

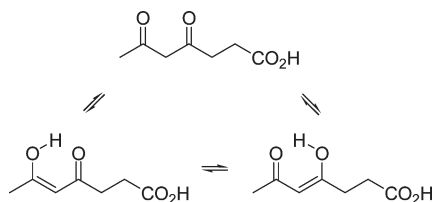
## Solution Structure of Succinylacetone, An Unsymmetrical $\beta$ -Diketone, As Studied by $^{13}\text{C}$ NMR and GIAO–DFT Calculations

Dominika Bal,\* Anna Kraska-Dziadecka, and Adam Gryff-Keller

Warsaw University of Technology, Faculty of Chemistry, Noakowskiego 3, 00-664 Warsaw, Poland

dbal@ch.pw.edu.pl

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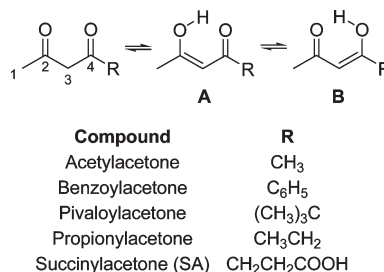
The enolization degrees of succinylacetone, an important heme biosynthesis inhibitor, have been determined in  $\text{CDCl}_3$  and water solutions using  $^1\text{H}$  NMR. The solution structures of SA have been investigated using a combined NMR/theoretical [GIAO DFT PBE1PBE/6-311++G(2d, p) PCM] approach. The populations of both enolic forms undergoing enol–enol equilibriums for SA and a series of unsymmetrical  $\beta$ -diketones have been established by a quantitative comparison of the experimental  $^{13}\text{C}$  NMR chemical shifts and calculated shielding constants. Moreover, using the same method and considering various trial structures differing in conformation and/or hydration of neutral SA molecule as well as its monoanion and dianion the structures of the most abundant species being present in the investigated water solutions have been deduced.

### Introduction

Theoretical calculations of NMR parameters and the experiment/theory correlation enable investigating various details of molecular structures and conformational equilibriums in gas as well as in condensed phases. In this paper, we intend to present a combined NMR/DFT study on succinylacetone (SA; 4,6-dioxoheptanoic acid) in  $\text{CDCl}_3$  and in water solutions of various acidities. This diketoacid is an effective inhibitor of heme biosynthesis and simultaneously a pathognomonic metabolite causing serious liver and kidney damage in organisms of children suffering from tyrosinemia type I, a severe genetic illness.<sup>1</sup> This compound was in the past a subject of various biochemical

and analytical investigations and numerous procedures of its determination for medical analyses were elaborated.<sup>2–6</sup> On the other hand, very few physicochemical investigations of this compound have been reported.<sup>7,8</sup>

### SCHEME 1. The Keto/enol Tautomerism of $\beta$ -Diketones



SA, being a  $\beta$ -diketone, exists in solutions as an equilibrium mixture of its diketone and ketoenolic forms undergoing tautomeric transformations, which are usually slow in

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TABLE 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Chemical Shifts of SA and Its Anions in Chloroform and Water Solutions

labels	chloroform		water				
	ketoenole	diketone	pH 2.5		pH 7.1		pH 11.5
			ketoenole	diketone	ketoenole	diketone	
$\delta(^{13}\text{C})/\text{ppm}$							
1	23.62	27.66	26.27	33.40	26.90	33.33	29.64
2	187.21	202.21	193.13	211.14	195.38	211.44	198.72
3	99.73	57.80	103.02	59.31	102.82	59.31	103.29
4	194.98	202.21	198.86	210.46	198.01	211.44	197.66
5	33.37	37.66	35.97	41.14	37.18	42.69	41.07
6	28.61	30.77	31.90	30.67	35.61	33.82	37.61
7	178.23	178.30	180.27	180.13	184.12	183.95	185.39
$\delta(^1\text{H})/\text{ppm}$							
1	2.020	2.230	2.068	2.267	2.093	2.281	2.142
3	5.510	3.623	5.754	3.864	5.741	3.866	5.250
5		2.801		2.911	2.601	2.827	
6	2.651	2.651	2.682	2.619	2.464	2.427	2.36–2.39

