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The hydrophobic effect in reaction mechanism studies and in catalysis by artificial enzymes[†,‡]

Ronald Breslow*

Department of Chemistry, Columbia University, New York, New York 10027, USA

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ABSTRACT: Water is an environmentally benign solvent, easily cleaned and with a harmless vapor, so there is great interest in using water in chemical manufacturing. However, water also has important special properties as a solvent, with the hydrophobic effect promoting rapid and selective reactions in addition reactions, atom transfer reactions, and substitutions. The presence of the hydrophobic effect can be determined with the addition of prohydrophobic and antihydrophobic materials to the water solution. Using such materials, it is possible to determine the detailed geometry of transition states for a number of classical reactions, including alkylation of phenoxide ions on oxygen and carbon. The factors involved, particularly the effect of antihydrophobic agents such as ethanol in lowering the free energy of non-polar reactants, have not been taken into account in previous well-known mechanistic studies. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: Diels-Alder; benzoin; carbonyl reductions; antihydrophobic additives; polyaziridines

INTRODUCTION

There is much current interest in the use of water as an environmentally benign solvent. Thus in the conference on Green Chemistry held in Washington DC in June 2005 there were very many papers describing various reactions in which water was substituted for more normal organic solvents. If the only advantage of water as a solvent were that it is easily purified and has a harmless vapor this could still make it an attractive solvent. However, we have seen that the hydrophobic effect that characterizes the interaction of water solvent with dissolved organic compounds can also have important consequences.

We first took advantage of this special character of water solvent in our work on biomimetic chemistry with artificial enzymes, in which the hydrophobic effect caused the substrates to bind into enzyme mimics.[1,2] More recently, we have seen that chemistry in water can be unique in the selectivity and rate accelerations it elicits.[3–11] The hydrophobic effect can also provide special information about transition states (TSs) of chemical reactions.[12–20]

**Correspondence to:* R. Breslow, Department of Chemistry, Columbia University, New York, NY 10027, USA.
E-mail: rb33@columbia.edu
[†]Based on the keynote address given by the author at the joint meeting of the 2nd International Conference on Green and Sustainable Chemistry and the 9th Annual Green Chemistry and Engineering Conference in Washington DC, June 2005.
[‡]This paper is in honor of Professor Norma Nudelman, an outstanding physical organic chemist and only the fourth woman elected as a Titular Member of the National Academy of Sciences of Argentina in its 150-year history. This article is published as part of the special issue *Festschrift for Norma Nudelman.*
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We will briefly describe our early evidence for such hydrophobic effects, then some recent work, to show how we can deduce the geometries of some TSs by modulating the hydrophobic effect. Finally, we will describe how mimics of the large molecules typical of natural enzymes can use the advantageous aspects of water as a solvent while overcoming some of its disadvantages.[21–27] The emphasis throughout will be on mechanism rather than on synthetic methodology development.

DIELS-ALDER REACTIONS

We had studied the catalysis of various reactions when substrates bind into cyclodextrins in water solution.[1,2] Molecular modeling indicated that beta-cyclodextrin, cycloheptaamylose, should be able to bind both a cyclopentadiene ring and acrylonitrile into the cavity. Thus we examined this system, and found that with cyclodextrin the Diels-Alder reaction of these components was indeed much faster than was the same reaction in a typical organic solvent.[3] However, when we omitted the cyclodextrin but kept water as the solvent we also saw a rapid Diels-Alder reaction, with a second-order rate constant about 60 times as large as that when isooctane was the solvent (Fig. 1).

Various tests[3–5] made it clear that we were seeing hydrophobic acceleration by the water solvent. For one thing, methanol as solvent accelerated the reaction by only 4-fold, not 60-fold, so solvent polarity was not the dominant factor with water. More importantly, we were able to accelerate the reaction in water using

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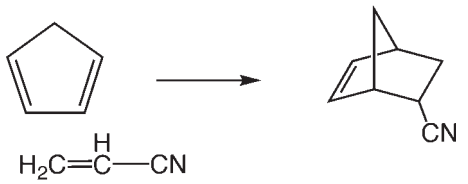


SOLVENT	RELATIVE RATE (30 °C)
ISOOCTANE	1.0
METHANOL	4.0
WATER	59.3

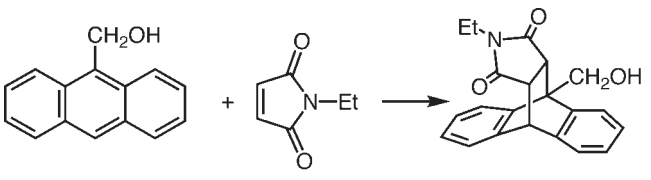
Figure 1. A Diels-Alder reaction with a large acceleration by water solvent

prohydrophobic additives such as LiCl, but antihydrophobic additives such as guanidinium chloride slowed the reaction. [Later we will describe how antihydrophobic additives let us learn the geometries of various TSs.] Similarly, water enormously accelerated the Diels-Alder reaction of *N*-ethylmaleimide with hydroxymethylanthracene (Fig. 2), and in this case the reaction in methanol was slower than that in isooctane, so hydrophobicity was the dominant factor in water, overcoming the solvent polarity effect.[4] Again pro- and antihydrophobic additives confirmed this picture (Fig. 3).

Perhaps the most remarkable finding in this early work was that the special effect of water was seen even when the reactants were suspended in the water, not dissolved in it.[4,5] As we pointed out,[1] this finding made water an attractive ''solvent'' even for synthetic reactions with reactants that have only limited solubility in the water.

