

Mechanistic studies using antihydrophobic agents

Ronald Breslow*, Richard Connors, and Zhaoning Zhu

Department of Chemistry, Columbia University, New York, N.Y.
10027, U.S.A.

Abstract: The hydrophobic effect—that decreases the solubilities of organic compounds in water and leads to aggregation of such species in water solution—can be diminished by various additives that we have termed **antihydrophobic** agents. When relatively small amounts are used, this may be the principal effect of such additives on solubilities and reaction rates. If so, quantitative studies can reveal the amount of hydrophobic surface that becomes isolated from the water solvent in a transition state; this is a direct guide to transition state structure.

This paper describes the evidence for this new approach to elucidating transition state structures for some reactions in water, and the relevance of this information to the situation in other solvents.

Introduction.

Molecules with apolar surfaces tend to aggregate in water solution because of the hydrophobic effect. This—the tendency of non-polar surfaces to avoid contact with water—is often ascribed to the strong self-association of water, but a quantitative study reveals that the major factor is the superior solvation of hydrocarbons by other hydrocarbons, rather than by water (1). Regardless of its cause, the hydrophobic effect can be invoked as a major determinant of the solubility of many organic compounds in water. Thus agents that increase the hydrophobic effect cause a decreased solubility of hydrocarbons in water, while agents that we have termed **antihydrophobic** cause an increase on hydrocarbon solubility. Such antihydrophobic agents are also denaturants of proteins and nucleic acids, whose three-dimensional structures are in large part the result of hydrophobic interactions between amino acid sidechains or nucleotide bases.

Some years ago we discovered a hydrophobic effect on certain Diels-Alder reactions that caused them to be faster in water solution (2). By the use of pro-hydrophobic and antihydrophobic agents we were able to show that the major cause of the rate acceleration was indeed the hydrophobic effect—packing of non-polar surfaces in the transition states of the reactions was favored in water, lowering the transition state energies and thus speeding the reactions (2-4). Others then extended these findings, both exploring the effects further (5) and using them to direct the course of some Diels-Alder reactions of synthetic interest (6). However, the Diels-Alder reaction is by no means the only process for which one might expect hydrophobic acceleration as non-polar surfaces aggregate in the transition states.

We examined the benzoin condensation (3, 7). It is much faster in water solution than in the usual alcohol solvents, but this of course does not prove that there is a hydrophobic effect operating. In any such comparisons one must remember that water

solvates charges more effectively than do organic solvents, and this can certainly contribute to the rate advantage. However, we showed that pro-hydrophobic salts such as LiCl increase the rate of the reaction, while the antihydrophobic additive LiClO₄ decreases the rate. This test—which we termed (7) "dichotomous salt effects"—is important to show the presence of a hydrophobic component in the rate; others who are interested in this area should also use such a test, not just cite rate effects when the solvent is switched to water.

Quantitative Studies with Antihydrophobic Agents. The Theory and Supporting Evidence.

Our work showed that there was packing of apolar surfaces in the transition states for the Diels-Alder and benzoin reactions, but it did not show exactly how much surface disappeared from contact with water. It seemed to us that we should be able to extract quantitative information from appropriate studies. In our first attempt (8), we examined the effect of antihydrophobic agents on the solubility of two benzyl alcohol derivatives, one carrying a methyl group and the other carrying a t-butyl group. We saw that the increased solubility induced by the addition of small amounts of ethanol correlated—when translated into free energy terms—with the calculated amount of hydrophobic surface in the solute. Our subsequent work (9, 10) has focussed on this relationship, and demonstrates that the change in free energy of solution in water induced by small amounts of cosolvents such as alcohols is indeed proportional to the amount of hydrophobic surface in the solute. The relationship is indicated in equations 1 and 2.

$$\delta\Delta G^\circ(2) = \rho_H \cdot \delta\Delta G^\circ(1) \quad (1)$$

$$\text{therefore } \log(S/S_0)_2 = \rho_H \cdot \log(S/S_0)_1 \quad (2)$$

Here the effect of small amounts of an alcohol co-solvent on the water solubility of solute 2 is related to the effect with solute 1 by the term ρ_H that is the ratio of their exposed hydrophobic surface areas. The relationship has been established so far for solutes of related structures, with similar hydrophobic surface types.

