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Photoisomerization of Azobenzenecarboxylic Acids and Their Potassium Salts: Evidence of Structural Volume Changes Associated with Hydrogen Bond Formation[§]

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The contractions determined by laser-induced optoacoustic spectroscopy (LIOAS) for the $E \rightarrow Z$ photoisomerization of the three isomers (para, meta, and ortho) of azobenzenecarboxylates in 0.01 M potassium phosphate buffer (pH 8) are rationalized in terms of the difference in the chromophore−water hydrogen bonds strength between the photoisomer and the parent compound. The relatively high concentration of K^+ is needed to solubilize the compounds by means of interrupting the intermolecular interactions. At this salt concentration a large portion of the anions is paired with potassium ions. Due to the reduced conjugation, a stronger interaction of the nitrogens' lone pairs and water is expected in the *Z* isomers, leading to the contraction upon isomerization. A higher K^+ concentration reduces the structural volume change as a consequence of its perturbing the hydrogen bond network. No structural volume change was observed for the $E \rightarrow Z$ photoisomerization of *meta*-azobenzenecarboxylic acid in a series of cyclic alkanes, consistent with the assignment of the changes to variations in the hydrogen bonds strengths, in view of the lack of those bonds in the cycloalkanes. Despite the relatively large error of the $Z-E$ energy differences derived from LIOAS, they are near those reported for nonsubstituted azobenzene and larger than those calculated for both free acid and lithium salts. With the LIOAS-derived structural volume changes ΔV_R and the quantitative relationship $\Delta S_R = (c_p\rho/\beta)_{ik}\ \Delta V_R/T$ $[(c_p\rho/\beta)_{ik} = 14$ kJ cm^{-3}, the ratio of thermoelastic parameters at the isokinetic temperature in water, ca. 300 K], a large entropic term, in turn determined by the specific solute−water interactions, is calculated for the $Z \rightarrow E$ thermal isomerization.

Introduction

The application of laser-induced optoacoustic spectroscopy (LIOAS) has afforded photophysical and thermodynamic parameters for the production of the first intermediate in aqueous solutions of the biological photoperceptors (all bearing chromophores undergoing cis−trans isomerizations as the primary photochemical step) phytochrome A and PYP,[1] as well as in detergent suspensions of sensory rhodopsin I,[2] and in membrane-embedded bovine rhodopsin.[3]

One of the unique features of LIOAS is its ability to deliver the structural volume change produced in a particular photoinduced reaction step.[4] Chromophore model studies with LIOAS should help understanding the molecular origin of the structural volume changes, in turn related to the entropy change of that step.

The relatively large contraction determined for the photoisomerization of a carbocyanine in aqueous solutions was explained on the basis of the change of the specific solute−solvent (hydrogen bridges) interactions around the photoisomer having a larger dipole moment than the parent compound.[5] The normal equation for electrostriction, based on the continuum theory for the solvent, could not explain the relatively large contraction observed.

For intramolecular electron transfer in a flexible donor−bridge−acceptor molecule in the *n*-alkanes series, the electrostriction[6] produced by the large change in dipole moment of the photoinduced ion pair explained in part the structural volume changes.[7] Intrinsic volume changes photoinduced in the flexible molecule had to be additionally implied. With a rigid donor−bridge−acceptor molecule experiencing a giant dipole moment change upon photoexcitation, electrostriction explained completely the structural volume changes observed.[8] At the same time it was realized that, due to the relatively large change in compressibility along the *n*-alkane series, the structural volume change should accordingly change strongly.[8] This fact and the low general solubility in *n*-alkanes induced us to try the cycloalkanes as a series of nonpolar solvents. We use this series of solvents again in the present report.

Recently, we found that the photoisomerization of 1,3,3-trimethylindolino-6′-nitrobenzopyrylspiran (SP) to its merocyanine form (MC) in an array of cyclic alkanes experienced a solvent contraction of −28 cm^3/mol attributed to a large electrostriction effect and a small change in the van der Waals volume.[9] The ring closure of the protonated merocyanine (MCH^+) to the SP form in aqueous solution showed an expansion of +11 cm^3/mol attributed to four factors, the main being the deprotonation and the reprotonation of the malonic acid (added to stabilize the merocyanine form).[9] These conclusions mean that in nonpolar and in polar solvents (in particular in water) the origins of the structural volume changes are entirely different, reflecting (as expected) the type of chromophore−solvent interactions.