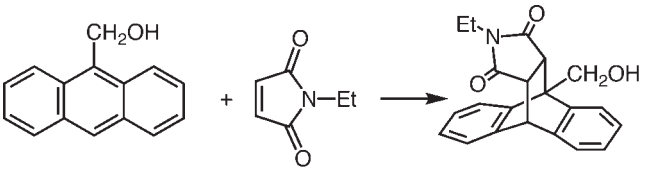
THE BENZOIN CONDENSATION

The conversion of two molecules of benzaldehyde to a molecule of benzoin, catalyzed by cyanide ion, has been extensively studied (Fig. 4). It is normally performed in



SOLVENT	RATE CONSTANTS (45 °C)
ISOOCTANE	800
1-BUTANOL	650
METHANOL	350
ACETONITRILE	100
WATER	22,500

Figure 2. A Diels-Alder reaction that is slowed by polar solvents, but greatly accelerated by water



SOLVENT	RELATIVE RATE
WATER	1.0
with LiCl (4.86 M)	2.5
with GuanidiniumCl (4.86 M)	0.32
with GuanidiniumCl (2.0 M)	0.56
with GuanidiniumClO_4 (2.0 M)	0.37
with $LiClO_4$ (4.0 M)	0.68

Figure 3. The reaction is accelerated in water by added lithium chloride, which increases the hydrophobic effect, but is slowed by the antihydrophobic guanidinium cation and perchlorate anion

ethanol solution. The rate-determining step under the usual conditions and concentrations is the addition of the carbanion of mandelonitrile to benzaldehyde, leading to kinetics first order in cyanide ion and second order in benzaldehyde. We predicted that this step would be subject to hydrophobic acceleration in water solution, and indeed that was so.[6] Under standard conditions, the reaction in water was 200 times as fast as the reaction in ethanol. Again a prohydrophobic additive LiCl accelerated the reaction, while an antihydrophobic additive lithium perchlorate slowed it.

Of course in this case, with an anionic substrate going to an anionic product, one must be concerned about electrostatic effects. In our later discussion of TS geometries we will show that the dominant effect here also rises simply from partial packing of the hydrophobic phenyl rings on each other in the TS.

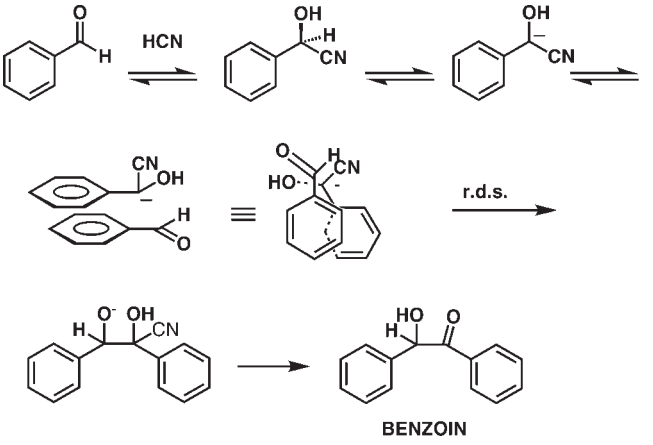


Figure 4. The benzoin condensation. Under our conditions the rate-limiting step is the addition of mandelonitrile anion to benzaldehyde with partial overlap of the two phenyl rings

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ATOM TRANSFER REACTIONS

Very recently we have taken up the study of hydride reductions of ketones and of epoxidations of olefins with reagents that can pack their hydrophobic surfaces onto substrate hydrophobic units. Again we find that important selectivities are achieved with such systems. In our earliest work we studied the selectivities achieved when ketones carrying hydrophobic groups **1a**–**1d** in competition with the methyl ketone **2** were reduced by lithium borohydride **5**, lithium phenylborohydride **6**, and lithium pentafluorophenylborohydride **7** to products **3** and **4**.[9]

As the data in Table 1 show, simple lithium borohydride **5** selectively reduced the methyl ketone in every case. Apparently the ketones **1a-1c** were deactivated by conjugative stabilization of the carbonyl group and steric crowding, while **1d** was also crowded. By contrast, hydrophobic phenylborohydride **6** overcame these effects in water, and selectively reduced the hydrophobic substrates **1a** and **1b**. In methanol solvent this selectivity reversal did not occur or occurred to a much smaller extent (**1b** with reductant **7**).

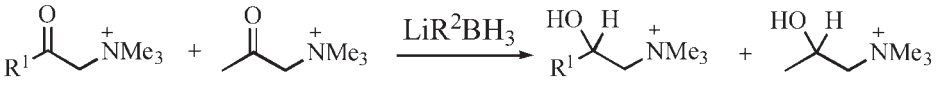
Compound **1c** showed a full reversal of selectivity in water only with reductant **7**, probably because the ketone carbonyl is somewhat twisted out of plane in substrate **1c**. From our other studies, we saw that the maximum effect in hydrophobic selectivity required that the carbonyl group be coplanar with the aromatic attached ring.[10] This presumably explains why an attached cyclohexyl group or a benzyl group in the substrate did not lead to hydrophobically induced selectivity.

In this work we also examined a steroid diketone **8** in which the carbonyl group at C-6 is deactivated to reduction because of conjugation (Fig. 5).[10] Thus with lithium borohydride **6** we observed a very large preference for reduction of the unconjugated carbonyl group at C-17 both in water and with added LiCl. By contrast, with reductant **7** in water there was a complete reversal of the selectivity. This is no surprise considering the results in Table 1, but it does indicate how the hydrophobic effect can produce selectivity in synthetically interesting examples. As expected, this selectivity reversal is suppressed when methanol is present in a mixed solvent.