For example, we have examined (9) the solubility of benzamide (1) and of N-phenylbenzamide (2) in water at 25 °C with and without the addition of 10 volume% 1,4-butanediol. The solubility of 1 increased 1.46 fold, while that of 2 increased 2.14 fold. From equation 2 this means that ρ_H is 2.14, comparing two phenyl groups with one. With 20% butanediol the ratio has the value of 1.95. In this case the well-known tendency for amides to adopt a trans conformation keeps the phenyl groups apart, so their surfaces are independently exposed to solvent. As another example, we compared the increased solubility of benzil (3) with that of benzaldehyde (4) in water on addition of 1,4-butanediol. Here too the phenyl groups of 3 are held apart, in this case by the dipolar repulsion of the two carbonyl groups, and the ratio of hydrophobic surfaces of 3 and 4—from the increased solubilities according to equation 2—is 1.96 ± 0.05 . By contrast, comparing benzoin (5) with benzaldehyde in this manner the ratio of surface areas is only 1.56 (10% butanediol) or 1.52 (20% butanediol). Here the two phenyl groups can partially overlap so as to shield some hydrophobic surface from solvent, and this measurement tells us how much such shielding there is. As we will see, the transition state in the benzoin condensation also has some shielding of the phenyls, but less than in the product. Later in this paper we will describe some studies on Diels-Alder reactions that further support the idea that equation 1 is followed.

In order to apply our method to reaction rates, we need to show that small amounts of apolar cosolvents have no significant effect on the rates of reactions that do not involve hydrophobic character. If so, as we have indeed found, then the cosolvent effect can be used to diagnose the amount of hydrophobic surface that is exposed in the transition state (activated complex) of the reaction compared with that in the starting materials and products. We find that the rate of the displacement reaction of hydroxylamine with sodium iodoacetate in water at 25 °C is unaffected by ethanol up to 20% v/v. In this reaction a pair of ions is produced during the displacement, so solvation could be important. However, as hoped the system just mobilizes whatever water is needed to solvate the charges, excluding ethanol from the solvation sphere if it is not useful. Such exclusion will have only a small entropy cost. As another example, the reaction rate for displacement on sodium chloroacetate by sodium thiomethoxide is also unaffected by 10 or 20% v/v ethanol in water. However, reactions that involve significant changes in the amount of hydrophobic surface exposed to solvent are slowed by antihydrophobic cosolvents.

