Interactions of Urea and Other Polar Compounds in Water[1]

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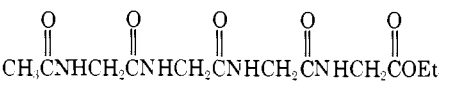
Abstract: The effects of a number of highly polar and relatively nonpolar cosolvents on the solubilities of uric acid and naphthalene in water are reported. The existence of a favorable polar interaction effect of urea and other polar, hydrogen-bonding cosolvents with uric acid, similar to that with acetyltetraglycine ethyl ester, is shown by (1) the much greater effectiveness of urea than of ethanol as a cosolvent, (2) the absence of a favorable effect of substitution of small alkyl groups for the protons of urea, amides, and guanidine hydrochloride, and (3) a decrease in the effect of urea with increasing temperature. Neither monofunctional nor bifunctional hydrogen bonding can provide the driving force for this favorable polar interaction effect of urea and guanidine hydrochloride. The available experimental data can be most simply described by the hypothesis that the driving force for the favorable interaction effect of cosolvents with both nonpolar and polar solutes arises from a more favorable *sum* of the free energies for cavity formation and for nonpolar interactions in the presence of cosolvents. With polar, hydrogen-bonding solutes the cosolvent must also be able to interact through hydrogen bonding in order to prevent a net *loss* of hydrogen bond interactions that will make an unfavorable contribution to the overall free energy of transfer from water to the mixed solvent. In other words, hydrogen bonding is necessary but generally does not provide the driving force for favorable polar interaction effects between small molecules in aqueous solution.

The development of a minimum number of empirical generalizations to describe intermolecular interactions in aqueous solution constitutes one approach toward the difficult problem of elucidating the nature of these interactions, which provide the driving force for the binding of small molecules to macromolecules, the maintenance of the three-dimensional structure of macromolecules, and the denaturation of macromolecules in aqueous solution. There are a number of more or less detailed theoretical treatments of solutions and interactions in water,[2-6] but none of them provide an altogether satisfactory description of these complex systems. On the other hand, there is a surprising shortage of experimental data describing the effects of systematic changes in the structure of solutes and cosolvents on the free energy of interactions in water, although such data must provide the basis for both empirical generalizations and more detailed theories.

There is strong experimental support for one empirical generalization, the existence of a "hydrophobic interaction,"[7] between relatively nonpolar molecules and groups in water.[8-11] It is still uncertain to what extent this interaction may be attributed to (1) attractive dispersion forces between solute molecules or groups,[12-15] (2) a "squeezing out" of such groups caused by the strong attractive forces of the solvent water,[4,9,10,16,17] and (3) structural changes in the water.[5,8,9] Urea also exhibits a favorable interaction with nonpolar solutes and substituent groups, in spite of its high polarity, but the mechanism of this interaction is no better understood than that of the nonpolar interactions.[9,11,18-21]

There is also strong evidence supporting a second empirical generalization, namely, that certain highly polar solutes interact favorably with each other in water. This has been demonstrated for the interactions of urea, guanidine hydrochloride, and amides with amides, such as the peptide acetyltetraglycine ethyl ester (ATGEE, **1**),[21,22] with other



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amides including amino acid side chains,[19,23,24] and with sucrose.[25] Polar nucleic acid bases and related compounds exhibit a favorable interaction with each other and with cosolvents of moderate polarity that is sometimes called a "stacking interaction,"[14,26-33] but it is not clear to what extent this represents a polar or a "hydrophobic" interaction.

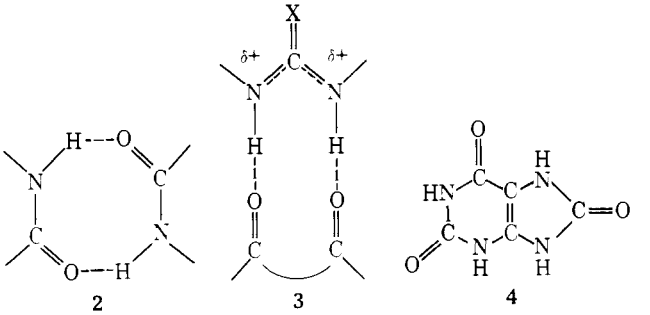
Both polar and nonpolar interactions provide the driving force for the denaturation of proteins and nucleic acids by urea and related compounds.[27,29,34-36] Experimentally, the polar interactions differ from the interactions with nonpolar solutes, as measured by solubility and activity coefficient effects, in three respects.

(1) The substitution of alkyl groups for protons on urea, guanidinium chloride, and amides results in a more favorable interaction with nonpolar compounds and a less favorable interaction with polar compounds.[21,27,35-37]

(2) Alcohols, dioxane, and related compounds generally interact favorably with nonpolar compounds but have a small or unfavorable effect with highly polar compounds, such as ATGEE.[21,38,39]

(3) The favorable effect of urea on nonpolar compounds increases with increasing temperature, whereas the effect on polar compounds such as ATGEE decreases with increasing temperature.[9,20,21,23,40]

Since there is experimental evidence that intermolecular monofunctional hydrogen bonds and bifunctional hydrogen bonds with the structure **2** have no appreciable stability in water,[41] it was suggested[21] that the explanation for the favorable polar interaction of urea and guanidine hydrochloride with ATGEE might lie in complexation through bifunctional hydrogen bonds with the structure **3**.



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We report here a study of the effects of urea and other cosolvents on the solubilities and activity coefficients of the polar molecule uric acid and the hydrocarbon naphthalene. Uric acid (**4**) is similar to ATGEE in that it is composed largely of polar -CONH- groups that would be expected to interact strongly with polar solvents but differs from

ATGEE in that bifunctional hydrogen bonding with urea through structure **3** is geometrically impossible, as revealed by inspection of molecular models. The observed favorable interaction of urea with uric acid confirms the existence of favorable polar interaction effects in water but shows that their driving force need not arise from bifunctional hydrogen bonding. At least two parameters must be postulated to describe the experimental results. The results are qualitatively consistent with a division of the observed solute–solvent interaction into one term that includes cavity formation and nonpolar interactions ($\Delta G^{cav} + \Delta G^{int}{}_{nonpolar}$) and a second term for polar interactions, $\Delta G^{int}{}_{polar}$, that is derived primarily from hydrogen bonding. We propose the simple generalization that the primary effect of almost all cosolvents is to solubilize and decrease the activity coefficient of nonionic solutes in water by making the first term more favorable and that the absence of a favorable effect of less polar cosolvents with polar solutes is simply a consequence of the poor hydrogen bonding ability of these cosolvents.

Experimental Section

All compounds were obtained commercially and (with the exception of reagent grade ethanol) were recrystallized or redistilled before use. Glass-distilled water was used throughout.

Solubility Determinations. An excess of the solid material was placed with solvent in screw-capped vials, which were sealed with a Teflon liner and dipped in paraffin. Mixing was accomplished by turning the vials end over end in a rotating rack immersed in a constant temperature bath.

To show that equilibrium had been reached the following two methods were used. In the first method, which was used with each cosolvent, one of two identical tubes was supersaturated with solute by warming. It was then equilibrated at the desired temperature so that equilibrium was approached from a supersaturated solution. The duplicate sample was equilibrated directly at the desired temperature. In the second method, the concentration of solute was redetermined after a second period of equilibration. It was found that in all cases equilibrium was reached within 25 hr at 25°, although a 48-hr equilibration period was normally used. Uric acid was shown to reach equilibrium in 24 hr at 40 and 54° and in four days at 5°. Naphthalene was shown to reach equilibrium in 6 days at 5°. The error in the reported values is estimated to be <4%.