HYDROPHOBIC EFFECTS AND TRANSITION STATE GEOMETRIES

In the cases described so far we have seen that the hydrophobic effects can be diminished by the addition of antihydrophobic materials such as guanidinium chloride, lithium perchlorate, and methanol. We showed that this effect reflected better solvation by incursion of the antihydrophobic substance between the water and the hydrocarbon surface.[28] The hydrophobic accelerations

Table 1. Ratios of products **3:4** formed in the competition reactions of quaternized β-keto amines **1** and **2** with substituted borohydrides under different reaction conditions[a,b,c] $\delta\Delta G^{\neq}$ (kcal/mol) for each reaction is in parentheses



1a: R^1 = Phenyl
1b: R^1 = 2-Naphthyl
1c: R^1 = 1-Naphthyl
1d: R^1 = Cyclohexyl

2

5: R^2 = H
6: R^2 = Ph
7: R^2 = C_6F_5

3 : **4**

	R^2	D_2O	4M LiCl/D_2O	CD_3OD
1a	H	44:56 (0.143)	47:53 (0.071)	31:69 (0.474)
1a	Ph	56:44 (−0.143)	64:36 (−0.341)	35:65 (0.367)
1a	C_6F_5	74:26 (−0.620)	84:16 (−0.983)	40:60 (0.240)
1b	H	53:47 (−0.071)	52:48 (−0.047)	35:65 (0.367)
1b	Ph	67:33 (−0.420)	72:28 (−0.560)	38:62 (0.290)
1b	C_6F_5	91:9 (−1.371)	95:5 (−1.745)	54:46 (−0.095)
1c	H	32:68 (0.447)	32:68 (0.447)	19:81 (0.859)
1c	Ph	40:60 (0.240)	42:58 (0.191)	30:70 (0.502)
1c	C_6F_5	60:40 (−0.240)	64:36 (−0.341)	23:77 (0.716)
1d	H	29:71 (0.531)	30:70 (0.502)	31:69 (0.474)
1d	Ph	32:68 (0.447)	32:68 (0.447)	32:68 (0.447)
1d	C_6F_5	20:80 (0.821)	20:80 (0.821)	16:84 (0.982)

[a] All reactions were carried to *ca*. 5% conversion.
[b] Experiments with **1a**, **1d**, **1f**, and **1g** were conducted at a concentration of 20 mM. Experiments with **1b** were conducted at a concentration of 6 mM. Experiments with **1c** were conducted at a concentration of 10 mM.
[c] Reported ratios are within an error of ±1% in at least duplicate runs.

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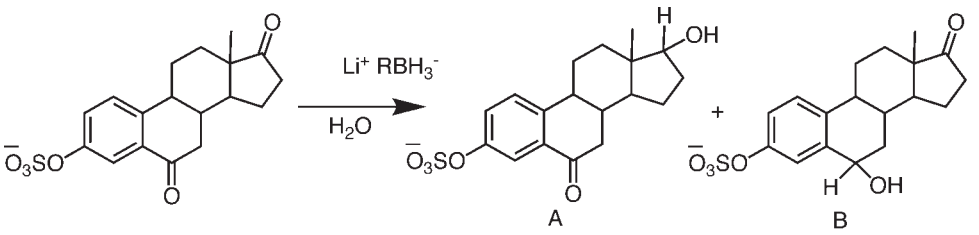

B:A ratio

R	D_2O	4M LiCl/D_2O	1:1 CD_3OD/D_2O
H	13:87	14:86	10:90
Ph	60:40	69:31	32:68
C_6F_5	78:22	85:15	46:54

Mark R. Biiscoe, Christopher Uyeda, and RB
Org. Lett. 2004, 6, 4331-4334

Figure 5. Hydrophobic overlap of reagent and substrate in water reverses the selectivity of the reduction

reflect the fact that the energy of TSs are not raised as much by moving the reaction from an organic into a water medium—relative to the increased energy of the starting materials induced by water—when the TSs, by packing, diminish the exposure of hydrophobic surfaces to the solvent. With TSs whose hydrophobic components cannot pack on each other to diminish water exposure, such as the TS for epoxidation by peracids,[11] no such effect was seen. Thus we asked the quantitative question: can we use hydrophobic effects to let us learn how much hiding of hydrophobic surface occurs in the TSs of various reactions?[20]

To diminish salt effects, we used neutral alcohols—ethanol and higher homologs—as the antihydrophobic agents. In general molecules with hydrophobic parts have limited solubility in water, reflecting the high free energy of a hydrocarbon/water interface, and added ethanol will increase their solubility. We needed to show that the resulting change in free energy with added antihydrophobic agents was proportional to the amount of exposed similar hydrophobic surface. To do this, we examined solubility changes of various compounds induced by antihydrophobic cosolvents.

Solubility is an equilibrium constant between free solute and dissolved solute, so the free energy change induced by a cosolvent—the $\delta\Delta G^\circ$—is proportional to the log of the ratio of the two solubilities, with and without the cosolvent. As Fig. 6 shows, we find that compounds with two non-overlapping phenyl rings show twice the $\delta\Delta G^\circ$ of those with only one phenyl ring. Note that it is not the water solubilities that are at issue, it is how those solubilities are altered by an added cosolvent. For instance, benzaldehyde and benzamide have very different water solubilities, but the ratio of solubility with

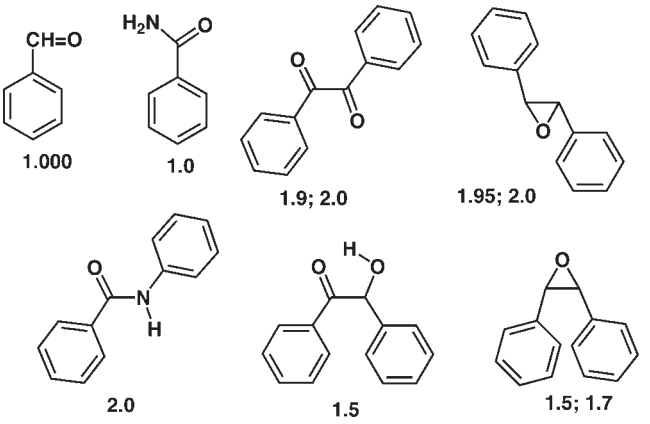

Figure 6. The change in the free energy of solution in water induced by antihydrophobic cosolvents is proportional to the amount of exposed phenyl surface

a cosolvent over solubility in water alone is the same for them, reflecting the phenyl/water interface.

