uncovered. This reflects the advantage of using difference spectroscopy in the present work. The relevant part of the binding site is reproduced in Figure 11, which may be related to Figure 10 by the occurrence of protons j and $Ala_1\alpha$ in both figures. The close approach of $Ala_2\alpha$ and j confirms that aromatic ring IV is folded in the complex to give a compact structure. The studies of Nieto and Perkins[9] had indicated that the binding site for this D-alanine residue is very restricted. The proximities of the methyl group to j, w, and f show the reason for this. Additionally, we observed an NOE indicating a distance of 2.1 Å from $Ala_2\beta$ to an unidentified proton, resonating as a broad singlet at 2.94 ppm, presumably a mannose hydroxyl proton.

The Binding Site for L-Lys in Vancomycin and Ristocetin A. The conclusions with regard to the binding site of the L-lysine residue of the tripeptide are less clear cut than for the binding of the D-Ala residues and are conventiently dealt with together for both antibiotics. The binding of the lysine residue is too weak to allow any distance measurements, but the collective data give a good indication of its position. The only protons that change chemical shift significantly on changing the peptide from Ac-D-Ala-D-Ala to Ac_2-L-Lys-D-Ala-D-Ala are s_6, b, and a_1 (see Figure 9). Relative to its position in the Ac-D-Ala-D-Ala/ristocetin A complex, the NH proton a_1 is deshielded by 0.73 ppm, suggesting stronger hydrogen bonding to the Lys carbonyl oxygen than to the acetyl carbonyl oxygen of Ac-D-Ala-D-Ala. The shifts of b and s_6 suggest that the lysine side chain is lying in the direction of ring I rather than in the direction of ring VII (see Figure 9). This conclusion is reinforced by a number of NOEs (seen after a 0.3-s preirradiation); in ristoscetin A, Lys ϵ-$CH_3CO \rightarrow$ bb, and in vancomycin, Lys ϵ-$CH_2 \rightarrow$ z.

The extension of the hydrophobic portion of the lysine side chain over ring I is reasonable in light of the hydrophobic nature of this area. The fact that the side chain is free to adopt a large number of conformations makes the binding more favorable in terms of entropy. Additionally, it implies that the antibiotics can bind with similar strengths to the mucopeptides of a number of bacterial species, irrespective of the variable nature of the antepenultimate residue.

Conclusion

The binding of both vancomycin and ristocetin A to Ac-D-Ala-D-Ala is remarkably efficient. In the case of vancomycin, the most striking result of the present work is to establish the formation of a "carboxylate anion binding pocket" upon complexation with Ac-D-Ala-D-Ala. This pocket has hydrophobic walls on two sides, formed from aromatic and aliphatic hydrocarbon groups, thus strengthening the hydrogen bonds that occur within it. An analogous pocket is established to occur in the complex between ristocetin A and Ac_2-L-Lys-D-Ala-D-Ala. However, in this case, both walls of the pocket are formed from aromatic hydrocarbon groups.

Such is the efficiency of both antibiotics in binding the cell-wall analogues that it seems highly probable that the structures have been refined for this purpose by the pressures of natural selection. The necessary pressures would have operated if the organisms producing the antibiotics (*Streptomyces orientalis and Nocardia lurida*) derived an advantage by an ability to kill Gram-positive bacteria in their immediate environment.

It is clear that, in cases where proton NMR spectra of both a drug and its receptor can be analyzed, NOEDs provide a powerful method for establishing the molecular basis of drug action, permitting in favorable cases the calculation of interproton distances in the complexes.

Acknowledgment. We thank SERC, Churchill College, and the SmithKline Foundation, U.K., for financial support.

Registry No. Vancomycin, 1404-90-6; ristocetin A, 11021-66-2; Ac-D-Ala-D-Ala-OH, 19993-26-1; α,ϵ-Ac_2-L-Lys-D-Ala-D-Ala-OH, 24570-39-6; vancomycin/Ac-D-Ala-D-Ala complex, 84174-46-9; vistocetin A/α,ϵ-Ac_2-L-Lys-D-Ala-D-Ala complex, 84174-47-0; Z_2-L-Lys-OH, 405-39-0; D-Ala-D-Ala-OCH_2Ph, 82748-54-7; Z_2-L-Lys-D-Ala-D-Ala-OCH_2Ph, 84192-54-1; H-L-Lys-D-Ala-D-Ala-OH, 33755-56-5; α-Ac-L-Lys-D-Ala-D-Ala-OH, 28845-97-8.