After equilibration, the phases appeared to separate readily upon standing in the water bath for 1 hr. Aliquots of the clear solution were removed with a Pasteur pipet and filtered through a plug of glass wool in another Pasteur pipet before spectrophotometric analysis. This procedure was carried out in the cold room for the experiments at 4°. Samples were withdrawn from experiments at higher temperatures with a warmed pipet containing a plug of glass wool in the stem. The pipet was broken above the plug and the contents were transferred to a warm test tube, from which aliquots were removed with a warmed pipet for dilution.

The solvents used with uric acid ($pK = 5.4$) contained 0.01 *M* hydrochloric acid to suppress ionization of the acid. Formic acid, 0.05 *M*, was added to solutions containing formamide, dimethylformamide, or acetamide to prevent an increase in pH caused by a small amount of hydrolysis of the amide during the equilibration period. It was shown that the solubility of uric acid is not significantly affected by the presence of 0.05 *M* formic acid or by changes in pH up to pH 4.0.

The concentration of uric acid was determined after suitable dilution in 0.01 *M* hydrochloric acid at 283 nm. The concentration of naphthalene was determined similarly from the absorbance in water at 276 nm. The measured solubilities of uric acid in 0.01 *M* hydrochloric acid and of naphthalene in water were shown to be constant in the presence of up to a tenfold excess of the solid phase. In order to minimize evaporation of naphthalene from aqueous solutions during dilution and mixing, an aliquot of the saturated aqueous phase (0.50 or 1.00 ml) was layered below a measured volume of water in a Teflon-stoppered cuvette. The stopper was replaced and the mixture was inverted several times. To ensure that the spectral properties of uric acid and naphthalene are not affected by the cosolvents the absorbance was checked on either side of the wavelength maximum in the solutions most concentrated in each cosolvent. In all cases, solvent-induced spectral changes were found to be negligible.

Table I. Solubility of Uric Acid and Naphthalene in Reference Solvents

Temp, °C	Uric acid[a] in 0.01 *M* HCl, g/l.	Naphthalene[b] in H_2O, g/l.
5	0.0173	0.1042
25	0.0313	0.0302
		0.0315[c]
40	0.0631	
54	0.117	

[a] Based on ϵ_{283} 1.2×10^4 [J. P. Phillips, J. C. Dacons, and R. G. Rice, Ed., "Organic Electronic Spectral Data," Vol. VII (1964–1965), Wiley-Interscience, New York, N.Y., 1971)]. [b] Saturated aqueous solutions were extracted with hexane and the concentration was determined spectrophotometrically at 275 nm based on ϵ 5.50×10^3. [c] L. J. Andrews and R. M. Keefer, *J. Amer. Chem. Soc.*, **71**, 3644 (1949).

The solubility of naphthalene in the presence of sodium salts of phenylacetic acid, cyclohexylacetic acid, and naphthylacetic acid was determined as follows. The filtered aqueous phase (1.0 ml) was added to a stoppered tube containing 4.0 ml of hexane and 0.5 ml of 0.1 *M* sodium hydroxide. The naphthalene was extracted into the hexane and the absorbance was measured at 275 nm. The solubility of naphthalene in the presence of pyridine was determined in a similar fashion except that the tubes contained 1.0 ml of 4 *M* hydrochloric acid instead of sodium hydroxide, and either 1.0 or 0.5 ml of aqueous phase was added in order to obtain an absorbance at 275 mμ between 0.35 and 0.80. In each series of determinations the solubility in water, S_0, was determined from the absorbance of naphthalene in hexane following extraction from water under the same conditions used for the mixed solvents.

An approximate value for the solubility of uric acid in the presence of 0.25 *M* benzyl alcohol was obtained as follows. To 4.0 ml of the saturated aqueous phase was added 4.0 ml of 1.2 *M* sodium bicarbonate to convert uric acid to the anion. The benzyl alcohol was removed from the aqueous phase by extracting three times with 5-ml portions of chloroform. The aqueous phase was brought to 25.0 ml with 1.2 *M* sodium bicarbonate and the uric acid was determined from the absorbance at 291.5 nm. The S_0 value was obtained by subjecting the solution of uric acid equilibrated in the presence of 0.01 *N* hydrochloric acid alone to the same extraction procedure. It was shown that 87% of the uric acid was recovered in the aqueous phase after three extractions with chloroform in the presence of bicarbonate under these conditions.

Results

The absolute solubilities of uric acid and naphthalene in the reference solvents are given in Table I. The solubilities in the presence of a series of cosolvents are given in Table II,[42] expressed as the ratio S/S_0 where S and S_0 refer to the solubility in the presence and absence of the cosolvent, respectively. Some representative curves showing the effect of cosolvent concentration on the solubilities of uric acid and naphthalene are given in the semilogarithmic plots of Figures 1 and 2, respectively. The solubility of uric acid increases steadily with increasing urea concentration up to 1.5 *M* (Figure 1) but then levels off and drops sharply at 2.0 *M* urea to a value of $S/S_0 = 0.2$, presumably as a consequence of the formation of an insoluble urea–uric acid complex.

The effectiveness of the different cosolvents at a concentration of 1.0 *M* in solubilizing uric acid and naphthalene is compared in Table III; data for the effect of 3.0 *M* cosolvents on the solubility of ATGEE[21] are included for comparison. Free energies of transfer from water to the cosolvent solution, based on $\Delta G_{tr} = -RT \ln (S/S_0)$, and parachor values for the cosolvents are also shown. The parachor

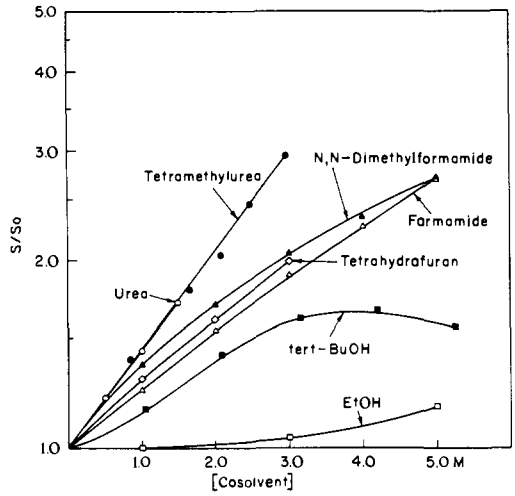

Figure 1. Representative data for the effects of cosolvents on the solubility of uric acid in water (containing 0.01 *M* hydrochloric acid) at 25°.