In Fig. 6 we also see some important differences. Benzil and benzoylanilide and E-diphenyloxirane all show two non-overlapping phenyl rings because of their geometries, but in benzoin and in Z-diphenyloxirane the two phenyl rings partially overlap and cover about 50% of one face of each ring, so the exposed surface corresponds to only a 1.5 phenyl ring (the equivalent of three exposed ring surfaces, not four).

In Fig. 7 we show the fundamental ideas behind our study of cosolvent effects on reactions in water. With a hydrophobic substrate **S** the cosolvent will increase its solubility, corresponding to the decrease in free energy. Similarly with the product **P**. We plot one possible

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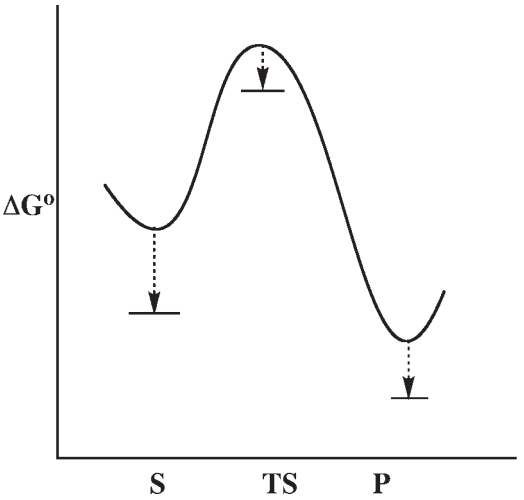

Figure 7. A free energy *versus* reaction curve for water solution (solid line) and with an added cosolvent that lowers the energies of starting materials, products, and transition state. The example shown is one in which the transition state is less hydrophobic, for example, if some hydrophobic surface is hidden by packing

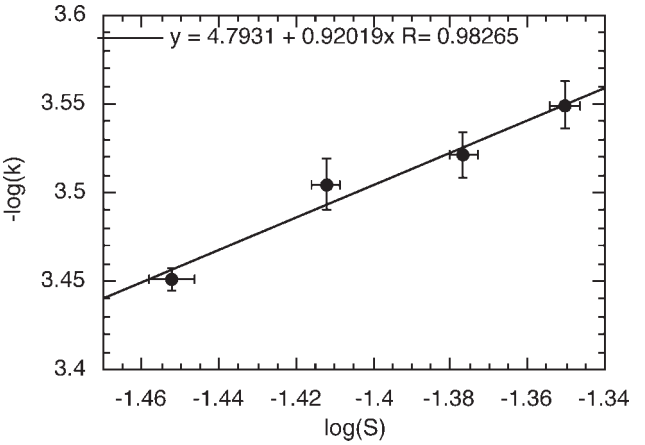

Figure 8. A plot of the log of the solubility (M) of cyclopentadiene in water and with 5%, 10%, and 15% v/v added ethanol *versus* the log of the rate constant for the Diels-Alder dimerization of cyclopentadiene ($M^{-1}s^{-1}$) in those solvents, all at 25°C. Increasing the ethanol concentration increases the solubility and decreases the rate, with proportional changes in the free energies. The slope of the curve indicates that *ca*. 92% of a cyclopentadiene surface is hidden from solvent in the transition state, corresponding to *ca*. one face of each ring

situation, in which a transition state **TS** for the reaction has less exposed hydrophobic surface than does the substrate. If this is all that matters, a point we will take up later, then the decrease in free energy of the activated complex at the transition point will be less than the decrease for the substrate, so the cosolvent will slow the reaction. Even more important, the slowing will indicate how much surface has been hidden in the transition state species. If there is no slowing, no hydrophobic surfaces have been hidden. If the hydrophobicity of exposed surfaces increases at the transition state—which we will discuss in the case of alkylation of phenoxide ions—the cosolvent will increase the rate.

To test these ideas, we first took up reactions in which no charges were involved, so the effect of the cosolvent on the water medium would reflect chiefly the solvation of hydrophobic surfaces, not a generalized change in polarity of the medium. As one example, we examined the Diels-Alder dimerization of cyclopentadiene in (quite dilute) water solution.[13,15] We saw (Fig. 8) that *ca*. 92% of one face of each cyclopentadiene is not exposed to solvent in the transition state, as expected for a face-to-face transition state. That is, the free energy of the transition state is lowered by cosolvent ethanol at various concentrations almost as much as the free energy of solution of one of the two cyclopentadienes is lowered. By a standard calculation of the transition state geometry, using AM1 and Macromodel, we predicted 76% coverage of each face, not quite as large as our measurement.[13–15]

In another example, we re-examined the Diels-Alder addition of a maleimide to an anthracene, in Fig. 9. Again in water with and without added ethanol we examined solubilities of substrates and product and the effect of the cosolvent on the reaction rate. We saw that in the transition state the maleimide covers *ca*. 27% of the total hydrophobic surface of the anthracene, completely sensible for such a reaction in which about half of one face is covered. However, in the product only 10% of the surface is no longer exposed (from solubilities), again a reasonable result.[15]