The fact that specific solute−solvent interactions are responsible for the structural volume changes in aqueous solutions had been already clear after the studies of the intra- and intermolecular photoinduced electron-transfer reactions of CN-

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substituted Ru complexes, forming strong CN−water bridges, in aqueous solutions and in the interior of reverse micelles.[10,11] LIOAS studies of these reactions in the presence of salts perturbing the degree of order of the water hydrogen-bridges network confirmed that the structural volume changes are determined by that order (sensed by the dissolved chromophore) and are related to the entropy change of the reaction.[12,13] Changes in hydrogen bond strengths have been recently invoked in order to explain the structural volume changes upon photoenolization, measured by a combination of time-resolved thermal grating and optoacoustics.[14]

As a continuation of these studies, we report now the results of LIOAS studies of the photoisomerization of water-soluble carboxy-azobenzenes which could be dissolved as acids in the series of cycloalkanes as well as carboxylates in potassium phosphate buffer at pH 8, offering the possibility of measuring the same (in principle) photoreaction in aqueous and nonaqueous media. The various factors determining the structural volume change in water and in nonpolar media are compared.

The spectroscopy and photochemistry of the azobenzenes have been studied by several research groups and have been reviewed by Rau.[15] It has been concluded that in general the $E \rightarrow Z$ photoisomerization of the azobenzenes in organic solvents after excitation in the n-π^* band occurs through the singlet state,[16] that the fluorescence yield is very low and that the triplet yield is extremely low.[15] These features make the azocompounds interesting for LIOAS studies because in these compounds photoisomerization to the perpendicular state is a clean reaction without competing energy-wasting steps.

Materials and Methods

Materials. Azobenzene-4-carboxylic acid (**1**H), azobenzene-3-carboxylic acid (**2**H), and azobenzene-2-carboxylic acid (**3**H) were prepared according to the literature[17] and purified by recrystallization (**1**H and **2**H) and preparative HPLC (**3**H). Identification of the compounds was performed by analyzing the NMR spectra recorded with a Brucker ARX-300 spectrometer and by comparison with the melting points reported in the literature.[18]

Freshly prepared samples were handled under green light. In every case the solutions were filtered through 0.2 μm filters before use. **1**H, **2**H, and **3**H are not soluble in water and, therefore, we used 0.01 M potassium phosphate buffer solutions (pH = 8.0). A series of experiments with **2**H were performed in 0.1 potassium phosphate buffer solutions (pH = 8.0). The absorbance of all samples at the excitation wavelength (500 nm) was A^{500} ca. 0.1. With the Fuoss equation[19] and a distance of closest approximation of the azocarboxylates and the potassium ions of ca. 9 Å, an ion-pairing association constant is calculated of K_{ass} ca. 50 M^{-1}, meaning that in 0.01 M buffer solution at least 33% of the azocarboxylates are forming ion pairs (the value represents a lower limit because the distance of closest approximation used is probably an underestimation). The calorimetric references Evans blue (Aldrich) in aqueous solutions and ferrocene (Aldrich) in cycloalkanes were used as received. Cyclopentane, cyclohexane, and methylcyclohexane (all from Aldrich, spectroscopic grade) were used as received, *cis*-decaline (Aldrich) was distilled, and water was deionized.

Fluorescence Measurements. A computer-controlled Spex-Fluorolog was used for the measurements of the absorbance-matched ($A^{500} = 0.1 \pm 0.005$) solutions of cresyl violet in ethanol and the azocompounds in 0.01 M KH_2PO_4 buffer.

Laser-Induced Optoacoustic Spectroscopy. The LIOAS setup has been already described.[2,3,5−13] For excitation, a Lambda Physik-EMG101 MSC excimer laser (XeCl) was used to pump a FL2000 dye laser with Coumarin 307 dye (25 ns pulses of 500 nm). A Pb−Zr−Ti piezoelectric transducer (Vernitron) was used to detect the pressure wave. The signal was amplified and subsequently recorded and averaged 100 times by a transient digitizer (Tektronix TDS 684 A) connected to a workstation. Considering that the lifetimes of the Z isomers are on the hours to days range, the amplitude (H) of the first acoustic wave was used, i.e., the difference between the first maximum and the first minimum, as a measure of the prompt heat. Low laser fluences (below 30 μJ) were applied in order to prevent multiphoton processes and large isomerization conversions.

The absorbances of the samples and references at the laser excitation wavelength were matched within 2% and recorded with a Shimadzu UV-2102 PC spectrophotometer before and after the LIOAS measurements. The absorbance of the sample solutions and that of the reference did not change with temperature in the range analyzed.

The LIOAS cuvette was thermostated with an accuracy of 0.1 K. A linear dependence with zero intercept of the LIOAS signal amplitude with laser fluence was observed up to 40 μJ.