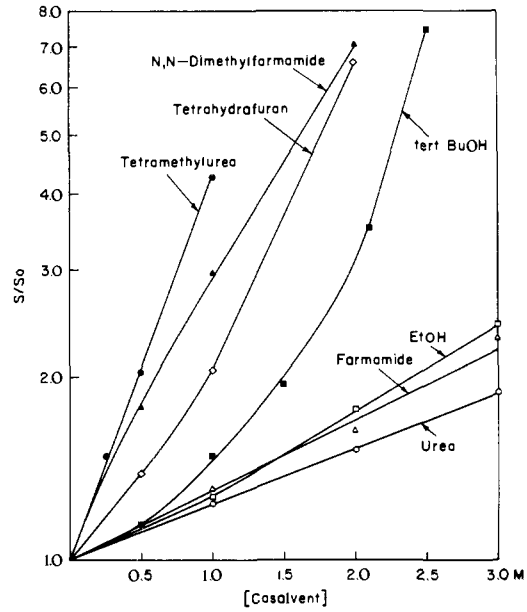

Figure 2. Representative data for the effects of cosolvents on the solubility of naphthalene in water at 25°.

was chosen as the most satisfactory method of comparing the molecular size of the cosolvents for which data or a reasonable method of estimation are available for all of the compounds examined.[43,44] For the larger alcohols and ether the solubilities were measured near the solubility limit of the cosolvent and extrapolated to 1.0 *M* assuming a linear increase of log (S/S_0) with concentration; this procedure probably gives a lower limit for the effects of these compounds in view of the upward curvature that was observed for related compounds in the plots of Figures 1 and 2. The curves for the sodium salts of substituted acetic acids exhibit a sharper upward curvature and the values for these compounds in Table III were estimated from the initial slopes.

The effects of temperature on ΔG_{tr} of uric acid and naphthalene to 1.0 *M* urea, tetramethylurea, and *tert*-butyl alcohol are summarized in Table IV. The effects of *tert*-butyl alcohol and tetramethylurea on the solubility of uric acid over the entire range of cosolvent concentration are shown in the semilogarithmic plot of Figure 3.

In Figures 4–6 the Gibbs free energies of transfer of naphthalene, uric acid, and ATGEE from water to water–cosolvent mixtures are plotted as a function of the parachor

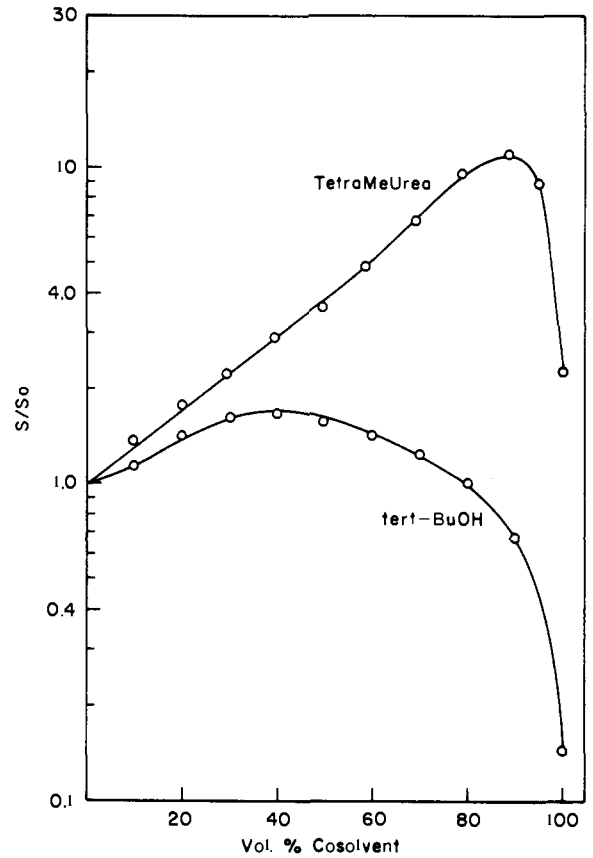

Figure 3. The solubility of uric acid in mixtures of water (containing 0.01 *M* hydrochloric acid) with tetramethylurea or *tert*-butyl alcohol at 25°. Volume per cent refers to (ml of cosolvent/ml of solution) × 100. The solutions were equilibrated for 3 days.

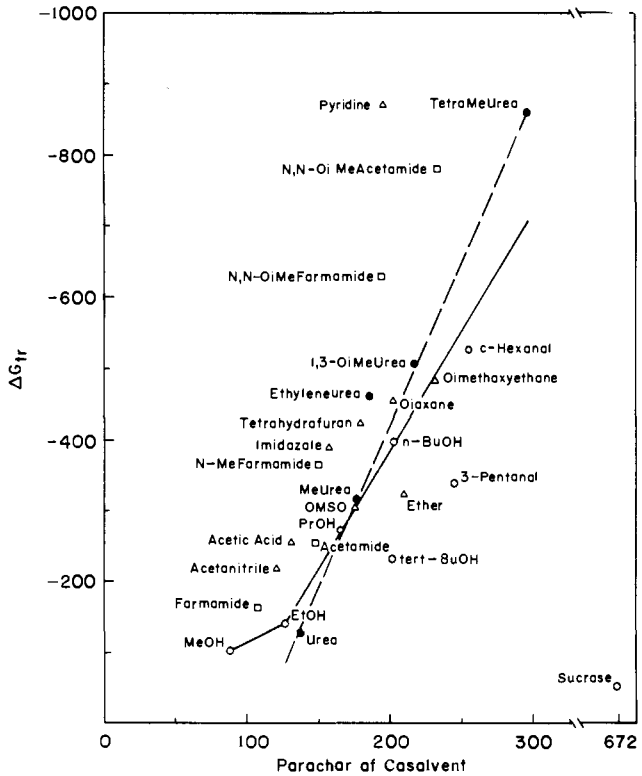

Figure 4. Values of ΔG_{tr} for naphthalene from water to 1.0 *M* cosolvents at 25°.

of the cosolvent. The effect of cosolvents is expected to increase as some function of their size and the parachor is an

Table III. Comparison of the Effects of Cosolvents on the Solubilities of Naphthalene, Uric Acid (1 *M* Cosolvent), and ATGEE (3 *M* Cosolvent) at 25°

Cosolvent	Parachor[a]	Naphthalene S/S_0	Naphthalene $-\Delta G_{tr}$, cal mol^{-1}	Uric acid S/S_0	Uric acid $-\Delta G_{tr}$, cal mol^{-1}	ATGEE[b] S/S_0	ATGEE[b] $-\Delta G_{tr}$, cal mol^{-1}
Urea	137	1.24	127	1.44	214	1.85	364
Methylurea	177	1.71	316	1.54	256		
Ethylurea	217			1.54	256		
n-Butylurea	297			2.28 (extrap)	489		
1,3-Dimethylurea	217	2.36	509				
1,1-Dimethylurea	217			1.67	302		
Ethyleneurea	186	2.18	462	1.60	279	1.46	224
Tetramethylurea	297	4.30	861	1.45	219	0.74	−178
Guanidine hydrochloride		1.18	98	1.49	236	3.5	742
1,1,3,3-Tetramethylguanidine hydrochloride		4.12	835	1.11	62	0.63	−273
Formamide	108	1.32	163	1.24	127	1.25	132
N-Methylformamide	151	1.85	365				
N,N-Dimethylformamide	194	2.96	630	1.34	173	0.89	−125
Acetamide	148	1.54	254	1.35	178	1.20	108
N,N-Dimethylacetamide	234	3.73	781			0.80	−132
Methanol	88	1.20	108	0.98	−11		
Ethanol	126	1.27	141	1.00	0	0.85	−96
n-Propyl alcohol	165	1.58	272	1.15	83		
n-Butyl alcohol[c]	203	1.96	398	1.47	228		
tert-Butyl alcohol	201	1.48	231	1.13	73		
3-Pentanol[c]	245	1.78	341				
Cyclohexanol[c]	255	2.44	528	2.0	412		
Benzyl alcohol[c]	258			45	2260		
Sucrose	672	1.08	45				
Dioxane	202	2.17	456	1.37	187	0.98	−12
Tetrahydrofuran	180	2.05	424	1.27	142	1.13	73
Diethyl ether[c]	210	1.72	321				
Dimethoxyethane	231	2.26	483				
Acetic acid	131	1.54	254	1.34	173	1.70	319
Citric acid	339	1.79	344				
Acetonitrile	122	1.45	219	1.17	93		
Imidazole	157	1.94	391				
Dimethyl sulfoxide	178	1.67	303	1.1	57		
Pyridine	197	4.36	870				
Sodium cyclohexylacetate[c]		1.27	142				
Sodium phenylacetate		1.27	142				
Sodium naphthylacetate		7.20	1170				
Sodium acetate		0.63	−278				