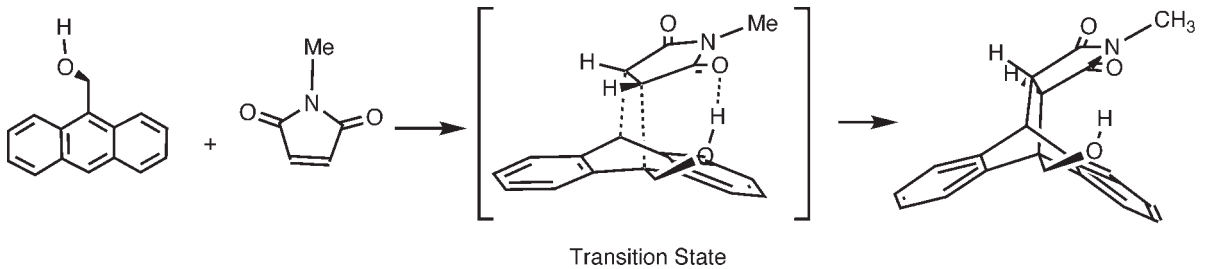

Figure 9. From the rate effects of various added alcohols on the Diels-Alder addition of *N*-methylmaleimide to 9-hydroxymethylanthracene we see that 27% of one face of the anthracene is covered in the transition state, but only 10% is covered in the product

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We also examined the effect of added ethanol, n-propanol, and 1,4-butanediol at various concentrations on the benzoin condensation (see Fig. 4), both its rate and the solubility of the benzaldehyde substrate.[15] We were concerned about the effect of the added alcohols on the energies of the cyanide ion, the mandelonitrile anion, and the initial benzoin anion product, but we found a good correlation line between the log of the solubility and the log of the rate constant in various aqueous alcohol solutions. Since the solubilities of benzaldehyde must reflect only changes in hydrophobic solvation, apparently that is also true of the rate constants. Perhaps the conversion of an anionic reagent to an anionic first product in the transition state cancels any ionic solvation differences. In any case, assuming this is so we determine that the two benzene rings in the transition state for the benzoin condensation are partially overlapping, covering only about 40% of one face of each ring. This is a sensible picture, since the mandelonitrile anion orbital must overlap with π^* of the aldehyde carbonyl, requiring an oblique approach.

In this case apparently a charged reagent anion producing a charged product anion is no problem with respect to our idea that the cosolvents act mainly to stabilize hydrophobic surfaces, but this charge compensation is unlikely to continue with other ionic reactions, such as alkylations of phenoxide ion or of aniline with a benzylic chloride. Even so, we examined such nucleophilic substitutions. We found that the reaction of *p*-carboxybenzyl chloride with phenoxide ion in water is 5% faster with added 20% v/v ethanol, while the reaction with *N*-methylaniline is 38% slower.[15]

The simplest idea here is that the phenoxide ion reaction has an unstacked transition state (Fig. 10) while that for the aniline has significant phenyl stacking (Fig. 11). This is reasonable. The phenoxide has a choice to use either the electron pair that is conjugated with the π system of the phenyl ring or instead to use one of the **n** unshared pairs. Using one of the **n** electron pairs leads to an unstacked geometry in which the π conjugation is unbroken. The aniline has no choice but to use the conjugated electrons. Our calculation of the preferred geometry for the phenoxide ion reaction TS supports this interpretation. However, there are many effects of the

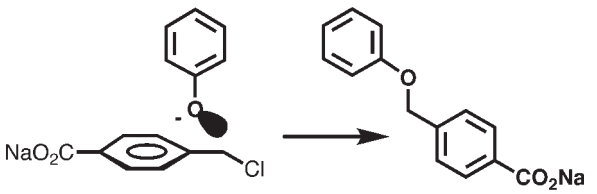

Figure 10. Alkylation of phenoxide ion by a benzyl chloride has a transition state with no loss of exposed hydrophobic surface, since the **n** electrons of the phenoxide oxygen are used

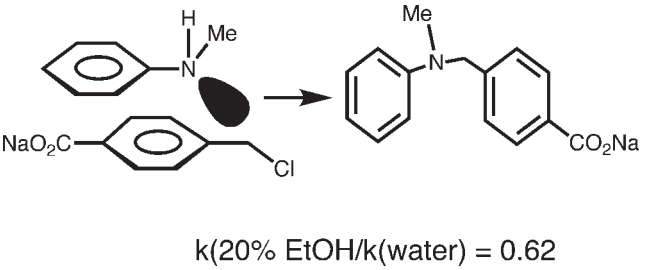

Figure 11. When an aniline is the nucleophile, the pi-conjugated nitrogen electrons are used, and the two phenyls pack and hide hydrophobic surface in the TS

cosolvent in addition to acting to solvate hydrophobic surfaces. For instance, we have seen that the chloride ion leaving group is more poorly solvated with added ethanol.

We used a number of techniques to determine what factors played a role in the cosolvent effects, with studies too complex to describe in detail here.[20] For example, we saw that DMSO is more effective than ethanol on a v/v basis in solubilizing hydrophobic species, but it has a much smaller effect on solvent polarity. Thus the rate effects of DMSO compared with ethanol could distinguish antihydrophobic solvation from charge solvation. Also, we dealt with the chloride ion solvation by replacing chlorine with a dimethylsulfonium ion, producing a neutral dimethylsulfide leaving group. We saw that the delocalized charge in phenoxide ion makes the phenyl ring less hydrophobic, so phenoxide ion becomes more hydrophobic as the oxygen is alkylated. Even in an S_N2 displacement the phenyl ring of benzyl chloride becomes less hydrophobic as partial positive charge is delocalized into the ring during reaction. Considering all these effects, Is the TS of Fig. 10 correct?

Our most convincing evidence came from a re-examination of the alkylation of 2,6-dimethylphenoxide (**8**) by *p*-carboxybenzyl chloride, in which the same reagents follow two different paths with different cosolvent effects (Fig. 12). Others had seen that in water, but not in other solvents, this reaction led to both *O*-alkylation and *para C*-alkylation, producing products **9** and **10**.[29,30] They had ascribed the *C*-alkylation, which is not seen with simple phenoxide ion, to steric hindrance of the oxygen by the methyl groups in **8** and to localized solvation of the oxygen by water, again blocking reaction there.

