

The Effect of Ordered Water on a Short, Strong (Speakman-Hadži) Hydrogen Bond*

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We have determined the structures of the sodium, tetrabutylammonium (TBA) and bis(triphenylphosphoranylidene)ammonium (PNP) salts of the bis(4-nitrophenoxide) anion by X-ray crystallography. The sodium salt is a dihydrate, with the water oxygens coordinated to the sodium cations, and one hydrogen from each water hydrogen bonded to one of the bridging oxygens of the anion. The TBA and PNP salts are anhydrous. Nevertheless the oxygen-oxygen distance is shortest in the sodium salt; 246.5 pm in the sodium salt, 247.5 pm in the TBA salt, and 249 pm in the PNP salt; suggesting that the hydrogen bond is not weakened by the water, and may be strongest in the hydrated salt. (All three compounds show Hadži type ii IR spectra, and are called Speakman-Hadži compounds in this paper.) The ²H chemical shifts of the bridging hydrogen in the three solids are 16.8 ppm for the sodium salt, 16.8 ppm for the TBA salt, and 16.5 ppm for the PNP salt. Again there is no evidence that the water weakens the hydrogen bond. These results can be understood by noting that the additional hydrogen bonds to the bridging oxygens decrease their proton affinity, but the mutual repulsion of the oxygens is also decreased. The net effect is to leave the hydrogen bond at least as strong as it was with-

* This paper is dedicated to the memory of Professor Stanko Borčić of the Faculty of Pharmacy and Biochemistry of the University of Zagreb.

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out the waters. It is competition and selective solvation by otherwise disordered water which weakens the hydrogen bond in aqueous solution. Enzymatic transition states contain little unoriented water, and, therefore, can contain Speakman-Hadži bonds. Several suggested Speakman-Hadži bonds in enzyme inhibitor complexes are described.

INTRODUCTION

J. C. Speakman seems to have first recognized that compounds containing a very short hydrogen bond between bases of similar or equal basicity form an identifiable class.^{1,2} D. Hadži first recognized that they generate similar and unusual infrared spectra (Hadži type ii spectra)³ and it was soon recognized that short hydrogen bonds and Hadži type ii spectra were complementary properties. Since then a large volume of experimental⁴⁻⁸ and theoretical⁹⁻¹¹ work on these substances, which we will call Speakman-Hadži compounds in this paper, has greatly clarified their nature, but not solved all the problems associated with them. (The cited papers provide an introduction to a very large literature.)

One of the remaining uncertainties is the manner in which water interferes with the formation of Speakman-Hadži bonds, and the circumstances in which it will interfere. The formation constants of hydrogen bicarboxylates are much smaller in aqueous than in nonaqueous solution,¹² and their isotopic fractionation factors are larger.⁸ Large formation constants in nonhydroxylic solvents (over 10^5 M^{-1} for the formation of the complex from separated constituents in favorable cases.)¹³ and small isotopic fractionation factors in such solvents⁸ are among the characteristics of Speakman-Hadži compounds, and both appear to be attenuated in dilute aqueous solution. An unusual, very low-field band for the bridging hydrogen is observed in the NMR spectra of such compounds, with δ values between 16 and 22 ppm.^{8,14} The chemical shift of the hydrogen biformate ion in aqueous solution is around 14 ppm,¹⁵ several ppm below the value anticipated in a nonhydroxylic solvent.^{8,14} On the other hand, protonated 1,8-bis(dimethylamino)naphthalene (proton sponge) maintains a very low-field NMR band, at $\delta = 19.5$ ppm, in aqueous solution.¹⁶ This band has a δ value of 18.5 ppm in acetonitrile solution,¹⁴ and 19.7 in solids.¹⁷ One might expect that the proton would become more nearly centralized as a hydrogen bond becomes stronger. Most of this evidence seems to suggest that water weakens a Speakman-Hadži hydrogen bond.

In the present paper we examine the hydrogen bis(4-nitrophenoxide) ion, a fairly typical Speakman-Hadži anion, in three crystalline environments; as a sodium salt, a tetrabutylammonium (TBA) salt, and as a bis(triphenylphosphoranylidene)ammonium (PNP) salt. The latter two are anhydrous.