Solvent Effects on Equilibria of Addition of Nucleophiles to Acetaldehyde and the Hydrophilic Character of Diols

Roger Bone, Paul Cullis, and Richard Wolfenden*

Contribution from the Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27514. *Received July 12, 1982*

Abstract: Equilibria of addition of water, methanol, methanethiol, ammonia, methylamine, nitromethane, and ethylene glycol to acetaldehyde have been compared in water and in chloroform, and the partition coefficients of reactants and products between the two solvents have been estimated by direct and indirect methods. Single additions of oxygen nucleophiles were found to proceed equally favorably in either solvent, whereas single additions of sulfur, nitrogen, and carbon nucleophiles proceeded much further toward completion in water than in chloroform. Equilibria of acetal formation, involving methanol or ethylene glycol, were somewhat more favorable in chloroform than in water. Reexamination of the vapor pressures of ethylene glycol and related compounds over water indicated that their hydrophilic character was greater than had been supposed.

Enzymatic transformations of carbonyl compounds and other unsaturated molecules commonly involve attack by nucleophiles at sp_2-hybridized carbon. During the action of papain, chymotrypsin, and triosephosphate dehydrogenase, for example, tetrahedral intermediates are believed to be formed during the generation and breakdown of an acyl–enzyme intermediate. With a slightly different strategy, adenosine and cytidine deaminases apparently catalyze substrate hydrolysis in part by stabilizing tetrahedral intermediates formed by direct addition of water across a C=N bond of the substrate. These enzymes are inhibited by small molecules that can adopt structures resembling these intermediates at the active site (for a recent review, see ref 1).

When substrates and inhibitors are bound at the active sites of enzymes, these small molecules are presumably stripped of much of the solvent water with which they were in contact. In considering catalytic devices that might be employed by enzymes whose reactions are thought to proceed through tetrahedral in-

(1) Wolfenden, R. In "Transition States of Biochemical Processes"; Gandour, R. D. Schowen, R. L., Eds., Plenum New York, 1978; pp 555–578.

0002-7863/83/1505-1339$01.50/0

termediates, and possible strategies for their inhibition, it would be of interest to know how equilibria of addition of various nucleophiles to carbonyl compounds are affected by the presence or absence of solvent water. Does the stabilization of intermediates in substrate transformation arise entirely from an arrangement of enzyme binding groups that is appropriate for specific attraction interactions with these intermediates or are these intermediates stabilized to some extent (relative to reactants) by the anhydrous character of the reaction environment?

The reported vapor pressure of ethylene glycol over water[2] is very much higher than might have been anticipated from the behavior of monohydric alcohols.[3] Thus, it would not be surprising to find anomalous solvation behavior in compounds of similar structure such as acetaldehyde hydrate and neutral tetrahedral intermediates in ester and amide hydrolysis.

To obtain further information about these effects, we have examined equilibria of addition of oxygen, sulfur, nitrogen, and carbon nucleophiles to acetaldehyde in water and chloroform. In the present study, chloroform was chosen as a reference solvent because it was found to be just sufficiently polar to dissolve all reactants at concentrations needed for accurate analysis by NMR spectroscopy. Although chloroform is capable of acting as a weak donor in hydrogen bonding, the only likely acceptor is acetaldehyde, a reactant common to all the equilibria examined; accordingly, results obtained in chloroform would be expected to remain valid in other solvents, in a relative sense. The vapor pressures of ethylene glycol, 1,3-propanediol, and ethanolamine over their dilute aqueous solutions have also been determined by isotopic methods, in order to reassess the anomalous solvation of polyols suggested by the early work of Butler and Ramchandani.[2]

Experimental Section

Materials. 1,3-[1-^{14}C]Propanediol and [1,2-^{14}C]ethanolamine–HCl were purchased from ICN Chemical and Radioisotope Division. [1,2-^{14}C]Ethylene glycol was purchased from New England Nuclear Corp. Deuterated solvents were obtained from KOR Incorporated. Anhydrous methanethiol was purchased from Matheson Gas Products. 1,1-Dimethoxyethane, 2-methyl-1,3-dioxolane, and 1,3-propanediol were obtained from Eastman Kodak Co. and used without further purification. Other chemicals were reagent grade or better. Acetaldehyde was fractionally distilled immediately before use. 1-Nitro-2-propanol[4] was distilled three times under reduced pressure (bp 86–89 °C (8 mm)); proton magnetic resonance spectra of the product showed no contaminating acetaldehyde or nitromethane even after several weeks. *N*-Ethylidenemethylamine[5] was freshly distilled before use (bp 27 °C (760 mm)).

General Procedures. Proton magnetic resonance determinations of distribution coefficients, and of the equilibrium of addition of ammonia to acetaldehyde in water, were made on a Varian EM 390 spectrometer operating at 90 MHz. Integrated intensities of peaks (Table I) corresponding to the solute and integration standard were based on the average of five measurements. Other equilibria of addition were examined on a Bruker WM 250 NMR spectrometer operating at 250 MHz and 20 °C. Dimethyl sulfoxide (Me_2SO) and *p*-dioxane were used as NMR integration standards. UV measurements of additions of methanol and ammonia to acetaldehyde in water were made on a Perkin-Elmer Model 124 double-beam spectrophotometer at 25 °C.