We saw that ethanol slightly *increased* the rate of *O*-alkylation, and DMSO even more so, but that both *decreased* the rate of *C*-alkylation, again the DMSO even more so. Since in both cases there is loss of phenoxide ion charge, and the need to solvate the chloride leaving group, the difference in cosolvent effects indicates that oxygen alkylation does not involve phenyl overlaps, just as we had earlier suggested, but that *C*-alkylation does involve such overlap (Fig. 13).

Even more strikingly, we saw that moving the methyl groups to the *meta* position in compound **11** still led to the

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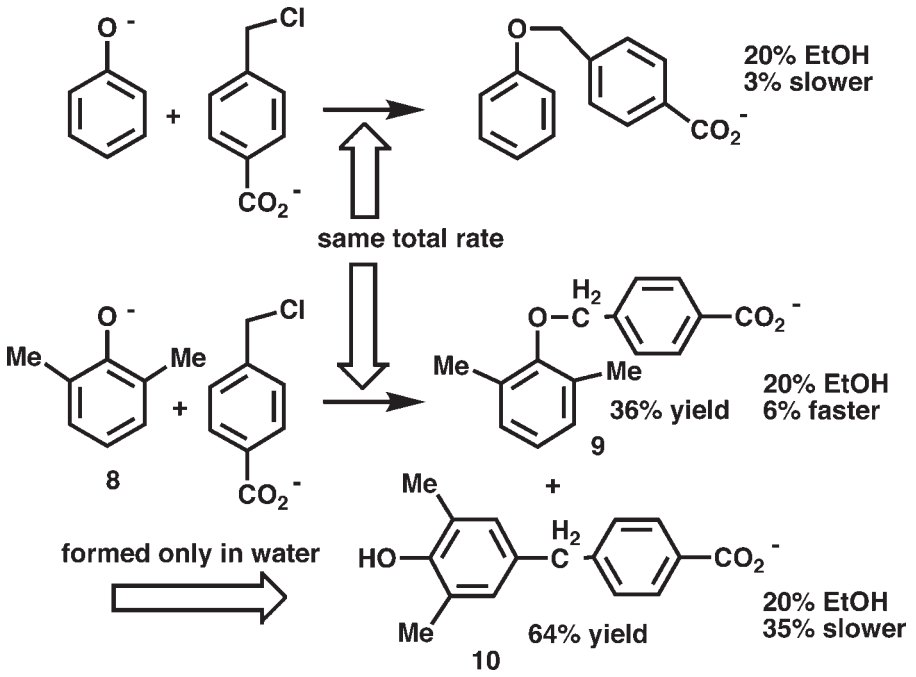

Figure 12. Simple phenoxide ion is alkylated only on the oxygen atom, but 2,6-dimethylphenoxide is alkylated on the *para* ring position as well. This *C*-alkylation occurs only in water solvent, and the effects of added ethanol indicate that *C*-alkylation, but not *O*-alkylation, involves hydrophobic packing in the transition state

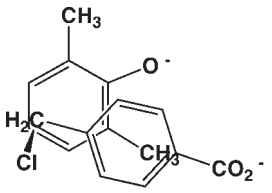

Figure 13. The ortho methyl groups promote hydrophobic packing and *C*-alkylation in water

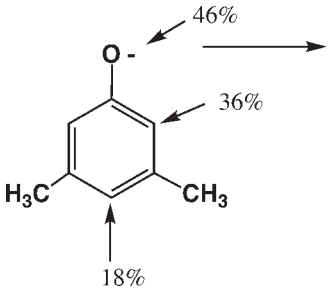

Figure 14. Even when the methyl groups are *meta* to the phenoxide oxygen there is ring alkylation by *p*-carboxybenzyl chloride in water solution

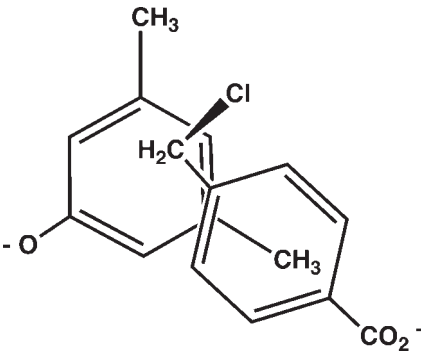

Figure 15. The *meta* methyl groups also add to the hydrophobic surface that promotes ring alkylation

C-alkylation not seen with simple phenoxide (Fig. 14). It is clear that the methyls do not supply steric hindrance as their primary role, but instead they extend the hydrophobic surface of the phenyl rings so as to promote hydrophobic stacking of the two reactants (Fig. 15). Supporting this, even a *para* methyl group on phenoxide ion is enough to promote some *C*-alkylation in the *ortho* positions, but other more polar *para* substituents have no such effect (Fig. 16).

From the use of the other approaches mentioned above, we were also able to show that with aniline as a nucleophile the alkylation by a benzylic chloride is a process with phenyl overlap (Fig. 11), as we had suggested. However, with reactions involving ions and their solvation it is in general critical to consider all the effects of cosolvents on rates in water, not just the modulation of hydrophobic solvation.