The sodium salt is a dihydrate, with the water hydrogen bonded to the basic oxygens. Nevertheless, in this orderly, crystalline environment, there is no sign that the water attenuates the characteristic properties of the Speakman-Hadži ion.

It has been suggested that short, strong, low-barrier hydrogen bonds (Speakman-Hadži bonds) play an important role in enzyme catalysis.^{18,19} Enzymes function in water, and contain water, but the contained water is thought to be organized and partially immobilized in the enzymatic transition state.²⁰ It is, therefore, of considerable interest to know what effect organized and immobilized water has on Speakman-Hadži bonds. These effects may be quite different from those found in dilute aqueous solution.

In Speakman-Hadži compounds the bridging hydron is distributed between the two basic sites. The two sites are only a few tens of pm apart, and the barrier separating the two structures is very low; probably not much above the lowest allowed vibrational level.⁸ This leads to a low zero-point vibrational energy, and to the low isotopic fractionation factors which have been alluded to. The reduction in zero-point energy which contributes 7–10 kJ/mol to the stability of these substances,²¹ is the proton analog of resonance energy. During early studies of these phenomena M. M. K. expounded this thought to Prof. Borčić, who was a wonderfully sceptical audience for new ideas. His response was »Don't ever tell that to undergraduates. I've spent years trying to teach them the difference between tautomerism and resonance.« Speakman-Hadži compounds do, indeed, bridge the gap between tautomerism and resonance.

EXPERIMENTAL

Materials

Sodium hydrogen bis(4-nitrophenoxide) dihydrate $(1(\text{H}_2\text{O})_2)^{22}$ was prepared by half-neutralizing 4-nitrophenol in concentrated aqueous solution. A warm, 1.4 M aqueous solution of 4-nitrophenol was half-neutralized with 1.1 M NaOH. Copious quantities of orange needles separated spontaneously on cooling to room temperature. The yield was somewhat increased by chilling. This material was purified by recrystallization from water, and readily grew into fairly large crystals. The identity of this material was confirmed by its infrared spectrum.²²

Tetrabutylammonium (TBA) hydrogen bis(4-nitrophenoxide) $(2)^8$ was prepared by combining 4-nitrophenol with TBA 4-nitrophenoxide. The latter was prepared by dissolving 4-nitrophenol in a two-fold excess of warm, aqueous, 40% TBA hydroxide (Aldrich Chemical Co.). TBA 4-nitrophenoxide precipitated as a hydrate on cooling. To prepare **2**, 1 g (2.5 mmol) of the simple salt and 0.35 g (2.5 mmol) of 4-nitrophenol were dissolved in 6 ml of a solvent made of 9 parts of 2-methyl-2-propanol to 1 part of water, by warming at a temperature not exceeding 50 °C. This solution was al-

lowed to cool slowly to room temperature. It deposited the crystalline product over a period of about two days.

Anal. Calcd. for $C_{28}H_{45}N_3O_6$: C 64.71, H 8.73, N 8.09%. Found: C 64.53, H 8.70, N 7.97%.²³

Strong, rapid cooling of the final solution of **2** produced a brown oil, which did not crystallise. Even with slow cooling, solvated, or contaminated crystals were sometimes obtained.

The identity and purity of subsequent preparations of **2** were determined from their IR and 1H -NMR spectra.

Bis(triphenylphosphoranylidene)ammonium (PNP) hydrogen bis(4-nitrophenoxide) (**3**) was prepared by dissolving **1** and a 50% excess of PNP chloride in hot, absolute alcohol; then cooling to room temperature. For every g of **1**, 10 g of ethanol was required. The product crystallized readily into well formed, cubic crystals. They were identified, and shown to be free of solvent, by their IR spectrum.

Anal. Calcd. for $C_{48}H_{39}N_3O_6P_2$: C 70.66, H 4.82, N 5.15, P 7.60%. Found: C 70.59, H 4.57, N 5.07, P 7.47%.²³

Analogues of $1(H_2O)_2$, **2**, and **3**, tetradeuterated in the 2,2',6,6' positions, were prepared from 4-nitrophenol-2,6- d_2 . This was prepared by deuterating phenol in acidic solution to get phenol-2,4,6- d_3 , then nitrating.