To separate the structural volume change from the thermal expansion in aqueous solutions, we used the several-temperatures (ST) method.[4] This method takes advantage of the strong temperature dependence of the thermal expansion coefficient, β, of water. Equation 1, readily derived as the ratio of the fluence-normalized LIOAS signal amplitude for the sample (H_n^{S}) and for the reference (H_n^{ref}) (of matched absorbances at the excitation wavelength) was used with the ST method.[4]

$$\frac{[H_n^{\mathrm{S}}]_r}{[H_n^{\mathrm{ref}}]_r} = \alpha + \frac{\Phi_{\mathrm{R}}\Delta V_{\mathrm{R}}}{E_\lambda}\left(\frac{c_p\,\rho}{\beta}\right)_T \tag{1}$$

Thus, the left term is linearly related to the ratio of thermoelastic parameters of the medium (the mass density ρ, the heat capacity c_p, and the thermal expansion coefficient β) and to the laser-wavelength energy per Einstein, E_λ (239.3 kJ/mol at 500 nm). The slope of the plot of the left term in eq 1 vs ($c_p\rho/\beta$) at various temperatures T affords the product of the quantum yield of the reaction and the structural volume change per mole of transformed molecules ($\Phi_{\mathrm{R}}\Delta V_{\mathrm{R}}$). The intercept of the plot contains information about the energy stored in species living longer than the pressure integration time. α is the fraction of prompt heat released within the pressure integration time.[4] Thus, the energy content of the energy-storing species, the Z azobenzene in our case, is $E_{\mathrm{P}} = E_\lambda\,(1 - \alpha)/\Phi_{\mathrm{R}}$.

The thermoelastic parameters of the 0.01 M phosphate buffer (pH = 8.0) solution were taken as identical to those of neat water. For the 0.1 M phosphate buffer (pH = 8) the ratio of the amplitudes of the LIOAS signals for Evan blue in buffer and in water was used to calculate the ratio ($c_p\rho/\beta$). The compressibility of water and of the 0.1 M buffer were considered identical in view of the fact that the speed of sound was the same in both media as determined by the arrival time of the sound wave to the detector after excitation of matched-absorbances solutions of Evans blue in water and in buffer.[5,20] We note that the ($c_p\rho/\beta$) range is more limited in 0.1 M buffer solutions than in 0.01 M buffer and water.

The thermoelastic parameters of nonaqueous media exhibit a poor dependence on temperature. In this case, the $\Phi_{\mathrm{R}}\Delta V_{\mathrm{R}}$ value was determined by using a series of cycloalkanes. Thus, for the solutions of cycloalkanes, eq 1 was used with ($c_p\rho/\beta$) different for each solvent in the series.[7,6]

Estimation of the Z Isomer Absorption Spectra. To obtain the quantum yield of the *E* → *Z* photoisomerization of compounds **1**K, **2**K, and **3**K the absorption spectra of both *E* and *Z* forms should be known. However, each *Z* form is in equilibrium with the corresponding *E* form and, therefore, the former cannot be easily isolated in the pure state and its absorption spectrum cannot be easily measured. To estimate the *Z* spectrum, we used the method developed by Fischer.[21] According to this method, aliquots of a ca. 10^{-4} M solution of the corresponding *E*-azobenzenecarboxylate in aqueous 0.01 M potassium phosphate buffer (pH = 8.0) were photoequilibrated by illumination with a lamp at 405 and at 448 nm for **1**K and for **3**K, and at 417 and at 448 nm for **2**K. The molar absorption coefficient at any particular wavelength was derived from the absorption spectra of *E* before and after photoequilibration at each of the two wavelengths.

Photoisomerization Quantum Yields. A 6.11×10^{-4} M solution of **1**K, 1.20×10^{-4} M of **2**K, and 1.7×10^{-4} M of **3**K in aqueous 0.01 M KH_2PO_4 buffer (pH = 8.0) was irradiated in each case in the n-π^* band at 500 nm at 20 °C with the laser system described in the previous section. For **2**K in 0.1 M phosphate buffer the concentration was 2.8×10^{-4} M. In all the experiments for quantum yield determination the total conversion was <5%. The increase in absorbance at 429 nm was used as a measure of the concentration of *Z* isomer (estimated $\epsilon^{429} = 1650$, 3134, and 1502 M^{-1} cm^{-1} for *Z*-**1**K, *Z*-**2**K, and *Z*-**3**K, respectively). The energy incident on the sample was measured with a Laser Precision Corp. RJP735 head connected to an RJ7100 meter.

Semiempirical Calculations. To determine the energy and dipole moment differences of the two *E*-*Z* isomers of the azobenzenecarboxylic acids and their metal salts, we carried out semiempirical calculations using the PM3 Hamiltonian.[22,23] For each isomer, the molecular geometry was optimized without any molecular symmetry constraints. Lithium was used as metal in view of the lack of parametrization for potassium.

Results

Fluorescence Yields. The emission quantum yields of **1**K, **2**K, and **3**K in the phosphate buffer resulted to be < 10^{-3} and that of **2**H in cyclohexane was ca.10^{-2}, in agreement with the literature data for other azobenzene derivatives.[15]

LIOAS Measurements in Aqueous Solutions. A typical LIOAS signal of **1**K at 22 °C is shown in Figure 2, together with that of the calorimetric reference Evans blue.