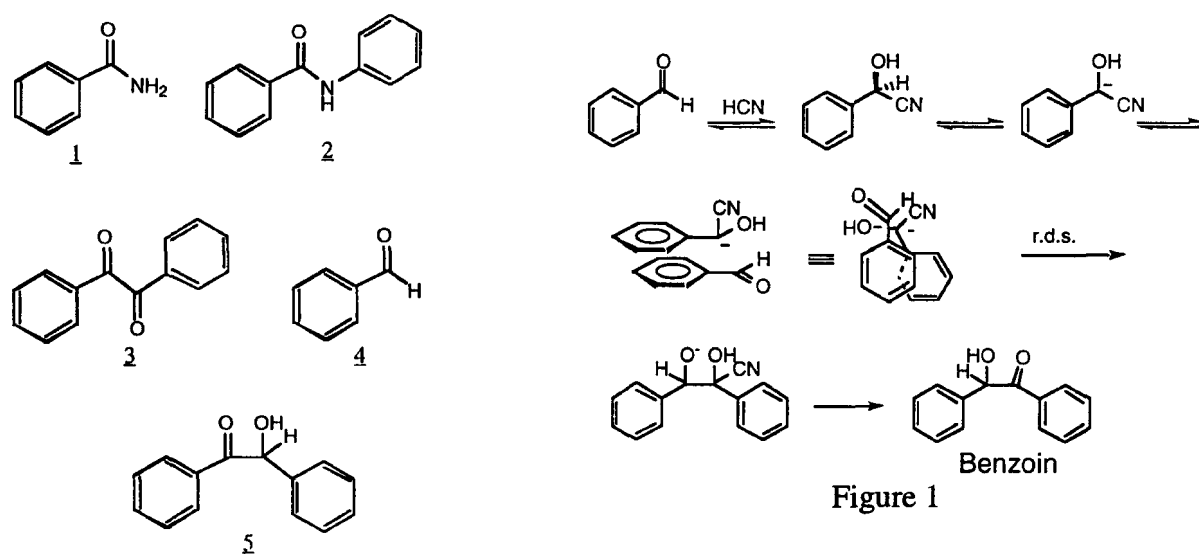


Figure 1

The Benzoin Condensation.

In the benzoin condensation (Figure 1) a benzaldehyde cyanohydrin anion reacts with a benzaldehyde molecule in the transition state, and our previous work (7) indicated that there is some overlap of the two phenyl groups. The free energy of the starting materials will be lowered by the effect of added ethanol on two exposed phenyl groups; if they were also fully exposed to solvent in the transition state, its energy would be lowered by the same amount, and there would be no rate effect. However, we see that the rate is slowed, indicating that some hydrophobic surface is hidden in the t.s. Equation 3 shows the expected relationship.

$$\log(k_0/k) = \underline{h}\Sigma\log(S/S_0) \quad (3)$$

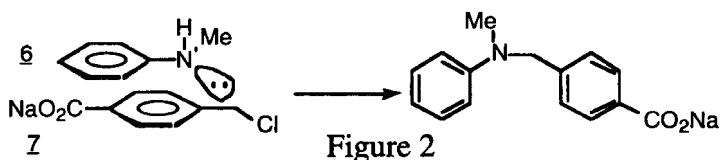
where k_0 is the rate constant in water, k is the rate constant in the presence of the antihydrophobic agent, the starting material solubility perturbations are summed over all reactants, and \underline{h} is a function of the amount of hydrophobic surface that becomes inaccessible to solvent in the transition state. In the benzoin condensation both reactants carry a phenyl group, and \underline{h} is the fraction of the total hydrophobic surface that becomes covered in the transition state. If the transition state had no exposed hydrophobic surface at all, the decrease of free energy of two reactants would make the $\log(k_0/k)$ show twice the effect of the $\log(S/S_0)$.

We had to perform the benzoin condensation at 65 °C, and the solubility studies with benzaldehyde were a problem at this temperature. Thus we used benzamide as a surrogate for the solubility effects on the starting benzaldehydes; our solubility studies at 25 °C showed that it had almost the same response to cosolvents. We determined the relative rates of the benzoin condensation at 65 °C in water (k_0) and with 10% v/v of ethanol, propanol, and 1,4-butanediol, and 20% ethanol. We also determined the relative solubilities of benzamide in water (S_0) and with the same cosolvents, and then used equation 3 to calculate \underline{h} . With 10% ethanol and 10% 1,4-butanediol we obtained S/S_0 of 2.30 and 2.54 respectively, which taken with their rate effects correspond to \underline{h} of 0.16, while with 20% ethanol and 10% propanol we obtained S/S_0 of 5.33 and 3.00, respectively, leading to \underline{h} values of 0.19. If each face of a benzene ring contributes 40% of its hydrophobic surface, with 20% for the edge, this \underline{h} would correspond to 40-47% coverage of one face of each phenyl group in the transition state.