the NMR time scale.<sup>9</sup> It is noteworthy that in the case of unsymmetrical compounds, two *cis*-enolic forms, A and B, are structurally different (Scheme 1). The A  $\leftrightarrow$  B interconversion processes are very fast, as the energetic barrier separating these two forms is very low.<sup>9–12</sup> There is little chance to investigate such an equilibrium using classical chemical methods or to freeze out the exchange in the NMR time scale still keeping the system in the liquid phase. In water solutions, this diketoacid can exist as a neutral molecule, monoanion, or dianion, dependent on the solution acidity. The first two of these forms are in tautomeric equilibria. Moreover, SA molecules undergo various fast conformational processes and probably a specific hydration. Thus the investigated system is rather complex, and a more involved interpretation of the NMR data supported by quantum-chemical calculations has required adopting some simplifying assumptions. Nevertheless, a lot of valuable information concerning the solution structure of SA could be obtained, as reported below.

## Results and Discussion

**Diketone–Ketoenole Equilibrium.** As it is well-known, the tautomeric keto–enol transformations of  $\beta$ -diketones are usually slow processes in the NMR time scale. As a result, the NMR spectroscopy is a method of choice for investigating such systems and has frequently been exploited.<sup>9</sup> The  $^1\text{H}$  as well as  $^{13}\text{C}$  NMR spectra of SA are simply the superpositions of the two solute forms present in the solution (Table 1). In the investigated case, making the spectral assignments has been a trivial task when using  $^1\text{H}$ – $^{13}\text{C}$  HMBC spectra. The populations of the two SA forms could be established by integrating the appropriate signals in  $^1\text{H}$  NMR spectra. This simple method appeared to be effective for SA in  $\text{CDCl}_3$ , as well as for SA and  $\text{SA}^-$  in water solutions. The results show that the ketoenole prevails in  $\text{CDCl}_3$  (diketone/ketoenole = 1/3), whereas the diketo form is more abundant in water solutions (diketone/ketoenole = 5 in solution of pH 2.5 and

7 in solution of pH 7.1). Such a solvent polarity effect was observed many times for other  $\beta$ -diketones.<sup>9,10,13</sup> It is noteworthy that in water solutions the equilibrium state is practically the same for SA and  $\text{SA}^-$  species.

**Enol–Enol Equilibrium.** The equilibrium populations of the enolic forms present in the investigated solution can be essentially established by using UV or IR spectroscopy or any other physical method connected with the sufficiently short time scale, provided that all the specific experimental limitations could be overcome. It is, however, possible to get the desired information using NMR spectroscopy as well. The method relies upon comparing the experimentally accessible averaged values of NMR parameters with the values estimated for individual enolic forms A and B. Such limiting values were usually deduced from the data for model compounds. Such an approach was used by Garbish et al.<sup>14</sup> to establish the enol–enol equilibrium of  $\beta$ -ketoaldehydes based on the chemical shift of aldehydic proton. The analogous procedure based on  $^{13}\text{C}$  NMR chemical shifts of the oxygen-bonded carbons was then applied to unsymmetrical  $\beta$ -diketones.<sup>15–17</sup> The method based on  $^{17}\text{O}$  NMR chemical shifts was also proposed. This method seems to be potentially the most reliable due to large spectroscopic effects accompanying the considered transformation.<sup>17–19</sup> Unfortunately, the method cannot be used in the case of water solutions or solutions in other oxygen-containing solvents. Finally, it was shown that the deuterium isotope effects observed in  $^{13}\text{C}$  NMR can also be used as a source of information on enol–enol equilibrium.<sup>19,20</sup> The whole area of applications of NMR spectroscopy in investigations of tautomeric equilibria was recently thoroughly reviewed by Claramunt et al.<sup>9</sup>