[a] Based on experimental or calculated[43] values. A correction of +3.2 ± 0.5 for the amide group was obtained from the difference between the observed values for formamide and acetamide and values calculated from the sum of the H—C, C=O, C—N, and N—H bond parachors.[43] The corresponding correction for urea is presumably more than one times and less than two times this value; the parachor for urea was accordingly calculated to be 137 based on an assumed correction of 4.5. [b] Reference 21, 3 *M* cosolvent. [c] Extrapolated from solubility measurements at concentrations of cosolvent below 1.0 *M*.

Table IV. Effect of Temperature on $-\Delta G_{tr}$ for Uric Acid, Naphthalene, and ATGEE from Water to 1.0 *M* Urea, Tetramethylurea, and *tert*-Butyl Alcohol

Temp, °C	$-\Delta G_{tr}$, cal/mol^{-1} Urea	Tetramethylurea	*tert*-Butyl alcohol
	Uric Acid		
5	324	205	0
25	216	220	72.5
40	209	222	103
54	219		
	Naphthalene[a]		
4.5	119	664	73
25	127	864	232
	ATGEE[b]		
0	343[c]		
25	260[c]		
40	248[c]		

[a] The solubility of naphthalene at temperatures higher than 25° was not determined because of losses by evaporation from aqueous solution during filtration and pipetting. [b] Reference 21. [c] 2 *M* urea.

approximate measure of molecular volume; the parachor of water is 53.[43,44] The solid lines in the figures are drawn through the points for a series of simple alcohols (open circles) and the dashed lines through the points for most of the substituted ureas (solid circles). The scales of the three figures are the same, but it should be noted that the ordinate scale for ATGEE is displaced upward and the data for naphthalene and uric acid refer to 1 *M* whereas those for ATGEE[21] refer to 3 *M* solutions of the cosolvent. The concentration of 1 *M* for the naphthalene and uric acid data was chosen for the comparisons as a compromise between the desirability of examining highly aqueous solutions and of obtaining experimental data in the presence of sufficient cosolvent to give easily measurable differences in solubility. These free energies of transfer provide an approximate measure of the trends that are associated with changes in the size and structure of the cosolvents but do not give an exact measure of concentration-independent effects because of the nonlinear dependence on concentration of the effects of some cosolvents (Figures 1 and 2). It should also be noted that the points on the right-hand portion of the figures are displaced upward if the solubility data are plotted as a linear instead of as a logarithmic function of the cosolvent concentration and that there is a similar displacement if ΔG_{tr} is plotted as a function of the surface area,[4,31,45] rather than the volume, of the cosolvent.

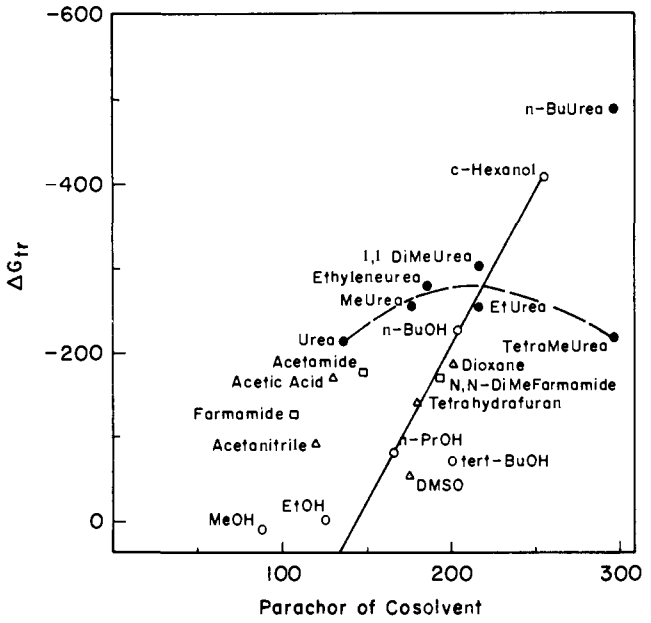

Figure 5. Values of ΔG_{tr} for uric acid from water to 1.0 *M* cosolvents at 25°.

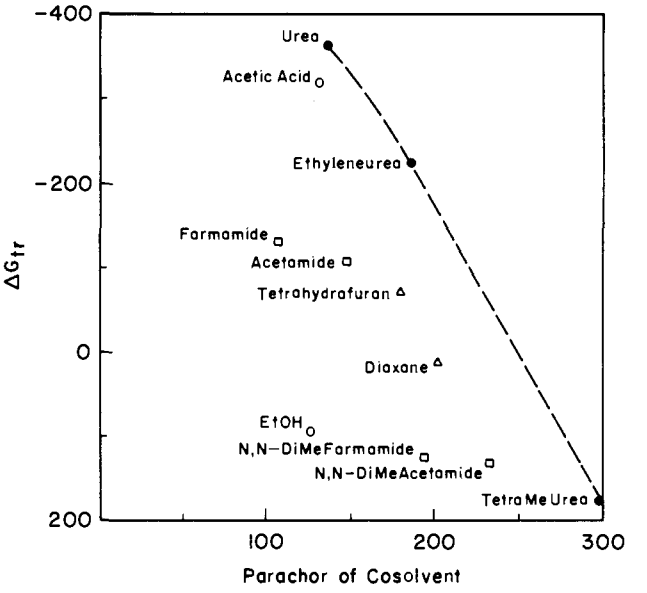

Figure 6. Values of ΔG_{tr} for ATGEE from water to 3.0 *M* cosolvents at 25°.[21]

Discussion

The following evidence supports the conclusion that uric acid exhibits a polar interaction with urea and other polar cosolvents, of the kind observed with ATGEE,[21] as well as a nonpolar interaction, such as is observed with naphthalene.

(1) Urea is the most effective organic cosolvent of its size for uric acid, whereas 1 *M* ethanol has no significant effect on the solubility of uric acid (Figure 5). This behavior is the same as with ATGEE (Figure 6), but for naphthalene the relative order is reversed and urea is the least effective cosolvent of its size. A similar difference is seen with guanidine hydrochloride, which is even more effective than urea with uric acid (Table III), and formamide shows a similar but smaller difference.

(2) Substitution of small alkyl groups for the protons of urea causes a dramatic increase in the favorable interaction of urea derivatives with naphthalene, decreases and eventually reverses the favorable interaction with ATGEE, and has very little effect with uric acid (dashed lines, Figures 4–6). Evidently, in the case of uric acid the increase in the favorable nonpolar interaction is offset by the loss of the polar interaction of unsubstituted urea upon alkyl substitution. A similar result is found in the amide series. Most of the favorable interaction of guanidine hydrochloride with uric acid is lost upon the replacement of 4 protons by methyl groups in tetramethylguanidine hydrochloride, as in the case of ATGEE, but with naphthalene the tetramethyl compound is nine times more effective than guanidine hydrochloride (Table III). The existence of a nonpolar interaction effect with uric acid is shown by the favorable effect of less polar cosolvents, such as the larger alcohols, and shows a similar increase with increasing size as in the case of naphthalene (solid lines, Figures 4 and 5). The large effect of *n*-butylurea with uric acid contrasts with the small effect of other alkyl substituents (Figure 5); a similar result has been found for protein denaturation.[36] The value of $-\Delta G_{tr}$ of 489 cal/mol is close to the sum of the values for urea and *n*-butyl alcohol of 214 and 228 cal/mol, respectively, suggesting that the effects of the urea and *n*-butyl groups of *n*-butylurea are roughly additive.