Distribution Coefficients. Both phases were saturated with countersolvent prior to extraction. Distribution coefficients between chloroform-saturated water and water-saturated chloroform were determined at 20 °C by two methods. For nitromethane, 2-methyl-1,3-dioxolane, 1,1-dimethoxyethane, and *N*-ethylidinemethylamine, 5 mL of solute (0.1–1 M in $CDCl_3$) was extracted with D_2O (5 mL), and the concentration of solute was determined in each phase by NMR. The distribution coefficient of ethylene glycol was determined as follows. Ethylene glycol (0.1–1 M in D_2O, 5 mL total volume) was first extracted with 250 mL of chloroform. The resulting chloroform phase was then extracted with 5 mL of D_2O. Portions (2 mL) of both the first and second D_2O phases were diluted with 0.5 mL of the integration standard Me_2SO (1 M in D_2O), and the concentration of solute was determined in each phase by NMR. The distribution coefficient was calculated from the final concentration of solute in D_2O after the first and second extraction (see Results). The distribution coefficient of ethanolamine was determined similarly except that 500 mL of $CHCl_2$ was used as the countersolvent and that solutions of solute and standard were adjusted to pD = 2 with DCl before NMR measurements. The distribution coefficient of methanethiol was determined similarly except that solutions (5 mL) of thiol (0.1–1 M in $CDCl_3$) were extracted with water, the water phase was back-extracted with $CDCl_3$ (5 mL), and Me_2SO in $CDCl_3$ was used as the integration standard.

Water-to-vapor distribution coefficients were determined as described previously[6] by using radioactive solutes ($1–10 \times 10^{-9}$ M) in the presence and absence of unlabeled solute at a final concentration of 1×10^{-3} M. The identity of the radioactive solute was verified by TLC on silica gel, with $CHCl_3$–CH_3CH_2OH (8:2) and visualizing the spots by autoradiography. The identities of the solutes were also confirmed by comparing pot and trap distribution coefficients with distribution coefficients of the authentic compounds.

Equilibrium Determinations. Equilibrated solutions were transferred from sealed vessels to Teflon-capped NMR tubes sealed with Parafilm the same day in which measurements were made. For slower equilibria, approximate half-times were determined to assure that equilibrium had been reached (at least eight half-times) before final measurements were made. In all cases except the two mentioned below, the product was clearly identified by its chemical shift, splitting pattern, and ratio of integrated peak intensities (Table I). In the case of ammonia addition in water, only a single proton resonance could be observed, but results obtained were consistent with those of a previous study.[7] In the case of acetaldehyde hydrate formation in chloroform, product peaks were assigned on the basis of their appearance upon addition of water to acetaldehyde in proportion to the concentration of water or acetaldehyde present.

In general, equilibrium constants were determined from the slope of a plot of adduct concentration divided by final acetaldehyde concentration vs. final nucleophile concentration, as shown in Figure 1 for methanol and methanethiol addition. The equilibrium constant was considered equal to the slope. For formation of the cyclic ethylene glycol adduct, the equilibrium constant was considered equal to the slope multiplied by the molarity of product water.

Table I. Proton Magnetic Resonances of Acetaldehyde, Nucleophiles, and Adducts

species	chemical shift in $CDCl_3$, ppm	chemical shift in D_2O, ppm
CH_3CHO	9.76 q	9.45 q
CH_3CHO	2.15 d	2.21 d
CH_3SH	2.02 d	1.81 d
CH_3SH	1.18 q	
$CH_3CH(OH)SCH_3$	4.86 q	4.08 q
$CH_3CH(OH)SCH_3$	1.48 d	1.27 d
$CH_3CH(OH)SCH_3$	2.14 s	1.94 s
CH_3OH	3.44 s	
$CH_3CH(OH)OCH_3$	1.32 d	
$CH_3CH(OH)OCH_3$	4.67 q	
$CH_3CH(OH)OCH_3$	3.36 s	
H_2O	3–5 (variable)	
$CH_3CH(OH)_2$	1.25 d	
$CH_3CH(OH)_2$	5.09 q	
$CH_3CH(OH)NH_2$		0.90 d
CH_3NO_2	4.18 s	4.18 s
$CH_3CH(OH)CH_2NO_2$	1.17 d	1.11 d
$CH_3CH(OH)CH_2NO_2$	4.38 m	4.32 m
$CH_3CH(OH)CH_2NO_2$	4.30 s	4.50 s
$CH_3CH(OCH_3)_2$	1.33 d	1.02 d
$CH_3CH(OCH_3)_2$	4.48 q	4.52 q
$CH_3CH(OCH_3)_2$	3.14 s	3.10 s
$CH_3CH(OCH_2-)_2$	1.26 d	1.22 d
$CH_3CH(OCH_2-)_2$	4.86 q	4.84 q
$CH_3CH(OCH_2-)_2$	3.79 m	3.76 m
Me_2SO	2.49 s	2.49 s
p-dioxane	3.53 s	3.53 s
$HOCH_2CH_2NH_3^+$		3.6 t
$HOCH_2CH_2NH_3^+$		2.9 t
CH_3CHNCH_3	1.75 m	1.66 (broad)
CH_3CHNCH_3	7.64 m	7.61 (broad)
CH_3CHNCH_3	3.08 m	3.13 (broad)