Methanesulfonic anhydride (13 g, 0.075 mol, Aldrich Chemical Co.) was dissolved in 60 g of D_2O , giving a 2.5 M solution of methanesulfonic acid-OD. Phenol (10 g, 0.11 mol) was added, and the resulting solution, which was homogeneous, was maintained at 60 °C for two days. Then the solution was cooled, and the phenol was separated by extraction with ether. The ether was dried with anhydrous sodium sulfate, and the ether was removed by evaporation. Analysis by 1H -NMR showed that the residue was phenol, 85–90% deuterated in the 2, 4, and 6 positions (a little short of equilibrium deuteration). It was nitrated without further purification.

Phenol was nitrated by the method of Poirier and Vottero²⁴, at 45 °C. (This temperature was important for optimizing the yield and purity of 4-nitrophenol.) After nine hours of reaction the ethanol solvent was removed under vacuum, and the organic fraction of the residue, a mixture of unreacted phenol and nitrated phenols, was separated from inorganic material by extraction into dichloromethane. The dichloromethane was allowed to evaporate, leaving a solid, brown residue. Phenol and 2-nitrophenol were removed from this solid by steam distillation. Contrary to a widespread impression, 4-nitrophenol also steam distills, though more slowly than 2-nitrophenol, so the distillate was periodically analyzed by thin layer chromatography, and the distillation was stopped when the concentration of 2-nitrophenol fell to less than 20% of its initial value. Pure 4-nitrophenol was isolated from the residue by recrystallization from water in a yield of 28.5%. When this procedure was applied to phenol-2,4,6- d_3 , the product was 4-nitrophenol-2,6- d_2 . Its identity and composition were determined by 1H -NMR spectroscopy. Its isotopic enrichment was the same as the phenol from which it was prepared.

Materials deuterated on oxygen were prepared by carrying out their preparation with O-deuterated solvents.

$1(H_2O)_2$ -2,2',6,6',O- d_5 was prepared from the corresponding D_2O solvated solid by passing laboratory air through it in a Buchner funnel for several days, until the

IR absorption of D₂O was no longer visible. Solid state ²H-NMR showed that the deuterium in the bridging position was not lost in this process.

Solid State NMR

Solid state NMR spectra were obtained with a Chemagnetics Infinity 400 NMR spectrometer, with a rotor spinning at the magic angle. In each case 1024 scans were made.

For ¹³C a standard cross-polarization, magic angle spinning pulse sequence was used.²⁵ The pulse delay was 10 s. The 90 deg. pulse width was 4.5 μs. The cross-polarization time was 5.0 ms. The acquisition delay was 20.0 μs. And the acquisition time was 20.480 ms. The observation frequency was 100.627 MHz. The proton decoupling and cross-polarization frequency was 400.148 MHz. And the spinning speed was 8 kHz. The spectral width was 50 kHz.

For ²H no cross-polarization was used. The pulse delay was 5 s. The pulse width was 4.5 μs. The acquisition delay was 20.0 μs. The acquisition time was 20.480 ms. The observation frequency was 61.425 MHz, and protons were decoupled at 400.148 MHz. The rotor was spun at 5 kHz. The spectral width was 200 kHz.

Crystallography

Crystals with dimensions between 0.1 mm and 0.5 mm and volumes between 0.016 mm³ and 0.08 mm³ were mounted on glass fibers and used for structure determination. Nitrogen evaporating from liquid nitrogen was used for cooling. Monitoring of standard reflections indicated no need to correct for sample decay or instrumental drift. Direct methods, supplemented by full-matrix least squares difference Fourier cycles gave preliminary structures containing all the atoms except the hydrogens.^{26,27} In the final refinement all the hydrogens except the bridging hydrogen were placed in ideal locations and treated as riding atoms. In the final refinement all these hydrogens were treated as isotropic, and given individual displacement parameters. The non-hydrogen atoms were refined with individual, anisotropic displacement parameters.^{26,27} The bridging hydrogen was, alternatively, treated either as a single atom with anisotropic displacement parameters, or as two half-hydrogens, each with a single, isotropic displacement parameter. The second treatment was more satisfactory and was adopted, except in the case of **3**, in which it was not possible to locate the bridging hydrogen reliably.

A Siemens SMART system was used to collect data for **1**(H₂O)₂ and **3**. An initial set of cell constants was calculated for each from reflections harvested from three sets of 30 frames. Final cell constants were calculated from large sets of strong reflections from the data collections. Random hemispheres of reciprocal space were surveyed to about 8.5 pm, and space groups were determined from systematic absences and intensity statistics.²⁶ Structures were determined using the SHELXTL V5.0 suite of programs.