Even so, hydrophobic solvation effects should not be ignored for any reaction in water. In the reactions of *C*-alkylphenoxides just discussed the earlier workers did not consider it, and it was not considered in the classic work by Winstein and Grunwald on solvolysis reactions in

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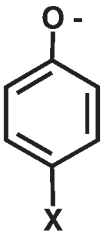
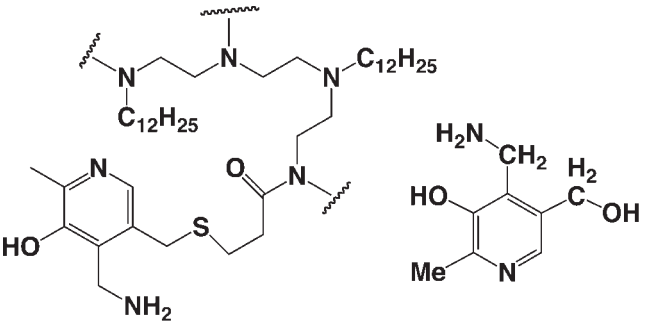


X = Me 16% ortho alkylation

X = MeO, Cl, CN, NO_2 only O alkylation

Figure 16. A *para* methyl group is enough to promote ring alkylation of phenoxide ion by *p*-carboxybenzyl chloride in water, but other non-hydrophobic substituents have no such effect



The Hybrid Pyridoxamine

Figure 17. An artificial transaminase enzyme with a pyridoxamine unit linked to a polyaziridine with attached hydrophobic chains

water.[31] They saw that the rate of solvolysis of *t*-butyl chloride in water was decreased with added ethanol, and concluded that this reflected poorer solvation of the product *t*-butyl cation and chloride anion by the less polar mixed solvent. Surely this played a role, but they did not consider the fact that *t*-butyl chloride itself is quite non-polar, and its water solubility is considerably increased by added ethanol. Thus the added ethanol not only raised the energy of the transition state with its partially ionic character, it also lowered the energy of the starting material, increasing its solubility. Our estimate is that about 50% of the rate effects of ethanol seen by Winstein and Grunwald were actually caused by this stabilization of starting material, the rest being as they ascribed.[20]

REACTIONS IN WATER, BUT NOT EXACTLY

Enzymes are large molecules, and the water-soluble ones generally have a charged polar exterior for water compatibility, but a non-polar interior where the catalysis actually occurs. This has large rate advantages. Enzymes typically use general acids and bases as catalytic groups, and sometimes nucleophilic groups as in serine proteases, and in water these catalytic groups are all hydrogen bonded with water molecules. Substrates are also frequently solvated by water molecules at their reactive points. The solvent water must dissociate from substrate and catalyst groups before catalysis can occur, and this slows the reaction. Thus many chemical processes proceed more rapidly in non-protic media rather than in alcohols or water. However, water is normally needed as the medium in enzymatic reactions, since for many substrates a principal binding mode into the enzyme is hydrophobic—the substrate non-polar sections are taken into the non-polar enzyme interior so as to diminish water/hydrocarbon interfaces.

We have initiated a series of studies of artificial enzymes that can take advantage of this principle. In our initial studies we have used commercially available polyaziridines of a variety of sizes.[32–35] The first example was a cross-linked highly disperse polyaziridine with M(n) of 60 000 and M(w) of 750 000. We attached some pyridoxamines to it and as well-attached hydrocarbon chains ranging from methyls up to hexadecyls. With the long chains (Fig. 17) there was an appreciable interior hydrophobic core.

To describe the results briefly, we saw that the polyamine itself (with and without the hydrophobic core) was an effective acid-base catalyst of transaminations by the pyridoxamine (Fig. 18). Because of electrostatic repulsion, this polyamine titrates all the way between pH 3 and pH 13, so at pH 8 it is half protonated. This gives it the strongest general acids and general bases possible at the pH 8 of our studies, those with a pK at the operating pH. We also saw that with the hydrophobic core the k_{cat} for amination of pyruvic acid to alanine by the attached pyridoxamines was increased several hundred fold compared with the polymers with methyl groups, apparently reflecting the results of a non-aqueous reaction medium described above. The substrate binding reflected in K_M for the reaction with pyruvate was depressed by the hydrophobic core, since pyruvate is not hydrophobic itself. However, with hydrophobic phenylpyruvate substrate, forming phenylalanine, both k_{cat} and the binding constant were increased with the polymer carrying a hydrophobic core.

The result was that with phenylpyruvate the conversion to phenylalanine was promoted by a rate factor k_{cat}/K_M of 240 000-fold by a pyridoxamine in the polymer compared with the second-order rate constant for pyridoxamine in simple water solution at the same pH. However, there was no guarantee that the pyridoxamines in the polymer were located in the hydrophobic core. Thus we examined a related case in which the polymer had no attached pyridoxamines but did have the hydrophobic core.[25] In this case we used pyridoxamine coenzyme modified with a hydrophobic sidechain to bind reversibly into the polymer, along with the substrate. With phenylpyruvic acid we saw an acceleration of 725 000-fold with this

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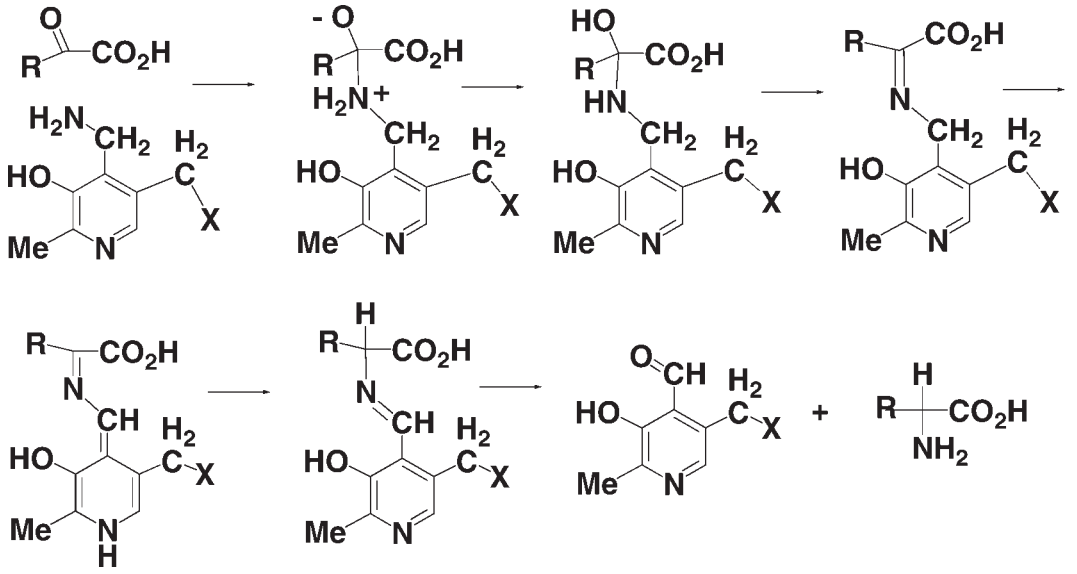

