the lithium salt. One may thus conclude that the barrier to the formyl group rotation in the free anion is lower than that for lithium salt. Nevertheless, the observed line broadening supports the proposed planar conformation of the free anion. An attempt has also been made to estimate the barrier to formyl group rotation for **1** in the same solvent. In ^1H NMR spectrum of **1** in DMF-d_7, H4 and H6 protons give a common signal. Carbon atoms C4 and C6 also give one signal that is slightly broadened. Probably, the internal hydrogen bond is broken in a fraction of the solute molecules because of competition of solvent molecules, which are strong proton acceptors. The barrier to the formyl group rotation in the species with intermolecular hydrogen bonding is expected to be lowered. Unfortunately, in the spectrum of **1** in DMF-d_7, recorded at −50 °C, C4 and C6 carbon atoms still give a broad common signal, which is additionally almost completely covered by the residual CHD_2 multiplet of the solvent. This, in practice, precluded the calculation of the barrier to rotation about the C2−C7 bond in **1**.

2-(2-Nitrobenzoyl)cyclohexane-1,3-dione 2. A successful interpretation of the results obtained for **1** prompted us to apply the similar procedure to investigate a more complex object, triketone **2**, which is much closer an analogue of NTBC than **1**. In this case, however, the use of 6-311G(2d,p) basis set during geometry optimization was unrealistic. Therefore, the smaller basis 6-31G** was applied at this stage of calculation and the larger one only during the magnetic shielding calculation. It seems that such a compromise remains without noticeable consequences, as judged from the test calculation of shielding constants for the carbons of **1** by two methods mentioned above. Both data sets are very close to each other. Corresponding values are shifted by about 1.8 ppm and excellently correlate ($\sigma'_i = 1.0063\sigma_i - 1.81$, where σ'_i was calculated in smaller basis, std dev = 0.12 ppm, $R = 1.0000$). The experimental carbon chemical shifts of **2** in $CDCl_3$ and the appropriate shielding constants calculated for both of its enolic structures are collected in Table 4. An excellent correlation between the experimental and the theoretical data was obtained using the least-squares method and eq 1: $x = 0.84$, $a = -0.9718$, $b = 174.6$ ppm, std dev = 1.36 ppm, and $R = 0.9997$. This correlation, together with some additional information coming from the proton-coupled ^{13}C NMR spectrum, allowed the unambiguous signal assignment in the carbon spectrum of **2** to be made. Moreover, this analysis has shown that triketone **2**, similarly as **1**, exists in $CDCl_3$ solution predominantly as the *endo*-enol.

The proton and carbon spectra of **2**, in which H4, H6, C1, C3, C4, and C6 have separate signals, indicate restricted rotation around the C2−C7 bond. In the calculated optimum structure of both tautomers of **2**, the carbonyl oxygen atom of the benzoyl group lies close to the plane defined by the C1, C2, C3, and C7 carbon atoms. This oxygen atom participates in the hydrogen bond with the enolic hydroxyl group. The phenyl ring is considerably twisted, relative to the mentioned plane, apparently due to steric reasons. Such a structure is very similar to that determined for **2** in the solid state.[10]

TABLE 4. Experimental and Theoretical ^{13}C Chemical Shifts for 2 and 3 in $CDCl_3$ Solution

carbon number	compound **2**				compound **3**[a]			
	δ_{exp}	δ_{calc}[b]	σ_{endo}[c]	σ_{exo}[c]	δ_{exp}	δ_{calc}[b]	σ_{endo}[c]	σ_{exo}[c]
1	193.9	192.7	−18.5	−19.5	194.1	192.7	−18.7	−19.5
2	112.8	111.4	65.0	65.5	112.7	111.5	64.7	65.7
3	195.6	197.3	−21.7	−32.1	195.8	197.4	−21.9	−32.4
4	31.8	32.3	147.3	142.2	31.6	31.9	147.9	142.3
5	19.1	20.2	158.8	159.2	19.1	20.1	159.1	159.3
6	37.5	37.2	141.5	141.0	37.3	36.6	142.4	141.2
7	197.7	198.4	−25.8	−18.2	196.3	196.7	−24.4	−16.7
8	136.6	138.9	35.9	40.9	139.7	141.8	32.7	37.9
9	145.5	144.8	30.5	31.2	145.5	144.9	30.5	30.3
10	123.6	123.7	52.5	51.6	121.1	122.8	53.3	52.7
11	129.6	127.1	49.0	48.2	132.0	130.8	45.1	44.1
12	134.1	135.0	40.8	40.7	130.8	131.0	44.7	44.7
13	126.7	125.2	51.0	50.0	127.7	125.3	50.9	49.5

[a] $\delta_{CF_3,exp} = 122.6$ ppm; $\delta_{CF_3,calcd} = 128.6$ ppm. [b] Chemical shifts calculated from eq 1. [c] Theoretical absolute values of shielding constants [GIAO DFT B3LYP/6-311G(2d,p) PCM].