(3) The effect of temperature on the interaction of urea with uric acid is characteristic of the polar interaction of urea with ATGEE and differs from that for nonpolar interactions (Figure 7). The favorable effect of *tert*-butyl alcohol and tetramethylurea on the solubility of naphthalene and of urea on the solubility of toluene and *n*-butane[20] increases with increasing temperature, as expected for a nonpolar "hydrophobic" interaction effect;[9] the temperature dependence for naphthalene and urea over the limited range of temperature examined is small but in the same direction. The effects of *tert*-butyl alcohol and tetramethylurea with uric acid also increase with increasing temperature as expected for a nonpolar interaction. In contrast, the polar interaction of urea with ATGEE and with uric acid exhibits a

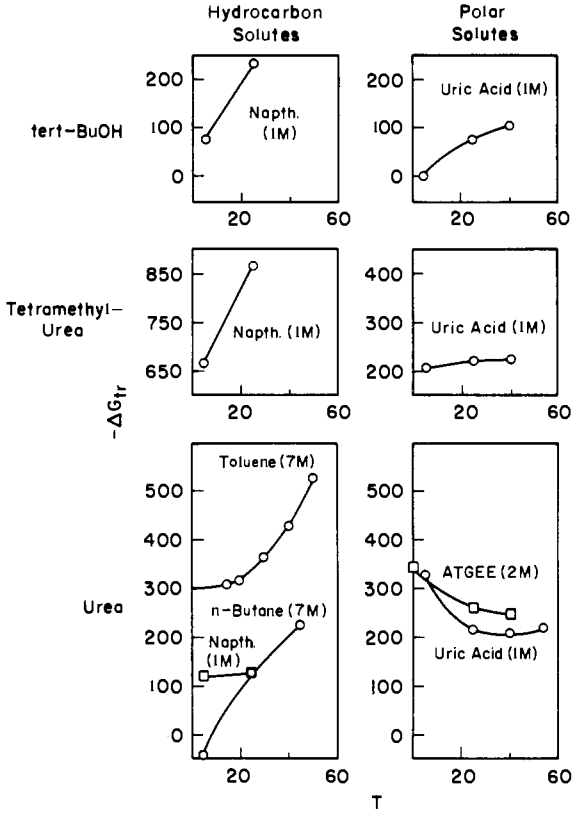

Figure 7. The effect of temperature on $-\Delta G_{tr}$ of hydrocarbon and polar solutes from water to the indicated cosolvent solutions. Concentrations of cosolvents are shown in parentheses. The data for toluene and *n*-butane are from ref 20 and for ATGEE from ref 21.

decrease with increasing temperature in the range 0–40°. The same temperature dependence is exhibited for the effect of urea on diketopiperazine.[23]

(4) The solubility of uric acid increases with increasing concentration of *tert*-butyl alcohol to a maximum value and then decreases sharply as the concentration of the solvent approaches 100% *tert*-butyl alcohol (Figure 3). Similar behavior is observed with tetramethylurea, with more than a tenfold increase in solubility in the presence of 90% cosolvent followed by a sharp decrease. The solubility curve does not exhibit the characteristic plateau region that is expected for the formation of an insoluble uric acid–tetramethylurea complex,[30] although this possibility has not been rigorously excluded. The results suggest that in water-rich solvents the addition of cosolvents results in a favorable interaction with uric acid that increases solubility, but that starting with pure organic solvent the addition of water provides a favorable polar interaction that increases solubility even more sharply.

It is evident that the uric acid molecule can interact favorably with both polar and nonpolar cosolvents. It is possible that the polar interactions are favored around the periphery of the molecule with its highly polar –CONH– groups and the nonpolar interactions are favored on the nearly planar faces of the molecule.

The observed favorable interaction effects may represent a decrease in the activity coefficients of individual solute molecules in the presence of added cosolvent, the formation of a weak stoichiometric complex of solute and cosolvent, or both. We refer to the observed effects here simply as a free energy of transfer from water to the water–cosolvent mixture.

These results cannot be described in terms of any single parameter scheme, such as a single scale of varying polarity for different solutes and cosolvents or the solubility parameter of regular solution theory.[46] In particular, at least two parameters are required to describe the experimental result that the order of effectiveness of the different cosolvents at low to moderate concentrations is entirely different and the effect of structural changes in the cosolvents is different or even reversed with naphthalene, uric acid, and ATGEE. For example, a one parameter scale of varying polarity could explain the solubilization of naphthalene, uric acid, and ATGEE by urea if all of these compounds are less polar than water, so that the addition of urea decreases the polarity of the solvent. The solubilization of naphthalene by ethanol could be explained similarly. However, such a scheme predicts that the solubility of uric acid should also be increased by ethanol, at least at low concentrations, and that the order of solubilizing effectiveness of all cosolvents at low concentrations should be the same toward these three solutes, contrary to the experimental results. Furthermore, no two parameters that exhibit a linear relationship to each other with changing cosolvent structure can describe the results, because the effects of any two such parameters could also be described by a single, combined parameter. The failure of a single parameter scale to explain the results is, of course, not unexpected for polar solutes in predominantly aqueous solution and corresponds to a value of Kirkwood's orientational or rotational entropy factor g that is larger than 1.0; large values of g are usually associated with hydrogen bonding.[47]

Hydrogen bonding cannot provide the *driving force* for the favorable polar interaction effect of aqueous urea and guanidine hydrochloride with uric acid. Bifunctional hydrogen bonding through structure **2** does not provide a significant favorable free energy of interaction in water[41] and is impossible for guanidine hydrochloride. The geometry of uric acid prevents hydrogen bonding through structure **3**. If the strong hydrogen bonding ability of water prevents a net favorable free energy of interaction from bifunctional hydrogen bonding between small molecules, it would be expected to do the same for monofunctional hydrogen bonding between amides or amide-like molecules, and no such interaction has been observed for *N*-methylacetamide.[41]

The results are consistent with the following, relatively simple model. Let us divide the solution process into three parts: (1) the formation of cavities in the solvent, (2) the development of relatively "nonpolar" solvent–solute interactions, in which we will include dispersion, dipole-induced dipole, and quadrupole forces, and (3) the development of "polar" interactions, which we will limit to interactions that involve the polarity of both the solvent and the solute, *i.e.*, primarily to solvent–solute hydrogen bonding in aqueous solutions. The overall Gibbs free energy of transfer of a solute from the gas phase to the solvent is then given by eq 1. The sum of the ΔG^{cav} and $\Delta G^{int}_{nonpolar}$ terms completely describes the solution of a nonpolar solute. Since we know ex-

$$\Delta G_{\substack{tr \\ g \to l}} = \Delta G^{cav} + \Delta G^{int}_{nonpolar} + \Delta G^{int}_{polar} \qquad (1)$$

perimentally that practically any organic cosolvent, including urea, can solubilize a hydrocarbon solute of moderate size, the cosolvent must have a favorable effect on the sum of the first two terms, thereby providing the increased driving force for solution. However, these terms are involved in the solution of a polar solute as well. The important consequence of this statement is that the presence of an organic cosolvent should make this same driving force available for polar solutes, since these terms are common to both kinds of solute.