(2) Butler, J. A. V.; Ramchandani, C. N. *J. Chem. Soc.* **1935**, 952–960.
(3) Hine, J.; Mookerjee, P. K. *J. Org. Chem.* **1975**, *40*, 292–298.
(4) Hurd, C. D.; Nilson, M. E. *J. Org. Chem.* **1955**, *20*, 927–936.
(5) Carter, G. B.; McIver, M. C.; Miller, G. J. *J. Chem. Soc. C* **1968**, 2591–2592.
(6) Wolfenden, R. *Biochemistry* **1978**, *17*, 201–204.
(7) Ogata, Y.; Kawasaki, A. *Tetrahedron* **1964**, *20*, 1573–1578.

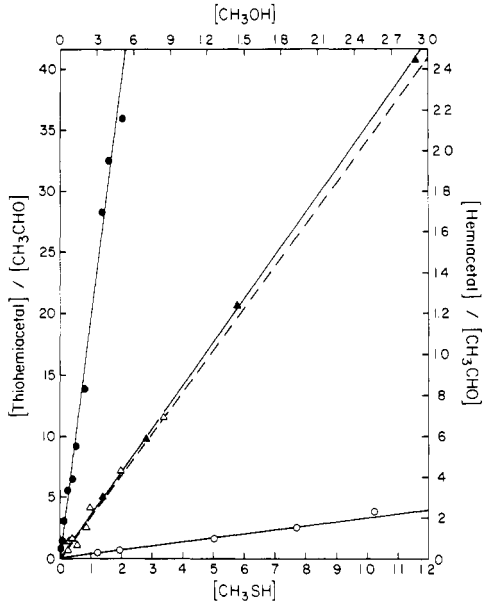


Figure 1. Final concentration of adduct divided by final concentration of acetaldehyde, plotted as a function of the final concentration of free nucleophile. Data are plotted for addition of methanethiol in water (closed circles) and in $CDCl_3$ (open circles) and for addition of methanol in water (closed triangles) and in $CDCl_3$ (open triangles, broken line).

Additions of methanol and methanethiol (0.1–1.0 M) to acetaldehyde (0.1–1 M) in $CDCl_3$ were examined by using as integration standards *p*-dioxane (for methanol) and Me_2SO (for methanethiol). In these experiments, methanethiol was bubbled through $CDCl_3$ to produce a concentrated solution. The equilibrium constant for methanethiol addition in D_2O was determined similarly, over a thiol concentration range 0.015–0.2 M. Methanethiol stock solutions had first been prepared by condensing the gas in a cold trap, mixing it was D_2O at 4 °C, and then allowing the solution to warm to room temperature with evaporation of excess thiol. Addition of methanol to acetaldehyde in water was examined by maintaining a constant initial acetaldehyde concentration (0.2 M) and varying the methanol concentration (0–3 M) while monitoring the acetaldehyde concentration by its UV absorbance at 278 nm. The final concentration of methanol and hemiacetal were calculated, correcting for the hydration of acetaldehyde as described previously.[8]

Solutions of the product 1-nitro-2-propanol (0.1–1 M), catalytic triethylamine (0.14 M), and the integration standard Me_2SO (0.2 M) were allowed to equilibriate in sealed tubes for 2 weeks to determine the equilibrium of addition of nitromethane to acetaldehyde in $CDCl_3$. Observed final concentrations of nitropropanol and acetaldehyde were used to determine the equilibrium constant. The concentration of nitromethane, whose resonance was obscured by one of the nitropropanol peaks, was assumed to be equal to the concentration of acetaldehyde.

Solutions of the product 2-methyl-1,3-dioxolane (0.03–0.3 M) and Me_2SO (0.2 M) were adjusted to pD = 3.0 with dilute DCl and allowed to equilibrate for 1 week to determine the equilibrium constant for addition of ethylene glycol to acetaldehyde to form the cyclic acetal in D_2O. The pD of these solutions remained within 0.2 unit of the original value.

Solutions of acetaldehyde (1–4 M) and standard *p*-dioxane were adjusted nearly to volume in order to examine the addition of water to acetaldehyde in $CDCl_3$, and then water (approximately one-tenth the acetaldehyde concentration) was added and the solution diluted to volume. The equilibrium constant was then determined from the observed concentration of reactants and products.