At $T_{\beta=0}$, a fluence-dependent negative LIOAS signal for **1**K, **2**K, and **3**K was observed, indicative of a contraction. The laser-fluence dependence of the LIOAS signal of **2**K at $T_{\beta=0}$ is shown in Figure 3, together with that of Evans blue.

Temperature-dependent measurements between 6 and 22 °C were carried out in order to evaluate the value of $\Phi_R \Delta V_R$. Figure 4 shows the plots of the ratio of fluence-normalized signal amplitudes for samples **1**K, **2**K, and **3**K and Evans blue vs the thermoelastic parameters of water. From the linear fits of the data taken for 0.01 M buffer the intercepts were $\alpha = 0.92 \pm 0.05$, 0.97 ± 0.05, and 0.77 ± 0.05 and the slopes were $\Phi_R \Delta V_R / E_\lambda = (-20 \pm 1) \times 10^{-4}$, $(-20 \pm 1) \times 10^{-4}$, and $(-17 \pm 1) \times 10^{-4}$ cm^3/kJ for **1**K, **2**K, and **3**K, respectively (Table 1). For **2**K at temperatures above 14 °C (data not shown) the mentioned ratio decreased, probably as a consequence of the variation with the temperature of the azobenzoate and potassium ions ion-pairing constant. For **2**K in 0.1 M buffer the values obtained were $\alpha = 0.97 \pm 0.05$ and the slope = $(-8 \pm 4) \times 10^{-4}$ cm^3/kJ.

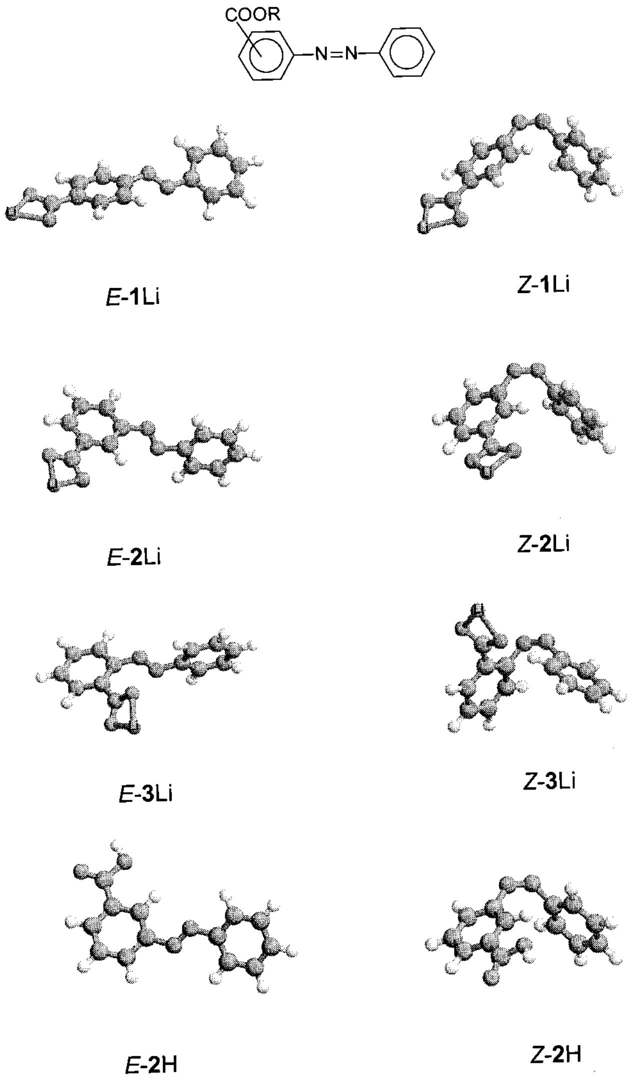

Figure 1. Computer plot of the PM3 optimized geometries for the *E*- and *Z*-*para* (**1**), *meta* (**2**), and *ortho* (**3**) lithium azobenzenecarboxylates and *meta*-azobenzene carboxylic acid **2**H. R = Li or H as indicated.

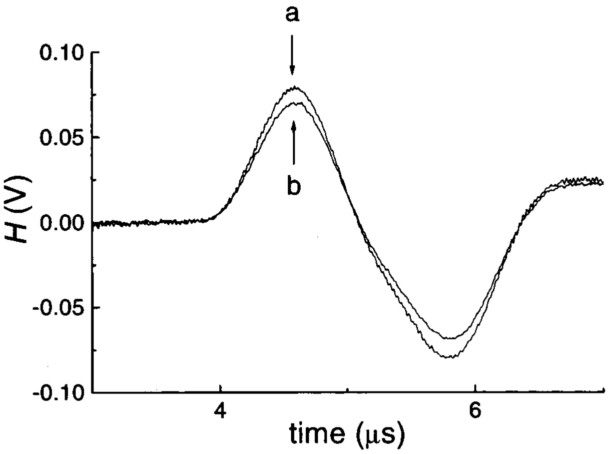

Figure 2. LIOAS signal for (a) the calorimetric reference Evans blue and (b) potassium azobenzene-4-carboxylate (**1**K) in aqueous 0.01 M potassium phosphate buffer (pH = 8.0) solution at 22 °C. Excitation wavelength: 500 nm.