Data was collected for **2** using an Enraf-Nonius CAD4 diffractometer. Cell constants were determined from a set of 25 well centered reflections in the range 15.0 < θ < 18.0, located by a random search routine. The data were corrected for Lorentz and polarization effects. The structure was determined using the TEXSAN suite of programs.²⁷

The important crystallographic parameters are collected in Table I.

TABLE I
Crystallographic Parameters^a

	1(H ₂ O) ₂ ^b	2	3
Emp. formula	C ₁₂ H ₁₃ N ₂ NaO ₈	C ₂₈ H ₄₅ N ₃ O ₆	C ₄₈ H ₃₉ N ₃ O ₆ P ₂
Molar mass (<i>M</i>)	336.23	519.68	815.76
Crystal system	monoclinic	monoclinic	monoclinic
Space group	<i>C</i> 2	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ / <i>n</i>
<i>a</i> /pm	2118.7(3)	853.4(5)	903.37(1)
<i>b</i> /pm	363.26(4)	3904(3)	2821.39(6)
<i>c</i> /pm	1032.5(1)	926.7(5)	1618.56(3)
β /°	117.23(1)	103.02(3)	97.351(1)
<i>V</i> /nm ³	0.7066(1)	3.008(6)	4.0914(1)
<i>Z</i>	2	4	4
<i>D</i> /(g/cm ³)	1.580	1.148	1.324
μ /cm ⁻¹	1.59	0.75	1.61
<i>T</i> /K	173(1) ^c	177(1)	173(1)
No. reflections	1510	7582	20742
Ind. reflections	782	3127	7197
No. parameters	122	339	574
Trans. range ^d	0.80–0.91	–	0.72–0.75
<i>G</i> (<i>F</i>) ^e	0.91	1.40	1.13
<i>R</i> ^f	0.032	0.044	0.047
Weighted <i>R</i> ^g	0.084	0.048	0.100

^a In each case the number in parentheses is the uncertainty in the last cited figure.

^b The Flack parameter²⁶ for 1(H₂O)₂ was 0.4(7).

^c The structure of 1(H₂O)₂ was also determined at room temperature. It is only slightly different from the 173 K structure.

^d An absorption correction was semiempirically determined for 1(H₂O)₂ and 3. No such correction was made for 2.

^e *G*(*F*) is the goodness-of-fit parameter. For 1(H₂O)₂ and 3 $G(F) = [\sum w(F_o^2 - F_c^2)^2 / (n - p)]^{1/2}$ where *w* is a weighting factor,²⁶ *n* is the number of independent reflections, and *p* is the number of parameters.

For 2 $G(F) = [\sum w(F_o - F_c)^2 / (n - p)]^{1/2}$, Ref. 27.

^f $R = \sum ||F_o| - |F_c|| / \sum F_o$, Ref. 26, 27.

^g The weighted *R* for 1(H₂O)₂ and 3 is that described in the SHELXTL manual.²⁶

The weighted *R* for 2 is somewhat different, and is described in the TEXSAN manual.²⁷

RESULTS

The structures of compounds 1(H₂O)₂, 2, and 3 are shown in Figures 1–3. Complete structures will be published elsewhere. In 1(H₂O)₂ the hydrogen bond is bisected by a rotational axis of symmetry, so that the two oxygens, and the two aromatic rings are identical, and the bridging hydrogen has to be symmetrically distributed between them. As expected, the solid state ¹³C-NMR spectrum shows only one set of aromatic carbon atoms. The best fit