Addition of ammonia to acetaldehyde in water at 25 °C was complicated by slow accumulation of byproducts that interfered with UV and NMR analyses. Solutions of NH_4Cl (1 M), Me_2SO (0.35 M), and acetaldehyde (0.2–0.8 M) were adjusted to pH 11.4 with sodium hydroxide at 4 °C, diluted to volume, and then adjusted quickly to 25 °C; both NMR and UV spectra were then recorded as rapidly as possible. The concentration of product was measured by NMR. The concentration of acetaldehyde was monitored by its UV absorbance at 278 nm at 5-min intervals over a period of 1 h. The concentration of ammonia was determined by subtraction, and the resulting equilibrium constants were extrapolated to zero time to yield a value with an estimated experimental error of 16% (Table III). At higher ammonia concentrations, byproducts accumulated too rapidly to allow the observation of acetaldehyde by UV. At lower concentrations, byproducts interfered with NMR measurements of acetaldehyde–ammonia.

(8) Lewis, C. L.; Wolfenden, R. *Biochemistry* **1977**, *16*, 4886–4890.

Table II. Distribution Coefficients of Acetaldehyde, Nucleophiles, and Adducts[a]

species	M_c/M_w	M_{vapor}/M_w
H_2O	1.18×10^{-3} [b]	2.51×10^{-5} [f]
NH_3	4.40×10^{-2} [c]	7.7×10^{-4} [g]
CH_3OH	4.25×10^{-2} [c]	1.91×10^{-4} [h]
CH_3SH	36 ± 14	1.23×10^{-1} [h]
CH_3NO_2	2.67 ± 0.23	
CH_3NH_2	0.10[d]	2.9×10^{-4} [g]
$HOCH_2CH_2OH$	$(2.70 \pm 0.6) \times 10^{-3}$	$(1.02 \pm 0.05) \times 10^{-7}$
CH_3CHO	1.29	2.69×10^{-3} [j]
$CH_3CH(OH)_2$	1.87×10^{-3} [e]	
$CH_3CH(OH)OCH_3$	5.04×10^{-2} [e]	
$CH_3CH(OCH_3)_2$	17.8 ± 1.7	
$CH_3CH(OH)SCH_3$	0.80[e]	
$CH_3CH(OH)CH_2NO_2$	0.20 ± 0.02	
$CH_3CH(OCH_2-)_2$	11.0 ± 1.5	
$HOCH_2CH_2CH_2OH$		$(4.5 \pm 3.0) \times 10^{-8}$
$HOCH_2CH_2NH_2$	$(3.3 \pm 0.6) \times 10^{-3}$	$(6.8 \pm 0.9) \times 10^{-9}$
$CH_3CH{=}NCH_3$	6.0 ± 0.9	

[a] Unless indicated otherwise, values were determined by direct measurement at 20 °C, each solvent having been saturated with the countersolvent. [b] Calculated from the solubility of water in chloroform. [c] Sandell, K. *Naturwissenschaften* **1964**, *51*, 336. [d] Felsing, W.; Buckley, S. *J. Phys. Chem.* **1933**, *37*, 779–786. Moore, T.; Winwill, T. *J. Chem. Soc.* **1912**, *101*, 1635–1676. [e] Calculated from equilibrium constants listed in Table III (see Results). [f] Calculated from the vapor pressure of water. [g] Reference 5. [h] Hine, J.; Weimar, R. D., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 3387–3396. [j] Pierotti, G. J.; Deal, C. H.; Derr, E. L. *Ind. Eng. Chem.* **1957**, *51*, 95–102; Supplement, Document no. 5782, American Documentation Institute, Library of Congress, Washington, D.C.

Results

Distribution Coefficients. Table II lists distribution coefficients observed in the present work, along with values determined in several earlier studies. In the extreme cases of ethylene glycol, ethanolamine, and methanethiol, for which distribution favors one solvent very strongly, the double-extraction procedure described in the Experimental Section was used. Apparent distribution coefficients were calculated by eq 1, where $K_{M\rightarrow L}$ = equilibrium

$$K_{M\rightarrow L} = \frac{BM}{LA - LB} \quad (1)$$

constant for transfer to less favored solvent, M = volume of more favored solvent used in both extractions, B = concentration of solute in more favored solvent after second or back extraction, L = volume of less favored solvent, and A = concentration of solute in more favored solvent after first extraction. For each of the solutes examined, solvent–solvent distribution coefficients remained constant over the range of concentrations studied. Similarly, water-to-vapor distribution coefficients of radioactive solutes were found to be identical in the presence and absence of added unlabelled solute, indicating the absence of apparent self-association in either phase. The identity of material transferred to the vapor phase was confirmed in each case by comparing its chromatographic and solvent distribution properties with those of the authentic solutes. Water-to-chloroform distribution coefficients of several of the adducts were determined indirectly, as indicated in Table II, from the observed equilibrium constants for the reaction in both solvents (see below) and the distribution coefficients of reactants and products. NMR analysis indicated that in distribution experiments, the concentration of countersolvent present in each phase was not detectably affected by the presence of solutes except at acetaldehyde concentrations in excess of 1 M. At these very high concentrations, which were attained only in measurements of covalent hydration in chloroform, concentrations of "excess" water transferred in this way were much lower than the concentration of aldehyde present in the chloroform-rich