LIOAS Measurements in Cycloalkane Solutions. To compare the values of ΔV_R in a nonaqueous medium, LIOAS measurements were performed in cycloalkane solutions. However, LIOAS signals could only be recorded for **2**H since **1**H

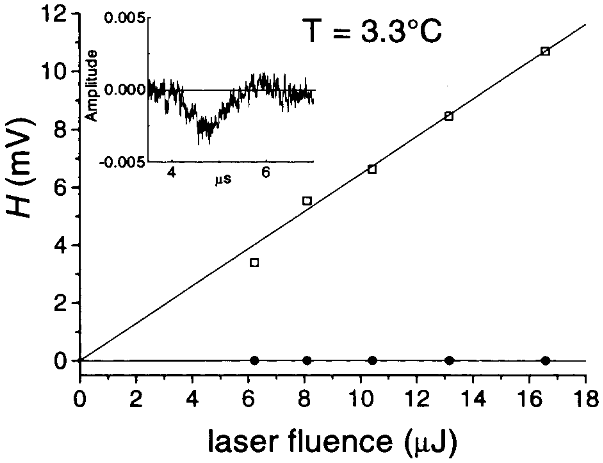

Figure 3. Laser-fluence dependence of the LIOAS signal amplitude in aqueous 0.01 M potassium phosphate buffer (pH = 8.0) solution of potassium azobenzene-3-carboxylate (**2**K) (open symbols) and for Evans blue (solid symbols) at $T_{\beta=0}$. Inset: LIOAS signal for the sample at $T_{\beta=0}$ showing the contraction.

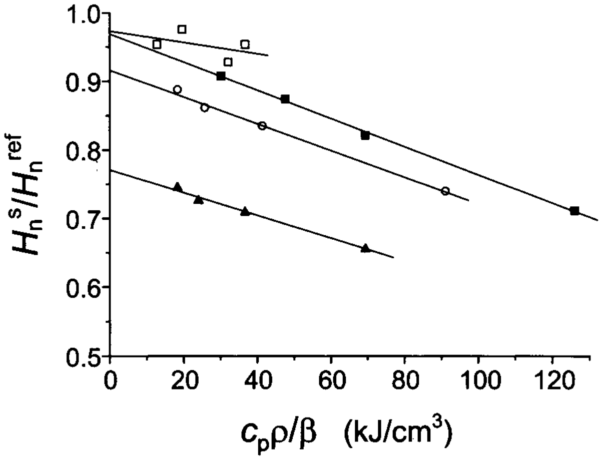

Figure 4. Ratio of energy-normalized LIOAS signal amplitudes for sample and reference (Evans blue) in aqueous 0.01 M phosphate buffer (pH = 8.0) solutions for (○) **1**K (22, 16, 11, and 7 °C), (■) **2**K (14, 10, 8, and 6 °C), (▲) **3**K (22, 17, 12, and 8 °C), and (□) **2**K in 0.1 M buffer (30, 18, 11, and 8 °C) vs the ratio of thermoelastic parameters. Each point is the average of two independent measurements. The ratio of thermoelastic parameters is different for both buffer solutions (see Experimental Section).

TABLE 1: Values of the Intercepts (α), Enthalpy Difference between the *Z* and *E* Isomers, (ΔH_{Z-E}), the Slopes ($\Phi_R\Delta V_R/E_\lambda$), and the Molar Structural Volume Changes (ΔV_R) Obtained from the Plots of Eq 1.

sample	α	ΔH_{Z-E} kJ/mol	$\Phi_R\Delta V_R/E_\lambda$ cm^3/kJ × 10^4	ΔV_R cm^3/mol
1K[a]	0.92 ± 0.05	120 ± 84[d]	−20 ± 1	−3.0 ± 0.5[d]
2K[a]	0.97 ± 0.05	45 ± 75[d]	−20 ± 1	−3.0 ± 0.5[d]
3K[a]	0.77 ± 0.05	239 ± 80[e]	−17 ± 1	−1.8 ± 0.3[e]
2K[b]	0.97 ± 0.05	60 ± 100[f]	−8 ± 4	−1.6 ± 0.9[f]
2H[c]	0.96 ± 0.05	60 ± 78[d]	0.8 ± 0.8	0.1 ± 0.1[d]

[a] Equation 1, aqueous 0.01 M potassium phosphate buffer (pH = 8.0) solution. [b] Equation 1, aqueous 0.1 M potassium phosphate buffer (pH = 8.0) solution. [c] Equation 1, cycloalkane solutions. [d] Calculated with Φ_R = 0.16. [e] Calculated with Φ_R = 0.23. [f] Calculated with Φ_R = 0.12.