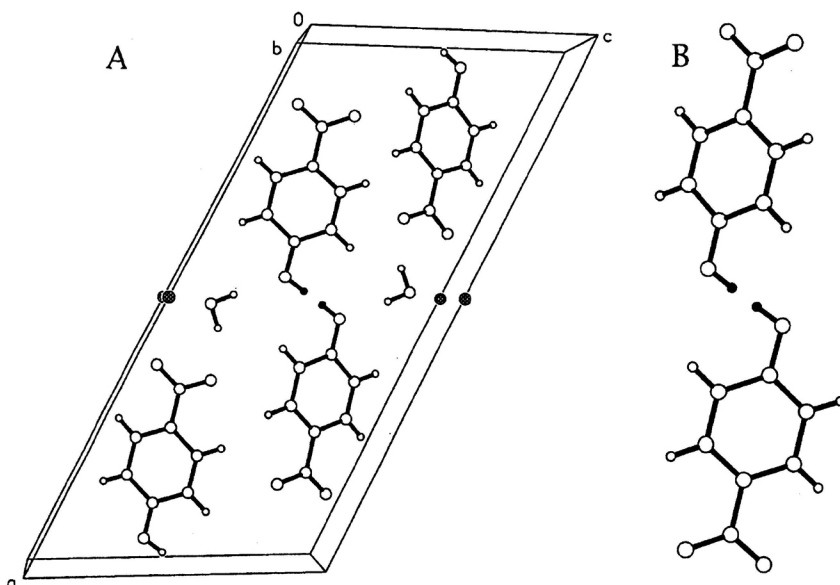


Figure 1. $1(\text{H}_2\text{O})_2$. The cross-hatched circles are the sodium ions; the solid circles are the half-hydrogens which form the Speakman-Hadži bond. (A) shows the unit cell. (B) shows an expanded view of the Speakman-Hadži ion.

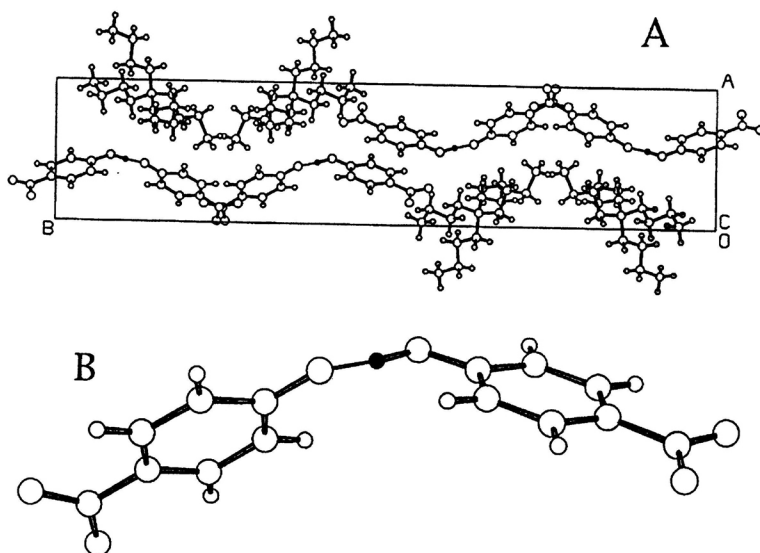


Figure 2. **2**. The filled circles are the bridging hydrogens. (A) shows the unit cell. (B) shows an expanded view of the Speakman-Hadži ion.