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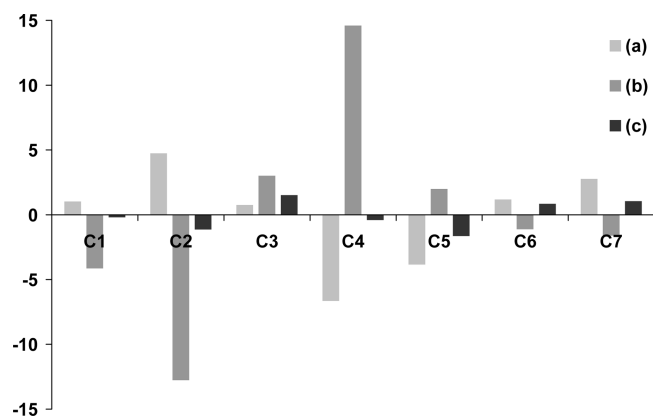
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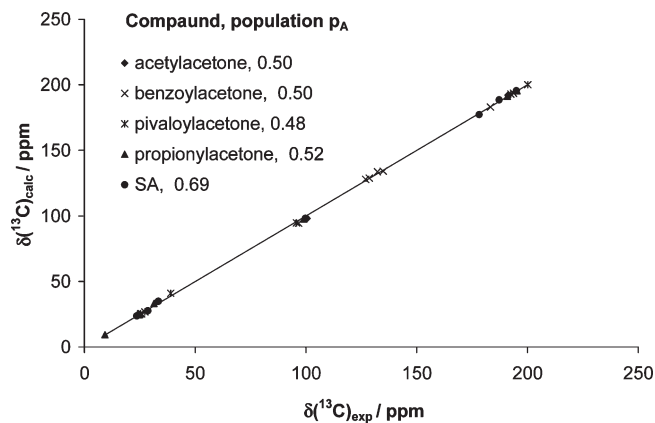
All the mentioned above investigations of the enol–enol equilibria assumed the knowledge of the limiting values of the spectral parameter carrying the desired information. Such limiting values were, however, experimentally accessible for model compounds but not for the investigated objects. This difficulty can be nowadays overcome by using theoretical methods. Reproducibility of the experimental values of NMR parameters by the GIAO–DFT calculations is already good enough to investigate systems in fast dynamic equilibrium by modeling the observable spectrum as a weighted average of the spectra of species undergoing exchange. In the case of  $\beta$ -diketones, such an approach was applied by Moon and Kwon,<sup>21</sup> who estimated the enol–enol equilibrium constants on the basis of  $^{17}\text{O}$  NMR chemical shifts. The authors used the GIAO-HF/6-31\*\* and the GIAO–DFT B3LYP/6-31G\*\* levels of theory in their calculations. Recently, Cornago et al.<sup>22</sup> used the same level of theory to investigate a series of curcuminoids and three model  $\beta$ -diketones on the basis of  $^{13}\text{C}$  NMR chemical shifts of the oxygen-bonded carbons. The analysis of all  $^{13}\text{C}$  NMR chemical shifts was previously successfully applied also in our laboratory to three triketones: 2-formyl-1,3-cyclopentanedione,<sup>23</sup> 2-formyl-1,3-cyclohexanedione, and 2-[2-nitro-4-(trifluoromethyl)benzoyl]-1,3-cyclohexanedione.<sup>24</sup>



**FIGURE 1.** Deviations of the experimental  $^{13}\text{C}$  chemical shifts of SA in  $\text{CDCl}_3$  solution from the regression lines representing the experiment/theory correlations under the assumption that: (a) only the tautomer A, (b) only the tautomer B, (c) the optimal composition of both tautomers, is present in the investigated solution.