Table III. Equilibrium Constants for Addition of Nucleophiles to Acetaldehyde

nucleophile	products	$K_{eq,w}$[a]	$K_{eq,c}$[c]	$K_{eq,c}/K_{eq,w}$
H_2O	$CH_3CH(OH)_2$	0.022[b]	0.027 ± 0.005	1.23
NH_3	$CH_3CHOH(NH_2)$	102 ± 16 (110[c])	(6.0[f])	(0.059[f])
CH_3OH	$CH_3CHOH(OCH_3)$	0.85 ± 0.02 (0.70[d])	0.83 ± 0.22	0.98 (1.13)
CH_3SH	$CH_3CHOH(SCH_3)$	190 ± 19	3.27 ± 0.33	0.017
CH_3NO_2	$CH_3CHOH(CH_2NO_2)$	4700[e]	267 ± 42	0.057
CH_3NH_2	$CH_3CH{=}NCH_3 + H_2O$	$>10^4$[e]	$>10^3$	0.055
$2(CH_3OH)$	$CH_3CH(OCH_3)_2 + H_2O$	1.58	14.2[e]	8.99
$HOCH_2CH_2OH$	$CH_3CH(OCH_2^-)_2 + H_2O$	16.8 ± 2	61.6[e]	3.67

[a] Expressed in units of M^{-1}, except for equilibria involving addition of methylamine and ethylene glycol, which are dimensionless. Values were determined at 20 °C in pure solvents unless otherwise indicated. w = water; c = chloroform. [b] Reference 8. [c] Reference 7. [d] Estimated at 25 °C (Guthrie, J. P. *J. Am. Chem. Soc.* **1973**, *95*, 6999–7003). [e] Calculated from distribution coefficients listed in Table II (see Results). [f] Calculated from distribution coefficients in Table III assuming the distribution coefficient of acetaldehyde–ammonia to be identical with that of ethanolamine (see Results).

phase, amounting to no more than 10%.

Equilibrium Constants. Figure 1 shows four sets of representative data, in which the concentration of adduct divided by the concentration of free aldehyde was plotted as a function of the concentration of nucleophile remaining after equilibrium had been achieved. Table III shows equilibrium constants determined in this work and in earlier studies. Several equilibrium constants were obtained indirectly (as indicated in Table III) by combining distribution coefficients of reactants and products with the equilibrium constant observed in the other solvent.

In view of the observed resemblance in distribution properties between ethylene glycol and 1,1-ethanediol (Table II), it seemed reasonable to infer that the distribution properties of acetaldehyde–ammonia were closely similar to those of 1,2-ethanolamine. In estimating the equilibrium constant for ammonia addition in chloroform (Table III), we assumed that the distribution coefficients of acetaldehyde–ammonia and of ethanolamine were identical.

Discussion

Distribution coefficients observed for reactants and products (Table II) appear in general to reflect the relative strengths and numbers of hydrogen bonds that might be expected to be formed with solvent water. In the exceptional case of nitrogen compounds, methylation hardly affects the observed distribution coefficient of ammonia from water to chloroform, an anomaly noted earlier in the water-to-vapor distribution of amines and amides;[6] in these compounds, relative water affinity cannot be judged by merely counting the number of hydrogens attached to nitrogen.

For polyfunctional compounds included in the present series, distribution coefficients tend to conform to additivity principles established through the correlations of Hansch and his associates.[9] The water-to-vapor distribution coefficient of aqueous ethylene glycol, reported by Butler and Ramchandani[2] as 2.5×10^{-6}, only a little lower than that of water itself, appears from the present findings to be much lower, 1.0×10^{-7}. The different between these experimental results can probably be attributed to the relatively insensitive and nonspecific interferometric method used in the earlier analysis.[2] The new value remains about 20-fold higher than expected from correlations based on the properties of monohydric alcohols.[3] For 1,3-propanediol, the water-to-vapor distribution is only about 10-fold higher than expected from the correlations of Hine and Mookerjee, and for ethanolamine the agreement is very close. The small remaining discrepancies can probably be attributed to weak intramolecular hydrogen bonding of vicinal diols in the vapor phase, for which spectroscopic evidence exists.[10-13] 2-Methoxyethanol has been reported to form an intramolecular hydrogen bond, with an equilibrium constant of 12.3, in carbon tetrachloride at 20 °C.[14] In view of these properties of vicinally substituted compounds, and of the considerably greater difficulty of forming an intramolecular hydrogen bond between *gem*-hydroxyl groups (or a hydroxyl and an amino group attached to the same carbon atom), it seems reasonable to infer that neutral tetrahedral intermediates with substantial lifetimes are likely to be solvated in water in a way that reflects the sum of the solvation properties of their constituent groups rather than in any anomalous way that might seem to have been suggested by the earlier observations on ethylene glycol.