It is interesting that the two phenyl groups in the benzoin condensation transition state are only partly occluded. Theory suggests that the cyanohydrin carbanion should add to the carbonyl group (Figure 1) from behind the π bond, overlapping with π^* . This brings the anion in at an angle relative to the benzene ring-carbonyl axis, so the two phenyl groups are not completely atop each other. Then in the product there is further coverage, with ca. 25% of the hydrophobic surface of each phenyl group not exposed to solvent. With the above assumption that a benzene face contributes 40% of its hydrophobic surface, which is of course only an assumption, this would correspond to 60% coverage of one face of each phenyl in the product benzoin. Regardless of the precise numbers, it seems clear that in this reaction the coverage of hydrophobic surfaces in the transition state is less than that in the final product. This proved not to be true in some Diels-Alder reactions discussed later.

Displacement Reactions.

Greater surface coverage is expected and seen in some other reactions. For example, the reaction (Figure 2) of N-methylaniline (**6**) with 4-(chloromethyl)benzoate anion (**7**) shows an \underline{h} of 0.36 (taking benzaldehyde solubility data as the reference for the phenyl groups in **6** and **7**), showing that the two phenyl groups essentially occlude each other in the transition state. That is, taking the same assumption as above—that each phenyl face contributes 40% of the total hydrophobicity—this value of \underline{h} corresponds to 90% coverage of one face of each phenyl group in the transition state. This is expected for overlap of the sp^3 hybrid orbital of the nucleophile with the σ^* orbital of the halide, which aligns the phenyl groups (Figure 2).



This simple tool for obtaining information on transition state structures can also give some surprises. For example, nucleophilic reaction of sodium thiophenoxide with the same **7** used above shows no rate effect of 10 or 20% ethanol cosolvent. Similarly, the reaction of sodium phenoxide with **7** shows no cosolvent rate effect. Thus in these transition states there is no packing of one phenyl on the other, and the transition states have just as much solvent exposed surface as do the starting materials.

One possible explanation of the difference between these nucleophiles and N-methylaniline, which did show a large cosolvent effect, could be stereoelectronic. The aniline will attack with an unshared pair of electrons on nitrogen that is also part of the π system, so the transition state involves a geometry (Figure 2) that forces the two

phenyls to shield each other. However, the phenoxide ion might well attack with unshared oxygen electrons that are not part of the π system, but are in the plane of the benzene ring. This would lead to a transition state (Figure 3) with a very different geometry from that for the aniline reaction, one in which the two phenyls are not occluded. As a test of this idea, we examined the reaction of 2,6-dimethylphenoxide ion (8) with 7. Here the attack by oxygen electrons in the benzene plane is sterically blocked, and now we see a very large slowing of the reaction by ethanol. Taking account of the cosolvent effects on solubilities of a phenyl derivative and of 1,3-dimethylbenzene, the effect corresponds to essentially complete coverage of the faces of the nucleophilic and electrophilic phenyl rings. The methyl groups have completely changed the geometry, and mechanism, so that now the attack is by the phenoxide electrons that are part of the π system, as was the case for the reaction with N-methylaniline. Apparently the reaction in the absence of methyls does indeed use a normal displacement mechanism, but as in Figure 3.

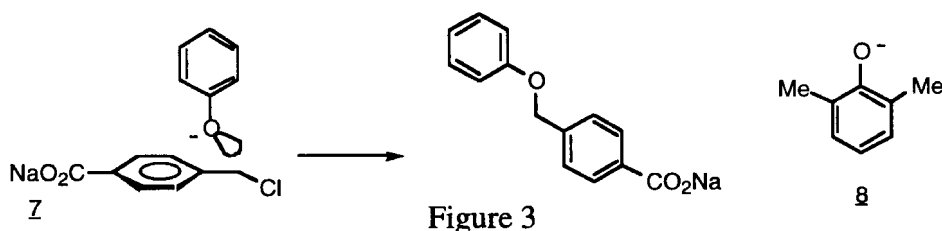


Figure 3

However, the situation is different with thiophenoxide ion as a nucleophile. Here the addition of two methyl groups ortho to the sulfur does not change the cosolvent situation: the reaction rate of substitution on 7 is unaffected by added ethanol with 2,6-dimethylthiophenoxide ion as well as with unsubstituted thiophenoxide ion. This suggests that the absence of a cosolvent effect here reflects yet a different cause from that involved in the phenoxide reactions. This could be a single electron transfer (SET) mechanism with the more easily oxidized sulfur derivative, in which electron transfer to form thiophenoxy radical, the benzyl radical, and a chloride ion occurs without there being significant hydrophobic packing of the two reactants. Further evidence will be needed to establish the complexities involved here with certainty. However, the cosolvent effects do furnish important evidence for details of the mechanisms that are not otherwise obvious.