Consider next the effect of the ΔG^{int}_{polar} term for polar solutes. There is no evidence that the ΔG^{int}_{polar} term is appreciably more favorable in aqueous urea or other polar cosolvent mixtures than in water. We have seen that the formation of hydrogen-bonded complexes of uric acid with urea and related compounds provides little or no net driving force for solubilization. Furthermore, if one compares the magnitude of the effects of cosolvents on naphthalene and on uric acid (Figures 4 and 5 and Table III), the striking experimental result is not so much that there is an especially large favorable effect of polar cosolvents with uric acid, but rather that the favorable effect of alkyl-substituted and relatively nonpolar cosolvents on naphthalene is reduced or abolished with uric acid. Naphthalene and uric acid are similar in size, with parachor values of 311 and (approximately) 296, respectively. Furthermore, although relatively nonpolar cosolvents interact still less favorably with the highly polar ATGEE molecule, the magnitude of the polar interaction effect of polar cosolvents with ATGEE is generally *smaller* than with uric acid, in spite of the larger size of ATGEE.

These results suggest that nonpolar cosolvents and alkyl substituents with a diminished ability to form hydrogen bonds give rise to a net *unfavorable* polar interaction term upon transfer of a polar, hydrogen-bonding solute from water to the cosolvent mixture; *i.e.*, ΔG^{int}_{polar} is less negative for such cosolvent mixtures than for water or for aqueous urea. Thus, it appears that the difference between the effects of cosolvents on naphthalene and on uric acid is better described as a *decreased* effectiveness of relatively nonpolar cosolvents than as an *increased* effectiveness of polar cosolvents toward uric acid.

The free energy for the transfer of a solute from water to a water–cosolvent mixture, $\Delta G_{tr}(h \to c)$, is given by the difference between the values of $\Delta G_{tr}(g \to l)$ from eq 1 for the two solvents and may be expressed in terms of eq 2, in

$$\Delta G_{\substack{tr \\ h \to c}} = (\delta \Delta G^{cav} + \delta \Delta G^{int}_{nonpolar}) + \delta \Delta G^{int}_{polar} \qquad (2)$$

Table V. Transfer of Solutes from Water to Water–Cosolvent Mixtures

	Cosolvent	$\delta\Delta G^{cav} + \delta\Delta G^{int}_{nonpolar}$	$\delta\Delta G^{int}_{polar}$	ΔG_{tr}
Nonpolar solutes	Ethanol	Favorable	0	Favorable
	Urea	Favorable	0	Favorable
Polar solutes	Ethanol	Favorable	Unfav[a]	~0
	Urea	Favorable	~0	Favorable

[a] For internal peptide groups this term is so unfavorable that the overall ΔG_{tr} becomes unfavorable [C. Tanford, *Advan. Protein Chem.*, **24**, 42 (1970)].

which each $\delta\Delta G$ term represents the difference in the free energy of that component in the two solvents. Although it is difficult to evaluate these terms quantitatively or separately, what we have done is to suggest that the data can be described by two parameters, of which the first is the *sum* of the $\delta\Delta G^{cav}$ and $\delta\Delta G^{int}_{nonpolar}$ terms and the second is an additional polar interaction term $\delta\Delta G^{int}_{polar}$, that becomes important for polar solutes. A first approximation of the contribution of the first term may be obtained from ΔG_{tr} for a nonpolar solute, for which the second term is not significant. The effects of ethanol and urea on ΔG_{tr} for nonpolar and for polar solutes are summarized according to this description in Table V. The conclusion that emerges is that (a) the driving force for the favorable free energy of transfer of both nonpolar and polar solutes to water–cosolvent mixtures arises from the more favorable sum of the $\delta\Delta G^{cav}$ and $\delta\Delta G^{int}_{nonpolar}$ terms in such mixtures than in water, and (b) for polar, hydrogen-bonding solutes the $\delta\Delta G^{int}_{polar}$ term must be close to zero in order that a favorable ΔG_{tr} may be observed. In other words, hydrogen bonding does not provide the *driving force* for the favorable transfer of peptide groups from water to aqueous urea, but hydrogen bonding to urea is *necessary* in order that the overall free energy of transfer may be favorable. It must be emphasized that this conclusion in no way implies that monofunctional and bifunctional hydrogen bonding does not occur. In fact, the temperature dependence for the interaction of urea with polar solutes is in the direction expected for the exchange of an amide for a water hydrogen bond.[41] It has recently been reported that the circular dichroism spectra of polypeptides and proteins in urea and guanidine hydrochloride solutions provide evidence for the binding of these denaturants to an "extended helix" of the peptide chain.[48]

This hypothesis provides a simple description of the experimental facts and is not subject to many of the uncertainties and objections of more detailed theories; we suggest that it provides a useful way of thinking about interactions in aqueous solution and may provide a starting point for the experimental investigation of more detailed theories. It is similar to an earlier description of the observed interactions of uncharged molecules in water in terms of two parameters, a nonpolar effect and hydrogen bonding,[8,21] but differs from the earlier proposal in that $\delta\Delta G^{cav}$ and $\delta\Delta G^{int}_{nonpolar}$ provide the driving force for polar as well as nonpolar interactions and that hydrogen bonding is a necessary rather than a sufficient condition for favorable polar interactions. Our approach is also similar to the homomorph, nonpolar analog, and similar treatments that have been applied to less complex systems.[49]

There is considerable evidence, of which the simplest is the solubilization of nonpolar solutes like naphthalene, that the addition of almost any cosolvent to water makes the *sum* of the $\delta\Delta G^{cav}$ and the $\delta\Delta G^{int}_{nonpolar}$ terms more favorable, but it is difficult or impossible to evaluate these terms separately for most cosolvents at the present time. The large number of hydrogen bonds per unit of volume and area and the resulting high cohesive energy density and surface tension of liquid water make cavity formation especially difficult in this solvent,[4] so that it is reasonable to suppose that cavity formation will be easier upon the replacement of water molecules by a cosolvent, even a highly polar cosolvent, that provides a smaller average density of hydrogen bonds; in many cases these changes will be accompanied by changes in the arrangement and low frequency motions of solvent molecules in order to maximize hydrogen bonding in the presence of the solute.[2,9,20,50] However, there is also strong evidence that the $\delta\Delta G^{int}_{nonpolar}$ term can be more favorable in the presence of cosolvents. For example, the ionization of large, resonance-stabilized acids can be facilitated by the addition of organic cosolvents[13] and the favorable interactions of the theophylline anion and uncharged theophylline with uncharged solutes are very similar;[31] these results suggest that the free energy of interaction of large, polarizable anions with organic cosolvents or solutes through dispersion forces can be equally or even more favorable compared with the free energy of solvation of the anion by water. The fact that the surface tension of water is slightly increased by urea[51] is difficult to reconcile with a facilitation of cavity formation by urea, but the fact that the interfacial tension between water and hydrocarbons is decreased by urea[52] is consistent with a more favorable $\delta\Delta G^{int}_{nonpolar}$ for aqueous urea.