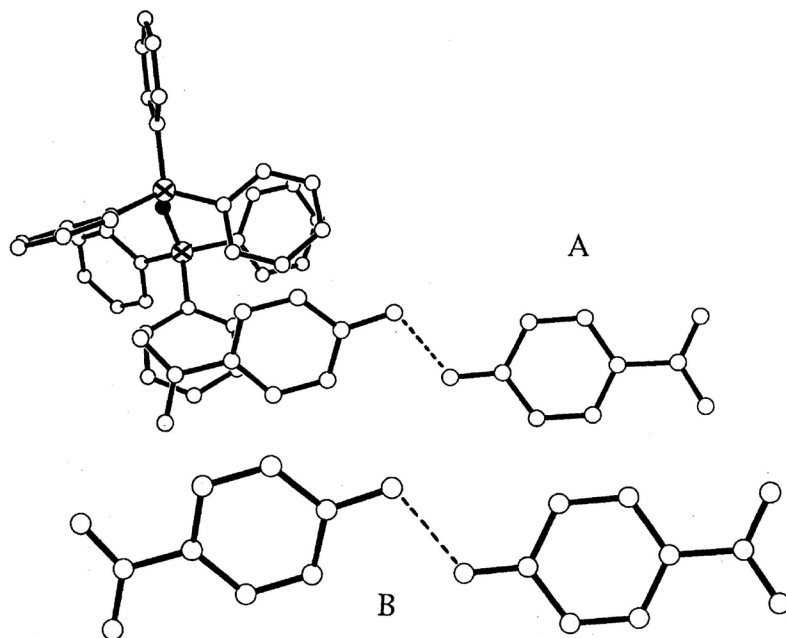


Figure 3. 3. The unit cell in this case is too large and complicated to be usefully displayed. It contains four cations and four anions. The hydrogens have been omitted for clarity, and because the bridging hydrogen could not be located reliably. The solid circle represents nitrogen, the crossed circles represent phosphorus. The dashed line is the Speakman-Hadži bond. (A) shows a single anion and a near neighbor cation. (B) gives an expanded view of the anion.

to the crystallographic data is obtained when the bridging hydrogen is represented by two half-hydrogens, separated by about 50 pm, and slightly displaced from the oxygen-oxygen axis, as shown in Figure 1.

Compound **1**(H₂O)₂ is a dihydrate, as indicated, the two waters being identical by symmetry. Each water oxygen is part of the coordination shell of a sodium ion. One of the water hydrogens is hydrogen bonded to a bridging oxygen, and the other is hydrogen bonded to an oxygen of a nitro group. The distance from the water oxygen to the bridging oxygen is 278.6 pm, so this appears to be a hydrogen bond of respectable strength, although it is much longer than a Speakman-Hadži bond. The hydrogen bond to the nitro group oxygen is somewhat longer, 284.0 pm.

In neither compounds **2** nor **3** are the two ends of the hydrogen bond related by symmetry, and the bridging hydrogen appears to be displaced toward one of the oxygens, so that there are identifiable phenol and phenoxide units in the anion. This is confirmed by the observation of two C-1 signals in the solid state ¹³C-NMR spectra of **2** and **3**. The best-fit location for the

bridging hydrogen in **2** is shown in Figure 2b. The unit cell for **3** is so large that the location of the bridging hydrogen does not appear significant. The splittings of the NMR signals are given in Table II. The crystals of **2** and **3** are anhydrous, as shown by their structures and confirmed by their IR spectra.

The best-fit oxygen-oxygen distances in the three hydrogen bonds are given in Table II, along with the statistical standard errors. The distance in the dihydrated sodium salt, $1(\text{H}_2\text{O})_2$, appears to be the shortest, but the uncertainties are such that this is not certain. It appears quite unlikely, however, that the oxygen-oxygen distance in the hydrogen bond of $1(\text{H}_2\text{O})_2$ is longer than that in the unhydrated salts.

The chemical shift has been determined for the bridging deuterons in compounds $1-(\text{H}_2\text{O})_2-2,2',6,6',\text{O}-\text{D}_5$, $2-2,2',6,6',\text{O}-\text{D}_5$, and $3-2,2',6,6',\text{O}-\text{D}_5$ by magic angle spinning, solid state NMR spectroscopy. The chemical shifts of the carbon-bound, ortho deuteriums were also determined in each of these substances. These chemical shifts are given in Table II. The apparent chemical shift for the hydrated sodium salt is equal to that for the anhydrous TBA salt, and slightly larger than that for the PNP salt. There is some uncertainty about the best reference for chemical shifts in solids, as the magnetic properties of solids vary.²⁸ In the present case solid zinc acetate- D_6 was used as a secondary reference. As usual, the primary reference was tetramethylsilane (TMS) in dilute solution in DCCl_3 . The protons (or deuterons) of zinc acetate were assumed to have a chemical shift of 2.0 ppm with respect to TMS. The carbon-bound deuterium was introduced to give a measure of the

TABLE II
Comparisons^a

	$1(\text{H}_2\text{O})_2$	2	3
O-O distance/ppm	246.5(4)	247.5(3)	249.2(5) ^b
C-1 splitting/ppm ^c	none ^d	6.9	9.6
Angle O-H-O/ ^o	169	176	—
δ (bridging D)/ppm	16.8	16.8	16.5
δ (ortho D)/ppm	6.5	6.8	6.4

^a The numbers in parenthesis are statistical uncertainties in the last cited figures.^{26,27}

^b This value was obtained by fixing the bridging hydrogen in a physically reasonable position. This position was found by removing the hydrogen altogether and finding the peak of the resulting electron density difference function. When the position of the hydrogen was refined in the usual way, the O-O distance was 249.4 pm and the hydrogen was found in an unreasonable position, not at the peak of its electron density distribution. When the structure was refined without the bridging hydrogen the O-O distance was 248.2 pm. We conclude that the data does not give a location for the hydrogen of useful accuracy, and the O-O distance is actually uncertain by close to one pm.