To avoid any doubts whether the differences between chemical shifts of the considered tautomers A and B are sufficient to define their populations well, we show the fits between the  $^{13}\text{C}$  NMR chemical shifts and the theoretical shielding data for SA in  $\text{CDCl}_3$ , achievable under three different assumptions. Figure 1 shows the deviations from the experiment/theory regression lines when assuming that either tautomer A, or tautomer B or the optimal composition of both tautomers is present in the investigated solution. To make the results obtained for the main object of our interest more credible, we have collected the similar experimental

data and performed the similar data analysis for a series of  $\beta$ -diketones (Scheme 1), namely acetylacetone, benzoylacetone, pivaloylacetone, and propionylacetone, investigated earlier by other authors.<sup>15–19,21,22</sup> The tautomer populations given in Figure 2 have been obtained by performing the data analysis for all the investigated objects simultaneously (see Experimental Section). The obtained fit between the experiment and theory is excellent (Figure 2).



**FIGURE 2.** Comparison of the experimental  $^{13}\text{C}$  chemical shifts and calculated shielding constants for ketoenolic forms of the investigated  $\beta$ -diketones in  $\text{CDCl}_3$  solutions and the determined populations  $p_A$  of the A ketoenolic forms (the estimated population errors are  $\pm 0.02$ ).

Thus, in accord to the most of the earlier reports,<sup>15–19,21,22</sup> we have found that in the investigated series in  $\text{CDCl}_3$  the populations of the A and B type enols are roughly equal (the equilibrium constant close to unity). There are, however, some exceptions, and SA is among them, in which one of the tautomers apparently prevails.<sup>25</sup> As it will be shown below, the same is observed for SA in water solutions. A rationalization of this result, however, does not seem to be straightforward.

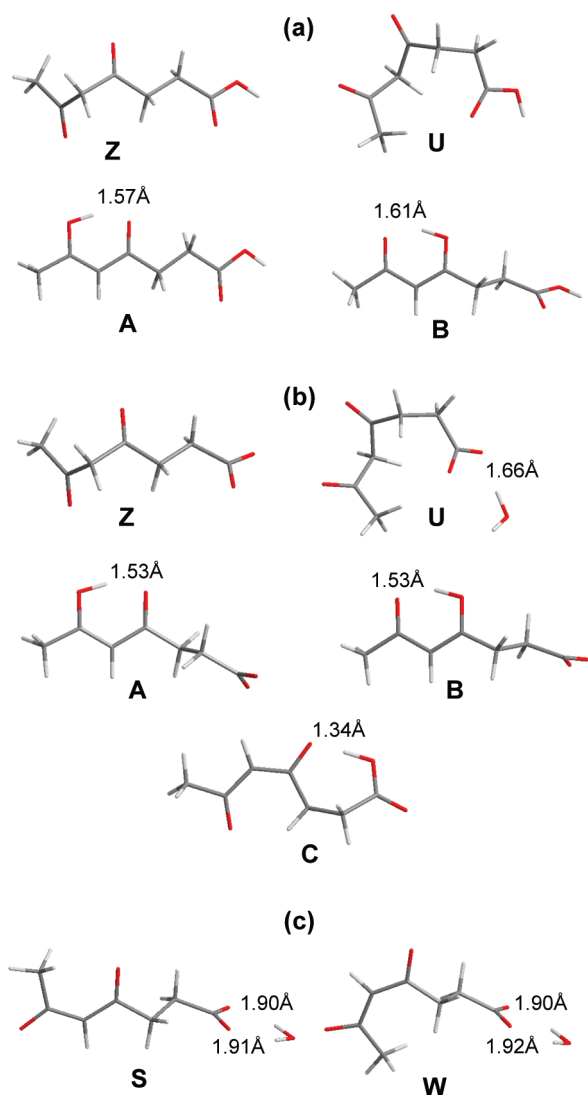
**Succinylacetone as Diacid.** The SA molecule possesses two relatively easily removable hydrogens, and so in water solutions this molecule can exist in three forms: SA,  $\text{SA}^-$ , and  $\text{SA}^{2-}$ , in proportions dependent on the solution acidity. The potentiometric titration of 0.01 M water solutions of SA yielded two acidity constants:  $\text{p}K_{0/1} = 4.5$  and  $\text{p}K_{1/2} = 9.9$ . These values were determined for relatively high solute concentrations, so they are not thermodynamic constants. Taking these values into account, we selected water solutions of pH 2.5, 7.1, and 11.5 for our NMR measurements. In such solutions, we deal exclusively with the neutral, monoanionic, and dianionic forms of SA, respectively. The  $^{13}\text{C}$  NMR chemical shifts for five easily recognizable species (SA and  $\text{SA}^-$  as diketone and ketoenole, and  $\text{SA}^{2-}$ ) encountered in water solutions of the selected acidities are collected in Table 1.