Enthalpy, Entropy, and "Nonclassical Hydrophobic Interactions." The negative enthalpy of the polar interaction effect of urea with uric acid and ATGEE in the range 0–40° is similar to that observed with other polar systems, such as the "stacking" interaction of nucleic acid bases and the self-association of dyes.[8,26,28,29,33,53,54] Experimentally, nucleic acid bases behave similarly to uric acid but exhibit somewhat less polar character. This is shown by the favorable effect on ΔG_{tr} of adding alkyl groups to ureas, amides, and alcohols and by a maximum in solubility at intermediate concentrations of cosolvents.[26,27,29,33,53,55]

It is a common practice to reach conclusions regarding the driving force and mechanism for interactions in aqueous solution from observed enthalpies and entropies of interaction. This procedure is hazardous, at best, and is frequently unjustified. It is well known that mutually compensating changes in enthalpy and entropy, which are often ascribed to changes in solvent "structure," frequently occur in aqueous solution with small changes in free energy.[8,56,57] These compensating changes in enthalpy and entropy are often large, unpredictable, and almost capricious and can lead to incorrect conclusions regarding the driving force for interaction. For example, if two molecules have "structure-breaking" groups on the surface that lead to a positive change in ΔH and ΔS of surrounding solvent molecules and these groups are shielded from the solvent when the molecules undergo association, a negative enthalpy change will be observed. This change could easily be identified incorrectly with a favorable enthalpy change resulting from the direct interaction of the two molecules with each other (*e.g.*, from dispersion forces) that provides the driving force for association. It is probable that the polar groups on nucleic acid bases, uric acid, and dyes frequently have a sufficient charge density, similar to that of moderately large ions, to induce a disruptive, "structure-breaking" effect on surrounding solvent molecules.[8,58] The poor correlation of changes in ΔG and ΔS with changes in ΔG for the interaction of nucleic acid bases of different structure is consistent with this kind of behavior.[32]

We have suggested that much or all of the driving force for both polar and nonpolar interactions in aqueous solution arises from a favorable ($\Delta G^{cav} + \Delta G^{int}_{nonpolar}$) term. This approach has the advantage that it does not require a separation of the enthalpy and entropy terms that arise from

changes in solvent structure and from the direct interaction of the molecules involved. It may be regarded as an extension of the "nonclassical hydrophobic interaction."[8] According to this view, changes in solvent structure and associated changes in thermodynamic parameters, while certainly important to a complete understanding of processes in aqueous solution, can occur readily with small changes in free energy and should not be casually identified with the driving force for such processes; in other words, they may frequently be secondary effects.

Other Effects. In spite of their large dipole moments,[59] tetramethylurea, *N,N*-dimethylacetamide, *N,N*-dimethylformamide, and pyridine have a much larger favorable interaction effect on naphthalene than do simple alcohols and ethers, including the cyclic compounds dioxane and cyclohexanol (Figure 4). Benzyl alcohol has a large effect on uric acid and sodium naphthylacetate has the largest effect on naphthalene of any compound examined, in spite of the unfavorable effect of the carboxylate group that is evident in sodium acetate (Table III). These large effects are not readily explained by classical hydrophobic interactions but are similar to the favorable interactions with ATGEE of phenol and salts that contain aromatic groups and to the rather small increase in the interaction of nucleic acid bases with unsaturated as opposed to aliphatic molecules.[26,27,60]

These interactions may be attributed to the $\delta\Delta G^{int}{}_{nonpolar}$ term that becomes more favorable for unsaturated systems as a consequence of dispersion, dipole-induced dipole, and possibly other interactions. Although the molar polarizability of cyclohexane is approximately the same as that of benzene (27 and 26 cm^3, respectively), the cyclohexane molecule is larger than benzene and when expressed on a constant volume basis the polarizability of benzene (0.292) is 15% larger than that of cyclohexane (0.254) and 42% larger than that of water (0.21).[61] Aromatic molecules may also be able to interact more favorably with adjacent molecules as a consequence of their planarity. The $\delta\Delta H^{int}{}_{nonpolar}$ term should be especially important for the interaction of small molecules with proteins, as a consequence of the strong dispersion interactions that are made possible by a correct fit into the close-packed, high density structure of proteins.[62]

Although the predominant effect of relatively nonpolar cosolvents is to increase the solubility of nonpolar solutes in water, other effects become important in some cases, especially for small molecules. Ben-Naim has shown, for example, that the predominant effect of organic cosolvents is to decrease the solubility of argon at low temperatures, although at higher concentrations of cosolvent and higher temperatures a solubilization is observed.[63] Similarly, urea *decreases* the solubility of the small hydrocarbon methane.[20] It is probable that the same phenomenon is responsible for (1) the nonlinear increase in the solubility of the larger naphthalene molecule with increasing concentrations of alcohols and tetrahydrofuran (Figure 2) and (2) the nonlinear dependence of $-\Delta G_{tr}$ for naphthalene and uric acid (Figures 4 and 5) and for nucleosides[29] on the parachor of the cosolvent in the alcohol series. In both cases the increase in solubility is small or absent with a low concentration or a small size of the cosolvent. The explanation of these effects is uncertain but it is likely that they reflect, at least in part, (1) a competition for interstitial sites in the aqueous solvent that is most important at low concentrations and when the solute or solvent is small and (2) a small $\delta\Delta G^{int}{}_{nonpolar}$ term for small solute and solvent molecules such as argon and methanol.

Relationship to Other Approaches. *The scaled particle theory* has proved remarkably successful in calculating the solubility and thermodynamic parameters for solution of nonpolar solutes in different solvents, including the "abnormal" values of these quantities in water.[6,64] According to this theory the free energy of cavity formation depends on the size and the number density of the solvent molecules, and the free energy of interaction of the solute and solvent is taken as the sum of the dispersion and inductive energies of interaction. The necessary parameters for the theory are obtained empirically and these parameters include any effects of factors such as the internal cohesion and "structure" of the solvent that are not explicitly included in the theory. According to the calculations of this theory, the lower solubility of argon in water than in benzene reflects more difficult cavity formation, by 820 cal/mol, and a less favorable interaction of this small solute with the aqueous solvent, by 280 cal/mol.[6] Cavity formation is still more favorable in *n*-hexane, cyclohexane, carbon tetrachloride, and most other solvents, and the interaction energy is generally more favorable with other solvents than with water.[64] Although we have not attempted a quantitative treatment of the complex systems that we describe here, these calculations suggest that it is reasonable to assign significant favorable contributions of both the $\delta\Delta G^{cav}$ and the $\delta\Delta G^{int}{}_{nonpolar}$ terms for solutions in water–cosolvent mixtures compared to water.