Figure 18. The mechanism of transamination by pyridoxamine units has many steps in which general acids and general bases can play a role

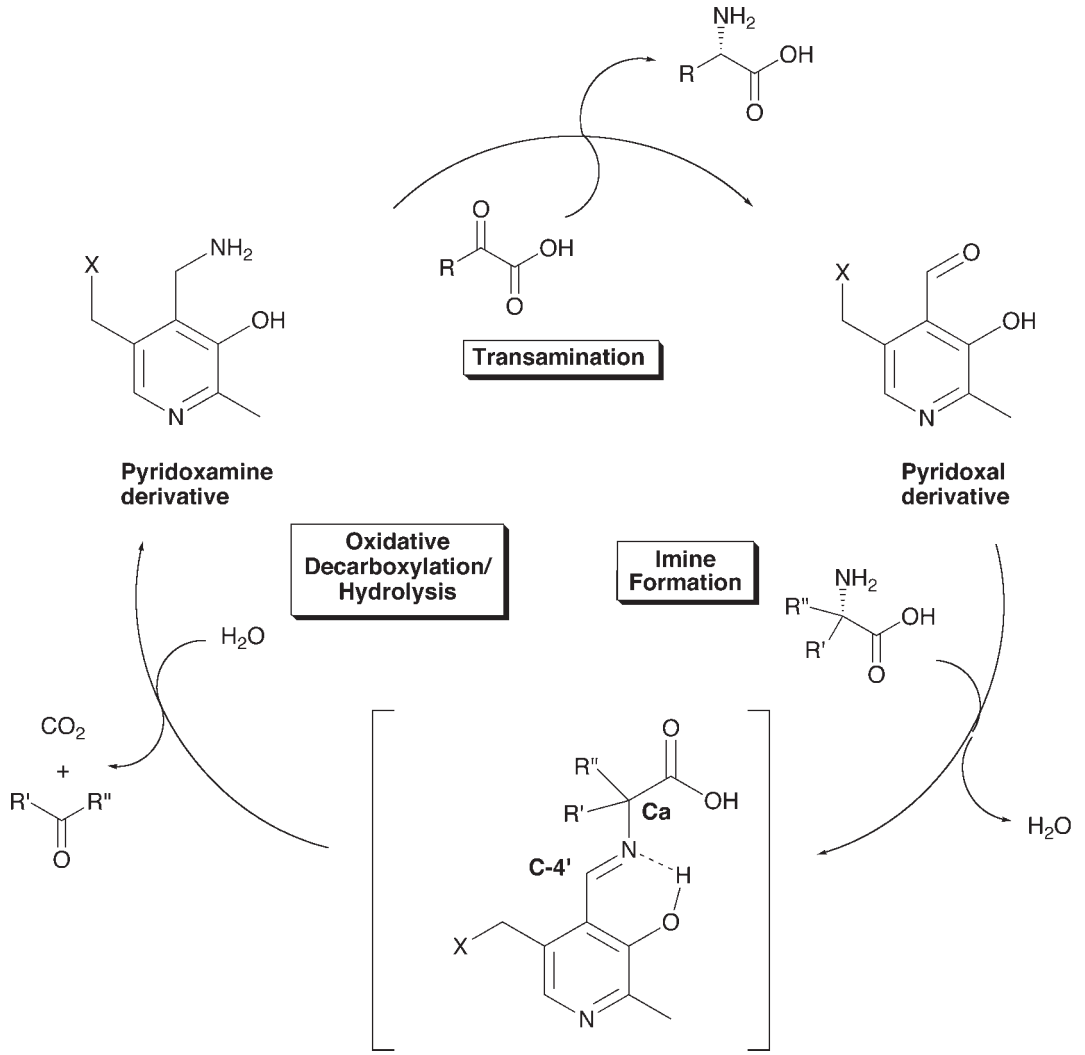

Figure 19. A catalytic transamination cycle. The pyridoxal derivative is converted to the pyridoxamine form by reaction with an α-methylated amino acid, to form the bracketed imine. This decarboxylates and protonates at C-4′ to form an imine of the pyridoxamine, which hydrolyzes to the pyridoxamine and a ketone. As many as 100 turnovers have so far been achieved

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system compared with the reaction without the added polymer.

Transamination by pyridoxamines converts ketoacids to amino acids, but the pyridoxamine itself is converted to pyridoxal. Biochemically this is converted back to the pyridoxamine form by a reverse transamination with a sacrificial amino acid, which becomes the related keto acid. However, chemically this is a slow and inefficient process, generally contrathermodynamic, and in model systems it has never worked well. Thus we devised an alternative, based on the unusual enzyme α-methylaminoacid decarboxylase. This forms a Schiff base (an imine) with pyridoxal, and then decarboxylates to generate the imine of pyridoxamine with a ketone, which hydrolyzes in water to generate the pyridoxamine. Using this process (Fig. 19) with sacrificial α-methylphenylglycine, we were able to achieve up to 100 turnovers of the overall transaminations of phenylpyruvate to phenylalanine by our hydrophobic polymer.[25,27]

This brief outline does not do justice to the detailed studies by my coworkers using these polymers as artificial enzymes. Their names are in the references, which I urge on interested readers.

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