By comparing the  $^{13}\text{C}$  chemical shifts of the appropriate carbons of the neutral diketonic form of SA and its monoanion (Table 1), one finds rather small changes, difficult to be commented upon using a qualitative approach. The same is true for the neutral ketoenol, its monoanion and, which is rather surprising, the SA dianion. Nevertheless,

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**SCHEME 2. Calculated Molecular Geometries of Various Forms of: (a) SA<sup>0</sup>, (b) SA<sup>-</sup>, and (c) SA<sup>2-</sup> Expected to Be Present in the Investigated Solution**



the existing differences do carry interesting structural information when treated quantitatively (see the next paragraph).

In the solution of pH = 11.5, i.e., at the highest applied pH, after a couple of hours, one can observe the appearance of decomposition products. Both <sup>1</sup>H and <sup>13</sup>C NMR spectra show that these are acetate, acetone, succinate, and levulinate forming in almost equimolar proportions. At elevated temperatures, this decomposition is complete within an hour.

**Solution Conformation and Specific Hydration.** It is obvious that flexible molecules, such as the molecule of SA, exist in solutions as a complex mixture of conformers undergoing mutual transformations. In water solutions, SA can additionally form various hydrogen bonded solute–solvent complexes. All these species influence the measured values of the chemical shifts to the extent proportional to their abundances. Including this whole “zoo” of the structures into the theoretical shielding calculations and data analysis is simply impossible. Nevertheless, it seemed worthwhile to consider

at least the species most likely to be present in the solution in high amounts.

We have found that theoretical reconstruction of <sup>13</sup>C NMR spectra of all the investigated water solutions, when only a single conformer for diketonic forms of SA<sup>0</sup> and SA<sup>-</sup>, A and B type enoles of SA<sup>0</sup> and SA<sup>-</sup>, and one of the four planar conformations of SA<sup>2-</sup> dianion were considered, was only moderately successful. The overall fit between the experimental and theoretical data achieved during the analysis was essentially acceptable (rmsd ~ 2.0 ppm), however, several data points deviated from the regression line by ca. 3 ppm and at least one by more than 4.5 ppm.

A substantial improvement of the fit for the ketoenol monoanion has been obtained when the contribution of the structure C (Scheme 2) was taken into account. The final analysis of the chemical shift data showed that the structure C of SA<sup>-</sup> was actually more abundant in the solution than the structures A or B (Table 2). Such a result may seem surprising, but it is to be stressed that the calculation shows that it is the structure C that has the lowest energy (2.1 kJ/mol lower than A and 2.0 kJ/mol lower than B). The hydrogen bond between carboxyl and carbonyl oxygens in C must be relatively strong, as judged from the calculated interatomic distances. The occurrence of the anion C in solutions does not obviously mean that it is formed by the proton loss from the enol –OH group. This anion form can appear after dissociation of the –COOH proton, followed by the stabilization of the anion structure as well.

**TABLE 2. Populations of Various Species Present in Water Solutions of SA as Determined by the Analyses of the Experimental <sup>13</sup>C NMR Chemical Shifts<sup>a</sup>**