Equilibria of single addition of oxygen nucleophiles (to form hydrates and hemiacetals) are found to be virtually unaffected by transfer from water to chloroform, whereas single additions of other nucleophiles occur less readily (Table III). These effects can be rationalized to some degree in terms of the relative distribution properties of constituent groups that are present in reactants and products. Hydroxyl groups are considerably more hydrophilic than are other nucleophilic groups included in the present comparisons. The same number of hydroxyl groups are present before and after addition of water (or of methanol) to acetaldehyde. When other nucleophiles such as methanethiol or nitromethane add to acetaldehyde, the adduct contains a hydroxyl group that was not present before addition; this presumably tends to enhance equilibrium constants for these additions in water. These findings accord with earlier suggestions[15,16] that differences in solvation may largely account for the well-known superiority of mercaptans to alcohols as nucleophiles in water. Distribution coefficients suggest that ammonia and primary amines are similar to each other in their hydrogen bonding capacities (Table II). When ammonia adds to acetaldehyde, the hydrogen-bonding capabilities of the amine portion of the product are therefore expected to be similar to those of the reactant ammonia, but a hydroxyl group is gained as a result of addition. Acetaldehyde–ammonia might thus tend to be formed more readily in water than in chloroform.

Other addition equilibria in Table III, which involve release of water as a second product, do not lend themselves to easy rationalization. For entropic reasons, addition of ethylene glycol appears to be more favorable in either solvent than diaddition of methanol; i.e., the effective molarity of alcoholic groups in acetal formation is higher if they are joined together. This property is commonly exploited in the preparation of benzylidene and isopropylidene derivatives of diols.

Peptide-related aldehydes, powerful inhibitors of proteases that contain nucleophilic cysteine or serine residues at their active sites,[17,18] are known to form thiohemiacetal[19-21] and hemiacetal derivatives with these nucleophiles. To what extent could the

(9) Hansch, C.; Leo, A. J. In "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979.
(10) Krueger, P. J.; Mette, H. D. *Can. J. Chem.* **1965**, *43*, 2970–2971.
(11) Penn, R. E.; Curl, R. F., Jr. *J. Chem. Phys.* **1971**, *55*, 651–658.
(12) Buc, H. *Ann. Chim.* **1963**, *8*, 409–430.
(13) Fishman, E.; Chen, T. L. *Spectrochim. Acta, Part A* **1968**, *25A*, 1231–1242.
(14) Kuhn, L. P.; Wires, R. A. *J. Am. Chem. Soc.* **1964**, *86*, 2161–2165.
(15) Lienhard, G. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1966**, *88*, 3982–3995.
(16) Kanchuger, M. S.; Byers, L. D. *J. Am. Chem. Soc.* **1979**, *101*, 3005–3010.
(17) Westerik, J. O.; Wolfenden, R. *J. Biol. Chem.* **1972**, *247*, 8195–8197.
(18) Thompson, R. C. *Biochemistry* **1973**, *12*, 47–51.
(19) Lewis, C. L.; Wolfenden, R. *Biochemistry* **1977**, *16*, 4890–4895.
(20) Clark, P. I.; Lowe, G.; Nurse, D. *J. Chem. Soc., Chem. Commun.* **1977**, 451–453.
(21) Brayer, G. D.; Delbaere, L. T. J.; James, M. N. G.; Bauer, C. A.; Thompson, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 96–100.

unusual stability of these enzyme–inhibitor complexes be due to a simple lowering of the microscopic dielectric constant at the active site, as compared with bulk solution?. The present results indicate that such effects on equilibria of binding should be negligible for hemiacetal formation and markedly adverse for thiohemiacetal formation. The observed strength of binding, all the more remarkable in view of these observations, can therefore be ascribed to specific forces of attraction that are present at the active site.

Acknowledgment. This work was supported by Research Grant PCM-7823016 from the National Science Foundation.

Registry No. CH_3CHO, 75-07-0; NH_3, 7664-41-7; CH_3OH, 67-56-1; CH_3SH, 74-93-1; CH_3NO_2, 75-52-5; CH_3NH_2, 74-89-5; $HOCH_2CH_2OH$, 107-21-1; $CH_3CH(OH)_2$, 4433-56-1; $CH_3CH(OH)OCH_3$, 563-64-4; $CH_3CH(OCH_3)_2$, 534-15-6; $CH_3CH(OH)SCH_3$, 84418-46-2; $CH_3CH(OH)CH_2NO_2$, 3156-73-8; $CH_3CH(OCH_2-)_2$, 497-26-7; $HOCH_2CH_2NH_2$, 141-43-5; $CH_3CH{=}NCH_3$, 14777-29-8; $HOCH_2CH_2CH_2OH$, 504-63-2; $HOCH_2CH_2NH_3^+$, 22852-66-0.