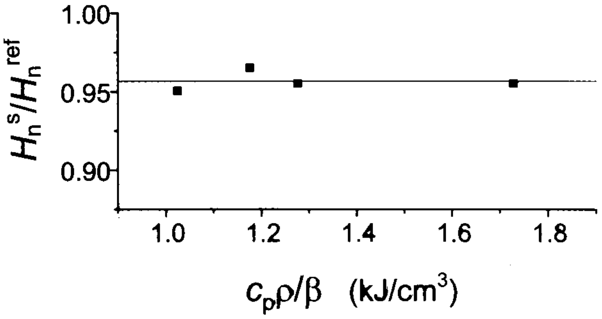

Figure 5. Ratio of fluence-normalized LIOAS signal amplitudes in cycloalkane solutions at 14 °C for sample (**2**H) and reference (ferrocene) vs the ratio of thermoelastic parameters of the solvent (from left to right: cyclopentane, cyclohexane, methylcyclohexane, and *cis*-decaline). Each point is the average of two independent measurements.

TABLE 2: Calculated PM3 Heats of Formation, Dipole Moments, and N-N Distances for the *E* and *Z* Isomers of Azobenzenecarboxylic Acids 1H, 2H, and 3H and the *E* and *Z* Isomers of Lithium Azobenzenecarboxylates 1Li, 2Li, and 3Li

compound	ΔH_0^f kJ/mol	dipole moment D	N−N distance Å
	Azobenzenecarboxylic Acids		
E-1H	6.3	2.8	1.230
Z-1H	16.7	2.9	1.216
E-2H	6.7	2.5	1.231
Z-2H	16.7	1.6	1.215
E-3H	18.4	2.0	1.228
Z-3H	21.8	3.6	1.215
	Lithium Azobenzenecarboxylates		
E-**1**Li	−22.6	2.8	1.230
Z-**1**Li	−12.8	5.7	1.216
E-**2**Li	−21.8	3.1	1.228
Z-**2**Li	−0.3	6.4	1.216
E-**3**Li	−3.6	3.4	1.227
Z-**3**Li	−3.9	4.1	1.216

and **3**H were not soluble in these media. In view of the linear correlation for temperatures up to 14 °C in aqueous solutions, the experiments were carried out in cycloalkanes at 14 °C. A linear fit of the plots of the fluence-normalized signal amplitudes for **2**H and for ferrocene used as the calorimetric reference vs ($c_p\rho/\beta$) for cyclopentane, cyclohexane, methylcyclohexane, and *cis*-decaline (Figure 5) yielded an intercept α = 0.96 ± 0.05 and a slope $\Phi_R\Delta V_R/E_\lambda = (0.8 \pm 0.8) \times 10^{-4}$ cm^3/kJ.

Photoisomerization Quantum Yields. With the concentration of formed *Z* isomer (determined by UV spectroscopy by measuring the photoinduced increase in 429 nm absorbance) and the total fluence absorbed by the sample, calculated using the Lambert−Beer law and the measured incident total fluence, the quantum yield for the *E* → *Z* transformation of both **1**K and **2**K in 0.01 M phosphate buffer resulted to be Φ_R = 0.16 ± 0.01 and 0.23 ± 0.02 for **3**K, similar to the values reported for the *E* → *Z* photoisomerization quantum yields of azobenzene at 436 and 439 nm in various solvents upon excitation within the n-π^* band (Φ_R from 0.2 to 0.3).[15,16,24] For **2**K in 0.1 M phosphate buffer, a slightly lower value of Φ_R = 0.12 ± 0.01 was obtained.

Semiempirical Calculations. The computer plots of the PM3 optimized geometries for the *E*- and *Z*-*meta*-azobenzenecarboxylic acid are shown in Figure 1. As expected, the calculated energies for all three geometrical isomers of the azobenzenecarboxylic acid (Table 2) indicate that the *E* isomer of **1**H, **2**H, and **3**H is more stable than the *Z* isomer by 10.4, 10.0, and 3.4 kJ/mol, respectively.[15] These are clearly rough estimations qualitatively in agreement with the expected larger stability of the *E* isomers, but much smaller than the 56 kJ/mol observed for azobenzene.[25] Table 2 also shows the N−N calculated bond lengths. At the PM3 level, the N−N distances for the *E* isomers are larger than those of the *Z* isomers (from 1.230 to 1.216 Å, from 1.231 to 1.215 Å, and from 1.228 to 1.215 Å, for **1**H, **2**H, and **3**H, respectively). These results are in agreement with a reduction in π conjugation in the Z isomers. In fact, in all three Z conformers, the planes of the benzene rings form an angle of about 80° (see Figure 1). This should cause a major distortion in the π conjugation, in particular for compounds **1**H and **2**H. Moreover, the E to Z isomerization is calculated to be accompanied by an increase in the dipole moment from 2.0 to 3.6 D for **3**H, by a decrease from 2.5 to 1.6 D for **2**H, and by a negligible change from 2.8 to 2.9 D for **1**H (Table 2).