Diels-Alder Reactions.

It is certainly clear that the two reactants in a Diels-Alder reaction will be face-to-face in the transition state, and that this should lead to hydrophobic acceleration of the reaction in water, and slowing when antihydrophobic cosolvents are added. Indeed we observed this many years ago (2-4). However, we have now applied our quantitative methods to this reaction.

The simplest process studied is the Diels-Alder dimerization of cyclopentadiene (Cp) in water, with and without alcohol cosolvents (10). We have measured the solubility of Cp in water and in 0.5/10, 1/10, and 1.5/10 (v/v) ethanol in water at 25 °C, and its dimerization rates under the same conditions. The data are plotted in Figure 4. From the slope, $2h$ has a value of 0.92 ± 0.12 , indicating that $46 \pm 6\%$ of each Cp is no longer solvent accessible in the t.s. This presumably indicates complete occlusion of one face of each Cp by the other, a reasonable transition state for endo addition. The errors are large relative to those in our other studies using this technique since here a 15% ethanol cosolvent induces a solubility increase of only 25% and a rate decrease of only 20%. The rate and solubility values themselves are accurate (standard error of the mean) to 2% and 1% respectively.

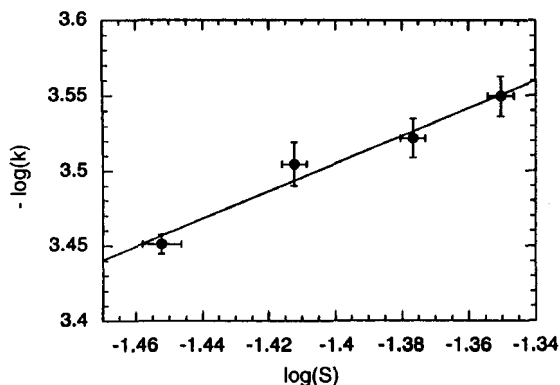
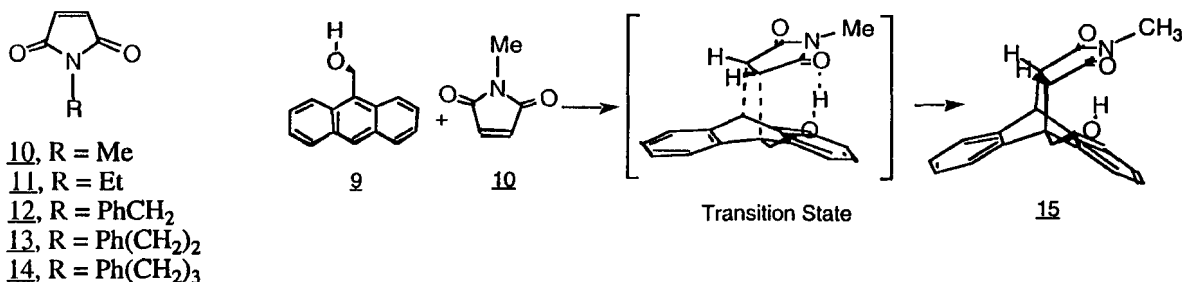


Figure 4. Cyclopentadiene dimerization second-order rate constant and cyclopentadiene solubility in water and in aqueous ethanol (5%, 10%, 15% v/v) plotted according to a transformation of equation 3. The slope has a value of $2h$.