2-[2-Nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione 3. To our knowledge, the only NMR data for **3** available in the literature are those reported by Kavana and Moran.[5] These data, however, concern the anionic form of this compound in water solution, and additionally, the carbon chemical shift reference is not given. Therefore, we decided to synthesize NTBC and to record its spectra. The magnetic inequivalence of C1 and C3 and C4 and C6, as well as appropriate CH_2 group protons, indicates that in chloroform the rotation around the C2−C7 bond is slow in the NMR time scale. The transparency of the phenyl proton signals allowed to determine all the chemical shifts and H,H as well as H,F coupling constants using a homemade Laokoon-like iterative program. The multiplets of the cyclohexane ring protons have been analyzed in the same manner as for **1** (see Supporting Information). Most carbon signals could be assigned to the appropriate carbon atoms on the basis of their chemical shifts, intensities, and C,F coupling constants. The signal of carbonyl carbon C7 could be identified, as in the proton-coupled spectrum, that it is split into a doublet owing to a spin−spin interaction with proton H13. All these assignments as well as the assignments of carbon atoms C1, C3, C4, and C6 have been independently made by the method described for **2**, using the results of the theoretical calculations. The analysis of the chemical shifts has shown that, similarly as in two remaining cases, in $CDCl_3$ solution, NTBC exists mainly in the form of *endo*-enol (for eq 1: $x = 0.82$, $a = -0.9693$, $b = 174.37$ ppm, std dev = 1.32 ppm, and cc = 0.9997). The theoretical calculations performed point out that the molecular geometry of **3** is analogous to that of **2**.

It is interesting that according to the calculation results the conformation of the free anion of NTBC (in DMSO solution) is similar to that of a neutral molecule, that is, C2 and all three carbonyl groups occupy roughly the same plane. Apparently, the internal hydrogen bond is not the dominant factor stabilizing this conformation.

The calculations for **3** were done using the same level of theory as in the case of **2** but with one additional constraint.

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Namely, during the geometry optimization, the rotation of the CF_3 group was frozen, assuming its orientation with respect to the aromatic ring plane found by the semiempirical method PM3. This constraint economized the computation time and, simultaneously, was believed to have a marginal impact on the calculation results. Looking through the data in Table 4, one can admit that the calculated chemical shift of the CF_3 carbon deviates from the experimental value. Actually, the data for CF_3 carbon was omitted in the appropriate correlation. We suppose the main reason of this discrepancy is the level of theory at the stage of shielding calculation, which is still too low, rather than the mentioned constraint during geometry optimization. It is well-known that the fluorine substituent is especially demanding as far as shielding calculations are concerned.

Comments on the Inhibition Mechanism of HPPD by NTBC. Recently, Moran et al.[5,6] have performed a comprehensive investigation of the interaction between HPPD and NTBC, proposed an almost complete mechanistic model of the interaction between this enzyme and its inhibitor, and discussed its biochemical consequences. To explain the kinetic data, the authors adopted a three-step mechanism of NTBC binding to the HPPD. After the pre-equilibrium binding step, the bidentate association of NTBC (in a form of *exo*-enol) with the active site ferrous ion of HPPD occurs, which is followed by the irreversible Lewis acid-assisted conversion of the bound enol to the enolate. However, some arguments used by the authors to rationalize the above mechanism cannot be accepted. The authors believe that their NMR spectra prove NTBC to exist in neutral water solution predominantly as the *exo*-enol tautomer.[5] Actually, in view of numerous literature data concerning various β-dicarbonyl and -tricarbonyl compounds (including results of this work), a simple differentiation of two types of tautomers by NMR spectra is unrealistic because of rapid tautomerization. In the case in hand, such a process demands not much more than a short distance proton displacement along the hydrogen bond. Moreover, neither the structure of *exo*-enol nor *endo*-enol ensures the equivalence of the C1 and C3 signals (nor C4 and C6 or appropriate proton signals). Such pseudosymmetry can arise only owing to the rapid, in the NMR time scale, rotation about the C2−C7 bond. Our results for **1**, **2**, and **3** obtained for $CDCl_3$ solutions, as well as numerous literature data,[8] suggest that the predominance of the *endo*-enol is more likely. Furthermore, what is most important, in the case of water NTBC solution, is the above problem becomes somewhat artificial, as this triketone, being a relatively strong acid ($pK_a = 3.1$),[9] at pH 7 exists almost exclusively in the form of the enolate anion (99.99%). Thus, some reinterpretation of the results of ref 5 and the HPPD−NTBC interaction model seems to be unavoidable. In light of our finding concerning strong solvation of the carbonyl oxygens by water molecules, it seems possible that one of the steps of the interaction between NTBC and HPPD, postulated by Moran et al.,[5] is actually the change in the solvation sphere of the triketone anion.