The results of the calculations performed for the salts **1**Li, **2**Li, and **3**Li (Table 2) are similar to those for the acids. However, for both the para and meta position isomers of the salts, the dipole moment increases upon $E \rightarrow Z$ isomerization. For all three position isomers the conjugation again decreases upon $E \rightarrow Z$ isomerization. It is clear that these are also rough estimations of the situation with the potassium salts in view of the very different behavior expected for the ion-pairing of the azobenzoates with Li^+ or K^+.

Discussion

For the three compounds a pK_a ca. 4 is expected.[26] Consequently, at pH 8 (required for solubility reasons) all three compounds are expected to be in the carboxylate form. Moreover, at the high K^+ concentrations ($>10^{-2}$ M), at least 33% of the carboxylates was calculated to be forming ion pairs with the K^+ ions (see Materials and Methods). Ion pairing is needed for solubilization purposes, as demonstrated by the fact that at buffer concentrations $<10^{-2}$ M the carboxy azocompounds do not solubilize. Most likely ion pairing interrupts intermolecular interactions between the carboxy groups and the lone pairs on the nitrogens.

Structural volume changes (ΔV_R) upon excitation of the compounds with 500 nm (n-π* band) were observed for all three compounds, i.e., potassium azobenzene-4-carboxylate (**1**K), azobenzene-3-carboxylate (**2**K), and azobenzene-2-carboxylate (**3**K).

The decrease in ΔV_R with temperature above 14 °C for **2**K (see Results) and perhaps above 22 °C for **3**K indicates that most of the effect observed originates in the isomerization of the ion pair, rather than in the isomerization of the free anion, taking into account that at a higher temperature the equilibrium of the ion pair is displaced to the separate ions. The high-temperature data (above 14 °C for **2**K and above 22 °C for **3**K) was not used for the calculations of the values of ΔV_R and energy contents of the photoisomers upon photoisomerization of the E forms of **2**K and **3**K.

From the intercepts of the linear fits using eq 1 (Figure 4) energy contents $E_P = E_\lambda (1 - \alpha)/\Phi_R = (120 \pm 84)$, (45 ± 75), and (239 ± 80) kJ/mol are obtained for the Z forms of **1**K, **2**K, and **3**K, respectively, all in 0.01 M potassium buffer by taking $\Phi_R = 0.16$ for **1**K and **2**K and $= 0.23$ for **3**K and considering negligible the energy loss by fluorescence ($\Phi_f < 10^{-3}$) (Table 1). A value of $E_P = (60 \pm 100)$ kJ/mol for **2**K in 0.1 M buffer is obtained with $\Phi_R = 0.12$. Bearing in mind that the α values are intercepts and that they are very large values (almost 100% prompt heat emission), the relative error of $(1 - \alpha)$ is very large and, consequently, the error of the calculated E_P values is equally large. A 5% error in the α value leads to 1 order of magnitude difference in the value of E_P. We have already pointed out the weakness of LIOAS for the determination of energy contents when either the quantum yield for the phototransformation or the energy difference between the product and parent compound are too small.[4]

Notwithstanding the errors in the LIOAS-derived energy levels, the difference in prompt heat (intercepts in Figure 4) is completely outside the experimental deviation. Therefore, the LIOAS values are larger and somehow opposite (the E-Z energy difference is larger for **1**K than for **2**K) than the calculated energy differences for the Li salts (Table 2). We speculate that most likely the differences are due to specific stabilization by the potassium cation of some of the forms, such as the E form of **1**K. The energy differences determined by LIOAS are, however, in line with the 56 kJ/mol difference reported between the E and Z forms of azobenzene.[25]

In 0.01 M buffer, with the slopes $\Phi_R \Delta V_R / E_\lambda = (-20 \pm 1) \times 10^{-4}$ and $(-20 \pm 1) \times 10^{-4}$ cm^3/kJ and again using $\Phi_R = 0.16$, a structural volume change $\Delta V_R = (-3.0 \pm 0.5)$ cm^3/mol of phototransformed species is calculated for **1**K and **2**K, i.e., an identical structural volume change for both compounds in aqueous solutions at pH 8. For **3**K and $\Phi_R = 0.23$, the value $\Delta V_R = (-1.8 \pm 0.3)$ cm^3/mol is obtained.

For **2**K in 0.1 M buffer, and using $\Phi_R = 0.12$, the lower value $\Delta V_R = (-1.6 \pm 0.9)$ cm^3/mol is obtained. Thus, a higher K^+ concentration reduces both the quantum yield and the molar structural volume change.