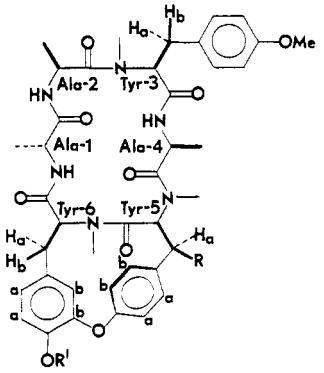
Solution Forms of Bouvardin and Relatives from NMR Studies. 6-*O*-Methylbouvardin

Robert B. Bates,[1a] Jack R. Cole,*[1b] Joseph J. Hoffmann,[1b] George R. Kriek,[1a] Gary S. Linz,[1a] and Sterling J. Torrance[1b]

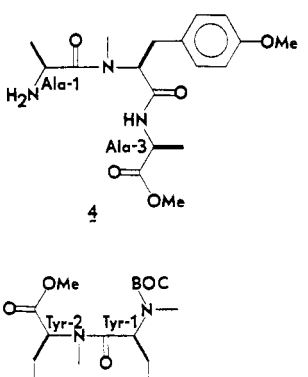
Contribution from the College of Pharmacy and the Department of Chemistry, University of Arizona, Tucson, Arizona 85721. *Received June 14, 1982*

Abstract: ^1H and ^{13}C NMR studies indicate that the predominant stereoisomer and conformer in solution for the potent natural antitumor agents deoxybouvardin (**1**), bouvardin (**2**), and the newly isolated and equally active 6-*O*-methylbouvardin (**3**) is that found in the solid state by X-ray diffraction. Unusual features in the spectra, all in the vicinity of the 14-membered ring, include an aromatic proton absorbing unusually far upfield at δ 4.35, a vicinal H–C–O–H coupling constant of 10.2 Hz, aryl carbons ortho to an ether oxygen absorbing at δ 124.2–125.9, and a geminal coupling constant of −20 Hz between the methylene protons in a tyrosine residue. A minor stereoisomer (~15%) separated by a 20.6 kcal/mol barrier is observed for **1**–**3**; variable-temperature ^1H NMR studies on model *N*-methyl peptides indicate this stereoisomer to differ in rotation about the Tyr-5 and/or Tyr-3 amide bond. Since the antitumor activities of six compounds differing in substitution on Tyr-5 and Tyr-6 do not vary appreciably while a change in Tyr-3 results in loss of activity, the rigid 14-membered ring portion of the molecule is not the active part but serves to get the rest of the molecule into the active conformation.

Deoxybouvardin (**1**) and bouvardin (**2**) are natural cyclic hexapeptides, possessing strong antitumor activity, and constituted



	R	R'
1	H_b	H
2	OH	H
3	OH	Me



from two L-alanines, a D-alanine, and three modified *N*-methyl-L-tyrosines.[2] Their most unusual structural feature is a 14-membered ring formed by oxidative coupling of the phenolic rings of two adjacent tyrosine units; this ring contains meta- and para-disubstituted benzene rings and a *cis*-peptide grouping. An X-ray study on **2** and spectral comparison of **1** and **2** gave their structures, but due to the complexity of their NMR spectra, very few of their NMR parameters were assigned. Analytical HPLC indicated the presence of two stereoisomers in chloroform solution,[3] but efforts to separate them on a preparative scale failed since they equilibrate at room temperature.

We report (a) the isolation of 6-*O*-methylbouvardin (**3**), an active minor component of *Bouvardia ternifolia* obtained during isolation of large quantities of **1** and **2** for biological testing, (b) ^1H and ^{13}C NMR studies on **1**–**3** which provide evidence on the shapes of the two major species which are observed in chloroform solution for each of these substances, and (c) structure–activity results which indicate how various portions of the molecule are involved in the activity.

Results and Discussion

6-*O*-Methylbouvardin was assigned structure **3** by comparison of its ^1H (Table I) and ^{13}C (Table II) NMR spectral parameters with those of **1** and **2** (see Figure 1 for ^1H NMR spectrum of **2**). The structure was confirmed by converting **2** to **3** with diazomethane.

The ^1H and ^{13}C shift assignments in Tables I and II for bouvardin (**2**) are consistent with extensive ^1H–^1H and ^1H–^{13}C decoupling results; the resonances of all carbons bearing hydrogens were umabiguously correlated with the resonances of those hydrogens. The assignment problems were thus reduced to which set of δ's and J's belonged to which alanine, which *N*-Me ^{13}C and

(1) (a) Department of Chemistry. (b) College of Pharmacy.
(2) Jolad, S. D.; Hoffmann, J. J.; Torrance, S. J.; Wiedhopf, R. M.; Cole, J. R.; Arora, S. K.; Bates, R. B.; Gargiulo, R. L.; Kriek, G. R. *J. Am. Chem. Soc.* **1977**, *99*, 8040.
(3) Hoffmann, J. J.; Torrance, S. J.; Cole, J. R. *J. Chromatogr. Sci.* **1978**, *17*, 287. The barrier between these forms is about 20 kcal/mol, which is the approximate boundary between configurations and conformations. We have found it more convenient to use the former terminology for them.

0002-7863/83/1505-1343$01.50/0