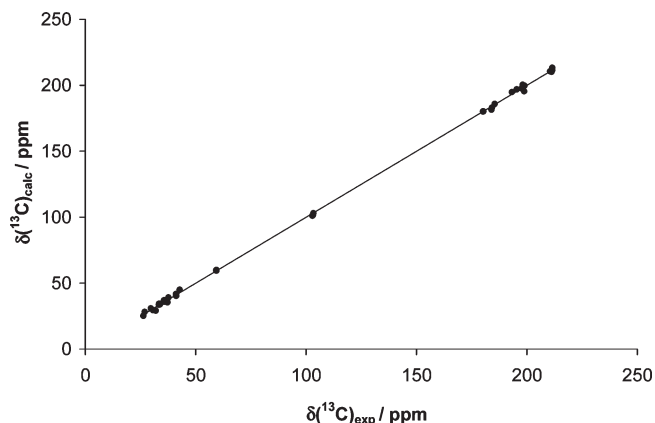
pH	species	ketoenole			diketone	
		A	B	C	Z	U
2.5	p(SA <sup>0</sup> )	65	35		24	76
	p(SA <sup>0</sup> ) <sub>t</sub>	65	35		26	74
	Δ	2	2		11	11
7.1	p(SA <sup>-</sup> )	14	31	55	33	67
	p(SA <sup>-</sup> ) <sub>t</sub>	12	31	57	34	66
	Δ	6	2	6	12	12
	species	W		S		
11.5	p(SA <sup>2-</sup> )	63		37		
	p(SA <sup>2-</sup> ) <sub>t</sub>	57		43		
	Δ	8		8		

<sup>a</sup>p(X): Values based on the data from the given solution. p(X)<sub>t</sub>: Values based on the simultaneous analysis of all the data. Δ: Errors estimated for the values from the preceding row.

In the case of diketonic forms of SA and SA<sup>-</sup>, the meaningful improvement of the agreement between the experiment and theory has been obtained when the U-type conformers (Scheme 2) were included into analysis. In water solution, such conformers also reflect the energy minima and one may speculate that they are stabilized by the high polarity of the solvent and/or specific solvation. The chemical shift data analysis shows that the contributions of the U-type conformations of the considered species to the equilibrium state are substantial (Table 2).

We have also examined whether including one or two water molecules hydrogen-bonded to the solute molecule into theoretical calculations (the discrete

solvation model<sup>26–30</sup>) can improve the experiment/theory agreement. The calculations performed were aimed at finding the direction and a rough estimate of the hydration effects only. Eventually, we have found out that including one H<sub>2</sub>O molecule hydrogen-bonded to the –COO<sup>–</sup> group does improve the overall experiment/theory agreement, although the calculated effect is not very large.



**FIGURE 3.** Comparison of the experimental <sup>13</sup>C chemical shifts and calculated shielding constants for SA in water solution of various pH.

When all the structures proposed above were taken into account, the overall experiment/theory agreement was much better than initially: rmsd = 1.38 ppm, with only one data point deviating remarkably (3.2 ppm) from the regression line (Figure 3). Even better fits were obtained when the data for neutral molecules, monoanions, and dianion were analyzed separately (Table 3). The apparent difference could be caused by the lower ratio of the number of data points to the number of unknowns. But it also seems likely that the differently charged species demand different scaling parameters (see Table 3). Anyway, the population parameters (Table 2) remain almost unaffected by the method by which the data were analyzed.

**TABLE 3.** Regression Parameters for Correlations of Various Sets of the Experimental <sup>13</sup>C NMR Chemical Shifts of SA in Water Solutions with the Shielding Constants Calculated Using GIAO–DFT PBE1PBE/6-311++G(2d,p) Method;<sup>a</sup>

data set	slope	$\sigma_{\text{ref}}$	rmsd	$d/u$
SA <sup>0</sup>	0.985	189.5	1.02	3.5
SA <sup>–</sup>	0.991	188.2	1.48	2.8
SA <sup>2–</sup>	1.005	186.8	1.07	2.3
SA <sup>0</sup> + SA <sup>–</sup> + SA <sup>2–</sup>	0.991	188.5	1.38	4.4
$\Delta$	0.001	0.2		

<sup>a</sup> $d/u$ : The ratio of the number of data points to the number of unknowns.;  $\Delta$ : Errors estimated for the values from the preceding row.