According to the continuum solvent model, a decrease in the dipole moment should lead to an expansion, an increase to a contraction, and different changes in dipole moment should be accompanied by different values of the volume change.[8] Although the calculated dipole moments for the E to Z isomerization of **1**Li and **2**Li are similar (Table 2), the expected structural volume change calculated with the continuum solvent model ($< 10^{-2}$)[27] is too small in comparison with the measured values. Some other factor should explain the experimental data. The values of ΔV_R in aqueous solutions should thus be explained by specific hydrogen bond formation between the potassium azobenzenecarboxylates and water. The reduced conjugation of the nitrogen lone pairs with the aromatic rings in the Z isomers should allow a stronger interaction between these pairs and water, resulting in stronger hydrogen bonds and affording a (similar) contraction in **1**K and **2**K. The lower value of ΔV_R for **3**K may have to do with some degree of intramolecular hydrogen bridging which impairs the solute–solvent interactions in this position isomer.

The decrease of the value of ΔV_R upon excitation of **2**K, for a larger K^+ concentration, may be explained by the destruction of the water hydrogen bridges network by the added cations.[12]

The decrease in the value of the isomerization quantum yield for **2**K, however, upon increasing the buffer concentration might be due to some localization of the excess K^+ on the nitrogen lone pairs that reduces the rotation around the (excited) double bond. This observation already sets the working limits of the present experiments, since it indicates that the range of variation of the K^+ concentration is small.

For cycloalkane solutions of **2**H, the linear fit using eq 1 and the variable $(c_p\rho/\beta)$ values affords an intercept $\alpha = 0.96 \pm 0.05$ (Figure 5) which yields an energy content of (60 ± 78) kJ/mol (Table 1), when using $\Phi_R = 0.16$, a value in the range of that determined for the $Z \rightarrow E$ isomerization of azobenzene in various organic solvents.[15,16] Again in this case a value with a large error is obtained, mainly determined by the large relative error in the value of $(1 - \alpha)$. Thus, the use of a Φ_R value between 0.1 and 0.3 leads to an E_p value within the experimental error. The same is true for the calculation of ΔV_R in the alkane solvent series (vide infra).

The solvent-dependent measurements resulted in errors in the slopes which were larger than their absolute values and, therefore, the slope can be regarded as zero (Table 1). This was expected in view of the very small change in the dipole moment for the *E* to *Z* isomerization (0.9 D at the PM3 level of theory) and thus the value calculated with the formula for electrostriction (as derived from the continuum solvent model applicable in the nonpolar organic solvents)[8] should be very small.

The only other reaction volume change reported for azobenzenes is a contraction of 12 and 32 cm^3/mol upon $Z \rightarrow E$ isomerization of 4-(dimethylamino)-4′-nitroazobenzene in CCl_4 and $CHCl_3$, respectively, obtained from the pressure influence on the $Z \rightleftharpoons E$ equilibrium.[28] The large contractions should most likely be attributed to the electrostriction produced by the expected large change in dipole moment upon isomerization of this particular azobenzene derivative.[8]

Consistent with the assignment of the structural volume changes to changes in the hydrogen bond strength, no structural volume change was observed for azobenzene-3-carboxylic acid (**2H**) in the cyloalkane solutions, since in this media no hydrogen bonds are expected. Electrostriction plays a negligible role in this case, since the dipole moment change upon photoisomerization is very small.

Based on the fact that the structural volume changes in aqueous solutions are due to changes in the specific solute−solvent interactions (hydrogen bonds), we apply the relationship between ΔS_R and ΔV_R found for other cases in which hydrogen bond changes also determined the values of ΔV_R, i.e., $\Delta S_R = (c_p\ \rho/\beta)_{ik}\ \Delta V_R\ /T$ [$(c_p\ \rho/\beta)_{ik} = 14$ kJ cm^{-3} is the value of the ratio of thermoelastic parameters at the isokinetic temperature in water, ca. 300 K].[12,13] With this relationship, values of $\Delta S_R = -0.15$ kJ mol^{-1} K^{-1} for **1**K and **2**K, −0.09 for **3**K (all three in 0.01 M buffer), and −0.08 for **2**K in 0.1 M buffer result. This means that at 14 °C the entropic term associated with the solute−solvent interactions is relatively large, $T\ \Delta S_R$ ca. −42 kJ mol^{-1} for the $E \rightarrow Z$ thermal isomerization of **1**K and **2**K and ca. −26 kJ mol^{-1} for the $E \rightarrow Z$ thermal isomerization of **3**K in aqueous 0.01 M buffer solution at pH 8, and ca. −22 kJ mol^{-1} for **2**K in 0.1 M buffer.

In view of the large errors associated with the calculated energy contents of the photoisomers (mainly due to the relatively small isomerization quantum yield) it makes little sense to use those energy differences to calculate the free energy of the thermal back isomerization in each case. The data, however, serve to assess that the entropic term associated with the thermal $Z \rightarrow E$ back reaction and determined by the solute−water interactions is always positive and relatively large.

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