^c For comparison, the difference between the C-1 signal of 4-nitrophenol and that of TBA 4-nitrophenoxide, measured separately in DCCl_3 , is 16.5 ppm.

^d No splitting was observable, even at 12 K; (unpublished measurements by R. G. Griffin).

effect of the different crystal environments, and referencing errors, on the chemical shifts of the bridging deuterons. The chemical shifts of carbon-bound deuteriums (also given in Table II) were within 0.2 ppm of their central value, which suggests a similar uncertainty in the intrinsic chemical shifts of the bridging deuteriums. With such uncertainties it is impossible to be certain which of the chemical shifts is the largest, but it is quite unlikely that the chemical shift of the hydrated sodium salt is smaller than those of the two anhydrous materials. We note, also, that there appears to be a correlation between the chemical shifts and the oxygen-oxygen distances.

Since the deuterium chemical shifts of deuterated Speakman Hadži compounds can be expected to be about 0.6 ppm lower than the proton chemical shifts of the corresponding hydrogen compounds,¹⁴ the bridging hydrogen of solid hydrogen bis(4-nitrophenoxide) salts should have a chemical shifts of around 17.3 ppm, a little larger than the values of 16.8 and 16.9 ppm which have been reported for it in acetonitrile solution.^{8b}

Compounds 1-3 all give Hadži type ii infrared spectra.

DISCUSSION

Bond lengths have long been thought to have an inverse relation to bond strengths,²⁹ and the downfield chemical shift of the bridging hydrogen of Speakman-Hadži compounds is thought to be related to the strength of the hydrogen bond.³⁰ Neither criterion indicates that the water molecules incorporated in the crystal structure of the hydrated sodium salt weaken its Speakman-Hadži bond, although they are also hydrogen bonded to the bridging oxygens. In fact, both the oxygen-oxygen distances and the chemical shifts suggest that the dihydrated sodium salt may have the strongest hydrogen bond of the three. Why the apparent difference between aqueous solutions and a hydrated solid?

We believe that, as suggested by Perrin and Thoburn,³¹ the reason lies in the disorder of the liquid. If 4-nitrophenoxide and 4-nitrophenol were independently dissolved in liquid water, the anion would cause a number of water molecules to be oriented in such a way as to stabilize the system. The effect would extend out into the liquid until the individual charge-dipole interactions fell below kT . If the phenol and the phenoxide formed a Speakman-Hadži anion, the negative charge would be distributed over the two oxygens. This would roughly double the number of water molecules within any given distance of the negatively charged atoms, but the magnitude of the individual interactions would be roughly halved. If the number of water molecules involved actually were doubled the solvent stabilization energy would be unchanged by the formation of the Speakman Hadži compound.

However the number of oriented water molecules would not be doubled, because of the exclusion of the solvent from the volume occupied by the phenol, because of the overlap of the two solvation shells, and because the range over which the smaller charges would be able to overcome thermal randomization would be halved. Thus the Speakman-Hadžić anion would be less stabilized by interaction with the solvent than a structure in which the charged and neutral components retained their identity. In dilute aqueous solution this effect is apparently large enough to prevent the formation of most Speakman-Hadžić anions, although many Speakman-Hadžić salts can be precipitated from more concentrated aqueous solutions.

A hydrated crystal is not analogous. The number of water molecules it contains is fixed, and they are not likely to be disordered. In the present case, if the proton were localized, there would be a full negative charge on one oxygen, and none on the other, in simple approximation. In that case, if the water-oxygen interactions are regarded as charge-dipole interactions, one of them would double in strength; but the other would be entirely lost. In contrast to the solution situation, where the stabilization of one full charge is larger than the stabilization of two half charges, in first approximation the stabilization of the Speakman-Hadžić compound by interaction with water in the hydrated crystal environment is the same as it would be if the proton and the charge were localized.