As in all our work using this method, one ambiguity here has to do with the amount of hydrophobic surface contributed by the faces and the edge of Cp respectively, and the extent to which a face-to-face transition state would interfere with edge solvation as well as face solvation. These are matters to be addressed in further developing this novel method for determining the geometry of transition states. We have calculated the transition state geometry for Cp dimerization using the AM1 program incorporated in the SPARTAN package, and the solvent accessible surface at a 3.0 Å contour from the van der Waals surface using Macromodel (10). This predicts 38% coverage of each Cp, which is on the edge of our estimated range above.

The reactions between 9-hydroxymethylanthracene (**9**) and various N-substituted maleimides **10-14** are also accelerated by hydrophobic effects (2-4). We examined the solubilities of **9-14** in water (S_0) and (S) in dilute aqueous solutions (1/10 v/v in water) of ethanol, isopropanol, and tert-butanol. We saw (10) that these solubilities, and those of some other hydrocarbon derivatives, showed an excellent correlation between the measured $\delta\Delta G^\circ$ of solution induced by the cosolvent and the calculated solvent-accessible surface areas of the solutes. This further confirms our critical idea that these free energy perturbations are proportional to the amount of surface exposed in a reactant, product, or transition state.



For N-methylmaleimide (**10**) the reaction with **9** is 17% slower in 1/10 ethanol in water, so $\log(k_0/k)$ is 0.08 ± 0.01 . For the same solvent, $\log(S/S_0)$ is 0.26 ± 0.01 for **9** and only 0.02 ± 0.001 for **10**. Thus h has a value of 0.29. Since the contribution of **10** to the $\delta\Delta G^\circ$ is only 8% that of **9**, this means that a little over one quarter of the surface of **9** has been covered by the maleimide in the transition state. This is a reasonable picture for this reaction.

$$\delta\Delta G^\circ_{\text{t.s.}} = 2.3RT(\Sigma\log(S/S_0)_{\text{reactants}} - \log(k_0/k)) \quad (4)$$

An equivalent treatment of the data is that of equation 4. This says that the ability of the cosolvent to lower the free energy of the transition state can be calculated from the rate and solubility effects. From equation 1 this can be used to derive a value of ρ_H , the surface area ratio for the transition state relative to starting materials. The value from the above data is 0.71, another way of expressing the 29% loss of available surface.

The final product has more accessible surface than the transition state. The adduct 15 between 9 and 10 has a $\log(S/S_0)$ of 0.233 in 1/10 v/v ethanol in water compared with water alone, corresponding to a solvent accessible hydrophobic surface area 90% that of 9. Some of this change relative to the ca. 70% in the transition state may reflect an increased hydrophobic contribution from the now saturated maleimide group, but it also reflects the more open structure of 15 compared with the transition state.

We have calculated (10) the transition state geometries for reactions of 9 with 10-14 using the AM1 program incorporated in the SPARTAN package, and related them to the perturbable hydrophobic surface area in the transition states for the reactions, from equation 4. There is a good correlation for transition states calculated with geometries that allow the hydrophobic components of the maleimides 10-14 to pack onto 9. Furthermore, the slope of the line is essentially the same as that for substrate solubility perturbation vs. calculated area. This indicates that solvation effects on hydrophobic surfaces in the transition states are similar to those in normal molecules, at least in this case.

Conclusions.

The relatively simple model used in these first studies does not make distinctions among hydrophobic surfaces of various types, e.g. aromatic and aliphatic. In the aromatic series, no distinction has been made between edge and face, and they are not likely to be equivalent. Furthermore, some of the correlations described here are with calculated geometries, subject to the uncertainties of the calculations. However, our conclusions that a full face of each Cp ring is covered in the t.s. for dimerization, and that about 25% of the anthracene surface is covered in the t.s. for N-methylmaleimide addition, are direct results of our solubility and rate measurements, and are quite reasonable. Furthermore, the finding that the two phenyl rings in the benzoin condensation transition state are only partially occluded, as stereoelectronic considerations predict, indicates that hydrophobic effects do not force a reaction into a transition state geometry that is abnormal.

More work will be needed to establish the fine points, and limitations, of this approach to the determination of transition state structures, but the early results are promising.

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