Experimental Section

Low-temperature ^{1}H NMR spectra of 2-formylcyclohexane-1,3-dione (**1**) in CD_2Cl_2 was measured using a spectrometer operating at 11.7 T. All other spectra were recorded using a spectrometer operating at 4.7 T. ^{1}H NMR spectra were recorded using the measurement conditions ensuring the receipt of nonsaturated and nonfolded spectra with sufficient digital resolution and signal-to-noise ratio. In the case of ^{13}C NMR, partially saturated spectra were recorded with the application of WALTZ 16 proton decoupling. For example, the following parameters were used to measure ^{1}H and ^{13}C (in parentheses) NMR spectra of **3** in $CDCl_3$, with a 4.7 T spectrometer: spectral width, 5000 Hz (12 500 Hz); acquisition time, 5 s (1 s); about a 30° pulse width, 6 μs (6 μs); number of scans, 1000 (10 000); and zero filling to 128 K (64 K) before FT. For $CDCl_3$ solutions, the solvent signals were used as the chemical shift reference: $\delta_{\mathbf{CH}Cl_3} = 7.26$ ppm for proton spectra and $\delta_{\mathbf{CDCl_3}} = 77.0$ ppm for carbon spectra. In the case of D_2O and DMSO-d_6 solutions, the spectra were calibrated relative to 3-(trimethylsilyl)-propionic acid-2,2,3,3-d_4 sodium salt used as the internal reference.

2-Formylcyclohexane-1,3-dione (1). The compound was prepared according to the literature procedure.[11] $Bp_{0.3} = 69-70$ °C. Pale yellow liquid changing color to brown after a few days even when stored at about 5 °C in a sealed ampule. No such change was observed when it was stored as a solid at about −15 °C. For NMR data, see Supporting Information.

2-(2-Nitrobenzoyl)cyclohexane-1,3-dione (2). The compound was obtained following the literature procedure[12] by *O*-acylation of cyclohexane-1,3-dione with 2-nitrobenzoyl chloride, followed by the rearrangement of the enol ester to the *C*-acyl isomer on treatment with sodium cyanide and triethylamine. Crude, beige triketone was dissolved in a water solution of sodium bicarbonate. The solution was stirred for a few minutes with charcoal at room temperature, filtered, and acidified with concentrated hydrochloric acid. A precipitated colorless solid was filtered, washed with water, and dried over P_2O_5 in a vacuum. Mp 143−144 °C (lit.[10] 135−137 °C). The carbon spectrum of **2** was identical with that given in the literature.[10]

2-(2-Nitro-4-trifluoromethylbenzoyl)cyclohexane-1,3-dione (NTBC; 3). The compound was prepared in the same manner as **2.** The synthesis of an appropriate benzoic acid derivative was started from the transformation of commercially available 2-nitro-4-trifluoromethylaniline into benzonitrile by the classical Sandmeyer method. Then the nitrile was hydrolyzed in 65% sulfuric acid to give 2-nitro-4-trifluoromethylbenzoic acid.[13] The obtained triketone **3** had a mp of 140−142 °C (lit.[14] 141−143 °C). For NMR data, see Supporting Information.

Alkali Metal Salts of 1. Formyldiketone **1** (2 mmol) and K_2CO_3, Na_2CO_3, or LiOH (2.1 mmol) were dissolved in 3 mL of distilled water. The solution, if colored, was stirred for a few minutes with charcoal at room temperature, filtered, and evaporated at reduced pressure. The residue was triturated thoroughly with ether to remove any traces of nonionic compounds (if present) and then with 5 mL of methanol. The solution was filtered out from insoluble inorganic salts, which were then washed with 2 mL of methanol. The combined filtrate was evaporated to dryness at reduced pressure. The residue was dried in a vacuum over P_2O_5, yielding white stable crystals. The obtained products were completely soluble in dry DMSO-d_6, and in their carbon NMR spectra, no other signals than what were expected were observed.

Theoretical Calculations. All theoretical calculations of the optimum geometries of the investigated species and NMR parameters were performed using the Gaussian 03 program,[15] employing the DFT-based approach with B3LYP functional and 6-311G(2d,p) basis set. Only during geometry optimization of the tautomers of **2** and **3** was the smaller basis 6-31G** used. Calculations of the NMR parameters were done by GIAO method. In all these calculations, the impact of the solvent was taken into account using the PCM of

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Tomasi et al.,[7,16] with the explicit treatment of hydrogens (radii taken from the UFF force field[17]) as implemented in the Gaussian program.

The comparison of the experimental ^{13}C NMR chemical shifts with the calculated isotropic shielding constants based on eq 1, the line-shape analysis of the appropriate spectral fragments, and the Laokoon-type analysis of some spectral patterns were performed using the nonlinear least-squares method and the homemade programs written in Fortran, exploiting the Newton−Rapson algorithm.

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Supporting Information Available: Calculated optimum geometries and free energies of the *endo*- and *exo*-enols of the investigated compounds **1**, **2**, **3** and the free anion and lithium salt of **1** in solutions. ^{1}H and ^{13}C NMR data for **1** and **3**. ^{1}H and ^{13}C NMR spectra of **1** and **3** in $CDCl_3$, and the lithium salt of **1** in DMSO-d_6. Calculated C7−H7 bond lengths and H7···O═C1 distances in the lithium salt and the free anion of **1**, Table S2. Influence of water solvation on carbon chemical shifts in acetone, Table S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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