The hydrogen bond in the dihydrated sodium salt may actually be stronger than that in the anhydrous salts, as the results suggest. The water molecules, placed as they are, reduce the electron density at the bridging oxygens, and, thus, reduce their affinity for the bridging hydrogen. But reducing the electron density at the oxygens also reduces their mutual repulsion. The two effects, together, appear to slightly strengthen the Speakman-Hadžić bond. In solution the formation constants for Speakman-Hadžić anions increase slightly as the strength of the acid is increased by electron-withdrawing substituents.³²

These results are relevant to the problem of enzyme catalysis, because enzymatic transition states are thought to be quite rigid, and to contain little or no unoriented water.¹⁹ Since transition states cannot be directly observed, it is not surprising that the most convincing demonstrations of Speakman-Hadžić bonds in enzymes occur in enzyme-inhibitor complexes designed to mimic transition states. For example, the complex of α -chymotrypsin with a peptidyltrifluoromethylketone has an apparent Speakman-Hadžić bond between a histidine nitrogen and an aspartate oxygen. Values of 18.7 and 18.9 ppm have been reported^{33,34} for the NMR chemical shift of the bridging hydrogen. The pK_a of the bridging hydrogen is 12, at least five units higher than the usual pK_a of protonated histidine.³⁴ The N-O distance in this complex is 252 pm,³⁵ about 55 pm less than the sum of the N and O van der Waals radii.³⁶ (The O-O distance reported for $1(H_2O)_2$ in this pa-

per, 246.5 pm, is about 57 pm less than twice the van der Waals radius of O.)³⁶ In another example, the complex of cytidine deaminase with the inhibitor, zebularine-3,4-hydrate appears to have a distance of only 245 pm between a hydroxyl oxygen of the inhibitor and a glutamate oxygen.³⁷ The proton affinity of the inhibitor hydroxyl group is reduced by coordination to a nearby Zn(II), so that it may well be similar to the proton affinity of glutamate. The reported O–O distance is similar to that in $1(\text{H}_2\text{O})_2$, and 59 pm less than twice the van der Waals radius of O.³⁶ When the inhibitor is replaced with an analog in which the hydroxyl group is replaced with hydrogen, its binding constant is reduced by over seven powers of ten.³⁸ The catalytic power of the same enzyme falls by eight powers of ten when the glutamic acid is replaced by an alanine.³⁹

We conclude that the presence of water in a well ordered environment need not attenuate the strength of a Speakman-Hadži bond, and need not interfere with the formation of such structural features in enzymatic transition states.

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SAŽETAK

Utjecaj uređene strukture vode na kratku, jaku (Speakman-Hadži) vodikovu vezu

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Određili smo strukture natrijeve, tetrabutilamonijeve (TBA) i bis(trifenilfosforaniliden)amonijeve (PNP) soli bis(4-nitrofenoksid)-aniona rentgenskom strukturnom analizom. Natrijeva sol je dihidrat s kisikovim atomima vode koordiniranim na natrijev ion, dok je jedan vodikov atom sa svake molekule vode vezan vodikovom vezom za premošćeni kisikov atom aniona. TBA i PNP soli su bezvodne. Ipak, udaljenost kisik-kisik je najkraća u natrijevoj soli; 246.5 pm u natrijevoj soli, 247.5 pm u TBA soli i 249 pm u PNP soli; što ukazuje da vodikova veza nije oslabljena molekulama vode i može biti najjača kod hidratizirane soli. (Sva tri spoja pokazuju Hadžijev tip II IR spektra, a u ovom se radu navode pod imenom Speakman-Hadžijevi spojevi.) ^2H kemijski pomaci premošćenih vodikovih atoma iznose 16.8 ppm za natrijevu sol, 16.8 ppm za TBA sol i 16.5 ppm za PNP sol. Ni ovdje nema dokaza da bi molekule vode slabile vodikove veze. Rezultati se mogu objasniti dodatnim vodikovim vezama s premošćenim kisikovim atomima koje smanjuju njihov afinitet prema protonu. Radi se o kompeticiji i selektivnoj solvataciji vodom koja, inače, svojom neuređenom strukturom slabi vodikove veze u vodenoj otopini. Enzimska prijelazna stanja sadrže malo neusmjerenih molekula vode pa stoga mogu sadržavati Speakman-Hadžijeve veze. Opisano je nekoliko veza u predloženim Speakman-Hadžijevim kompleksima s enzimskim inhibitorima.