## Conclusions

This investigation is an illustrative example of a quantitative interpretation of NMR data supported by DFT

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calculations. Our results show that nowadays the reproducibility of the experimental spectra already enables investigating systems in fast dynamic equilibrium, such as  $\beta$ -diketones undergoing fast enol–enol and conformational transformations, by modeling the observable spectrum as a weighted average of the spectra of species undergoing exchange. We have found out that in order to reproduce properly the <sup>13</sup>C NMR spectra of SA observed for solutions of various acidities, it was justified to take into account two and in one case three different structures for a given SA form. The proposed SA structures have been selected from many trial structures and reflect the minima on the appropriate energy surfaces. Of course, it is difficult to be sure that these structures represent all the important objects encountered in the investigated solutions, which especially concerns the hydrate structures. The data analysis performed in this work has shown once again that the conformational phenomena have to be considered when comparing the experimental chemical shifts and theoretically calculated shielding constants and, reversibly, a quantitative approach to the <sup>13</sup>C chemical shift data provides a potent tool to investigate systems in dynamic equilibrium.

## Experimental Section

Acetylacetone and benzoylacetone were obtained commercially, while SA<sup>31</sup> and pivaloylacetone<sup>32</sup> were prepared by the methods described in the literature.

The acidity constants ( $K = [\text{H}^+][\text{A}^-]/[\text{AH}]$ ) of SA were determined by potentiometric titration of 0.01 M solutions of SA using 0.1 M NaOH. The obtained pK values (pK<sub>0/1</sub> = 4.5 and pK<sub>1/2</sub> = 9.9) are the mean values of five measurements. NMR measurements were performed using a 500 MHz (11.7T) spectrometer equipped with a temperature controller. In the case of <sup>1</sup>H and <sup>13</sup>C NMR spectra, the 0.1 M CDCl<sub>3</sub> or 95% H<sub>2</sub>O + 5% D<sub>2</sub>O solutions were investigated. The water solution pH was adjusted by adding small amounts of NaOH<sub>aq</sub> and controlled with a pH-meter. All the spectra were recorded at 25 °C using the standard spectrometer software and parameter sets. <sup>13</sup>C chemical shifts were referenced to the TMS scale using the CDCl<sub>3</sub> signal ( $\delta(^{13}\text{C}) = 77$  ppm) or trimethylsilylpropionate-*d*<sub>4</sub> for water solutions.

All the theoretical calculations of molecular structures and NMR parameters were performed using Gaussian 03 program.<sup>33</sup> At both calculation stages, the density functional theory (DFT) with PBE1PBE functional<sup>34</sup> and 6-311++G(2d,p) basis set were used. The influence of the bulk solvent was taken into

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account using the polarizable continuum model (PCM) of Tomasi et al.<sup>35</sup> The cavity was defined according to the UFF scheme.<sup>36</sup> For calculating NMR parameters, the GIAO method was used.

To compensate, at least partially, for the deficiencies of the applied theoretical method and to eliminate problems with the chemical shift referencing, the scaling procedure was used during the data analysis. Namely, the experimental chemical shifts were expressed in terms of the shielding constants calculated for the structures present in the solution and undergoing fast exchange by the following equation:

$$\delta_i = a(b - p_A \sigma_{A,i} - p_B \sigma_{B,i} - \dots) \quad (1)$$

$a \approx 1$  and  $b \approx \sigma_{\text{ref}}$  were adjustable parameters, whereas  $p_A, p_B, \dots$  ( $p_A + p_B + \dots = 1$ ) were the populations of particular structures.

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The unknowns were derived from the experimental data analysis by a nonlinear least-squares optimization performed for a data set preferentially including the whole series of the investigated species. Such an approach is advantageous as the parameters  $a$  and  $b$  are common for the whole data set, and so the total number of unknowns can be reduced. The population errors were estimated with the aid of a Monte Carlo method described previously,<sup>37</sup> assuming the uncertainty of  $\delta$  and  $\sigma$  values to be 0.05 ppm and 1 ppm, respectively.

**Supporting Information Available:** Experimental  $^{13}\text{C}$  NMR chemical shifts and calculated magnetic shielding constants of carbon nuclei for ketoenolic forms A and B of the investigated  $\beta$ -diketones in  $\text{CDCl}_3$ . Details of computational results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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