According to the treatment of Sinanoğlu and Abdulnur the differences in the free energy of nucleic acid bases in water compared with other solvents result primarily from an increased difficulty of cavity formation in water that is a consequence of the unusually high surface tension and cohesive energy density of this solvent.[4] Although there can be no doubt that these are important factors,[65] the surface tension effect alone does not readily account for the thermodynamic parameters for solution of benzene and other nonpolar molecules in water[33] or the solubilizing effectiveness of urea, which increases the surface tension of water. It should also be kept in mind, with reference to "squeezing out" theories, that alcohols cause a sharp *increase* in the internal pressure of water.[66]

Finally, we wish to make a general comment on the uncritical use of cosolvent-induced changes in solvent "structure" as an *explanation* for changes in interaction free energies of solutes and cosolvent mixtures.[67] It is reasonable to expect that the creation of a cavity in water and the introduction of a relatively large solute molecule with few or no hydrogen bonding sites per unit of volume will restrict the number of energetically favorable rotational states and the number of positions in the system in which water molecules can exist, as well as librational motions of the solvent molecules, with corresponding losses of rotational and translational entropy that make the insertion of a solute molecule more difficult.[50,68] These losses of entropy should generally be smaller in the presence of cosolvents that have a smaller hydrogen bond density and a larger size compared to water. The difficulty arises from the previously mentioned compensation of ΔH and $T\Delta S$ upon changes in solvent structure, with little change in free energy, and from the tendency to insist that every *correlation* must represent a *cause*. Following the suggestion that urea has a "structure-breaking" effect on water,[69] a large literature has appeared that describes evidence for a small structure-breaking effect and implicitly or explicitly ascribes the favorable ΔG_{tr} of organic solutes from water to aqueous urea to this effect.[5,11,18,53,70] We submit that if urea had been found to be slightly "structure-making" an equally successful effort would have been made to "explain" its effects by this structural change. In fact, there is a considerable body of experimental evidence suggesting that many alkyl-substituted ureas and alcohols are considerably more "structure-making" than urea is "structure-breaking," and it has been shown here (Table III) and elsewhere that these compounds

have larger favorable effects on ΔG_{tr} for nonpolar solutes than does urea.[11,19,21,36-39,53,70,71] The insistence that a "structure-changing" effect, regardless of its direction, must reflect a causal relationship is illustrated by the recent conclusion that "both the structure-breaking and the structure-forming tendencies of the ureas, associated with the polar and nonpolar portions of the molecules, must contribute to their effectiveness as protein denaturants." [72]

This kind of confusion between correlation and cause has done nothing to further our understanding and, in fact, only serves to conceal our ignorance of the nature of the driving forces for interactions in aqueous solution. One reason for the popularity of "solvent structure" is that the addition of almost anything to water causes changes in its structure so that an "explanation" for any experimental result is immediately at hand. This advantage must, however, be tempered by the dictum that "A theory which is not refutable by any conceivable event is nonscientific." [73]

Supplementary Material Available. Table II will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-75-631.

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Communications to the Editor

Synthesis of Dimethylbisdehydrooxa- and -thia[13]annulenes. Configurational and Conformational Isomerism in Conjugated 13-Membered Heterocycles[1]

Sir:

There has been considerable interest in recent years in the synthesis of higher vinylogs of heterocycles of the pyrrole–furan–thiophene type. Such compounds may be diatropic ("aromatic") if they are $(4n + 1)$ membered and paratropic ("antiaromatic") if they are $(4n - 1)$ membered, provided the heteroatom can contribute two π-electrons to the delocalized system. Until now, the only monocyclic members to show ring current effects are the diatropic aza[9]-,[2] aza[13]-,[3,4] and aza[17]annulenes,[3] and their anions.[3,5] We now describe the synthesis of the stereoisomeric dimethylbisdehydrooxa[13]annulenes **13** and **19**,[6] and -thia[13]annulenes **14** and **20**. The di-trans sulfide **14** proved to be diatropic and is the first monocyclic nonnitrogeneous member of this series to show a ring current.

Treatment of **1**[7] in THF with 2 mole equiv of *n*-C_4H_9Li at −60° and reaction of the resulting bis ylid with 2 mole equiv of **3**[8] at this temperature (followed by warming to 20°) led to a mixture of **5**, **10**, and the corresponding di-cis stereoisomer[9a] (main λ_{max} (ether) 300 nm) (Scheme I). Coupling of the mixture with $Cu(OAc)_2$ in pyridine at 50° for ~1 hr gave 1.5% (based on **1** and **3**) of the relatively stable di-trans oxa[13]annulene **13**[9b] (pale yellow oil; *m/e* 196; λ_{max} (ether) 272 (ϵ 18,800), 345 nm (3400)) and 1.2% of the very unstable cis,trans isomer **19**[9b] (pale yellow oil which rapidly darkens; ^1H nmr ($CDCl_3$, 60 MHz), τ 3.2–4.6 m (olefinic), 8.05 s br (CH_3)).

Conversion of **2**[7] to the corresponding bis ylid by reaction in ether with 2 mol equiv of *n*-C_4H_9Li at 20°, followed by

Scheme I

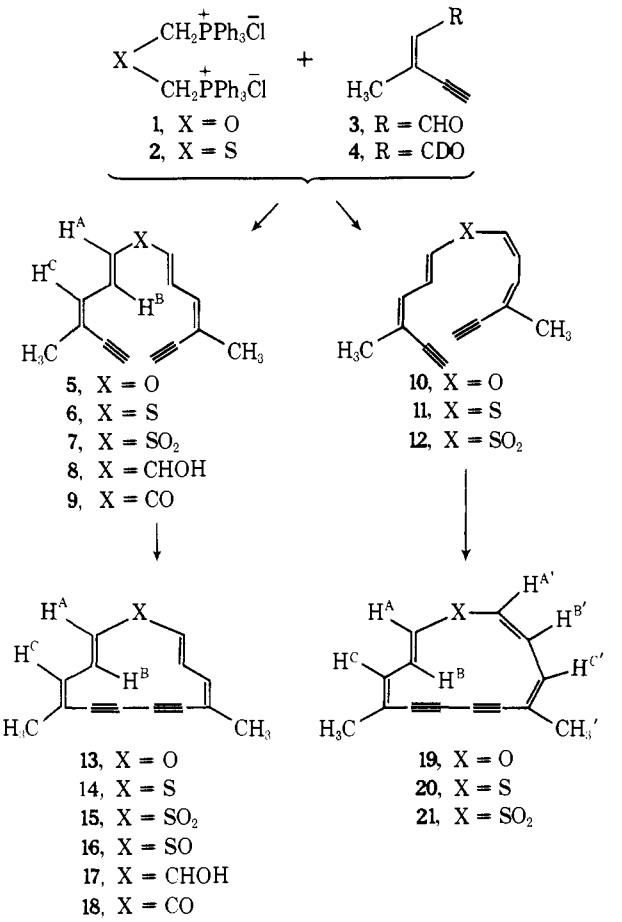

Table I. ^1H Nmr Parameters of **13–18** at 100 MHz in $CDCl_3$ (τ Values; Internal Standard, TMS; *J* Values in Hz in Parentheses)

Compd	H^A	H^B	H^C	CH_3
13, O	3.87 d (13.5)	5.09 dd (9.5, 13.5)	3.14 d (9.5)	7.90 s
14, S	4.49 d (15)	5.40 dd (9, 15)	2.80 d (9)	7.71 s
15, SO_2	5.05 d (14)	2.90 dd (5, 14)	3.07 d (5)	7.87 s
16, SO	5.28 d (16)	3.32 dd (5, 16)	3.10 d (5)	7.90 s
17, CHOH	5.52 d (15.5)	3.90 dd (5, 15.5)	3.32 d (5)	8.01 s
18, CO	3.93 d (17)	0.64 dd (9.5, 17)	3.74 d (9.5)	8.29 s
Δ (**13–5**), O	+0.56	+1.35	−0.60	−0.15
Δ (**14–6**), S	+0.85	~+1.8[a]	−0.93	−0.37
Δ (**15–7**), SO_2	+1.35	+0.52	−0.56	−0.08
Δ (**17–8**), CHOH	+1.26	+0.61	−0.41	−0.06
Δ (**18–9**), CO	+0.35	−1.71	+0.17	+0.28

[a] The H^B signal in **6** could not be located precisely.