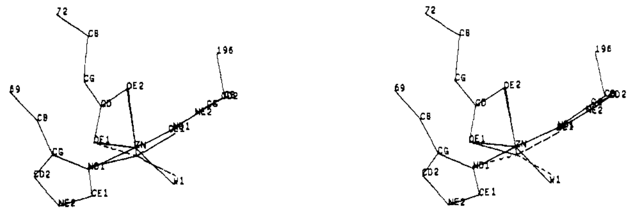

Figure 1. Stereogram of the metal binding site of carboxypeptidase A. The solid lines represent the side chains of His-69, His-196, and Glu-72 and bond to each ligand, including the water molecule (W1), for ZnCPA. The dashed lines are the bonds between Ni^{2+} and the Ni^{2+} ligands, superimposed on the Zn^{2+} structures.

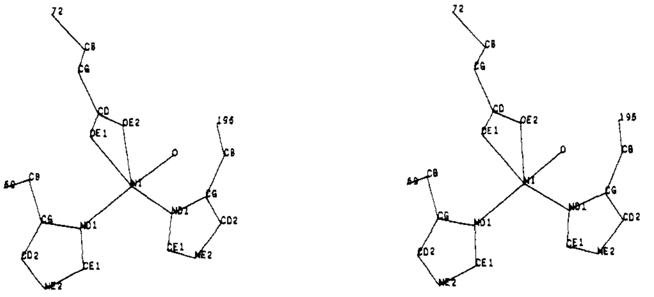

Figure 2. Model of the Ni enzyme in the same orientation as Figure 3. The water is marked O.

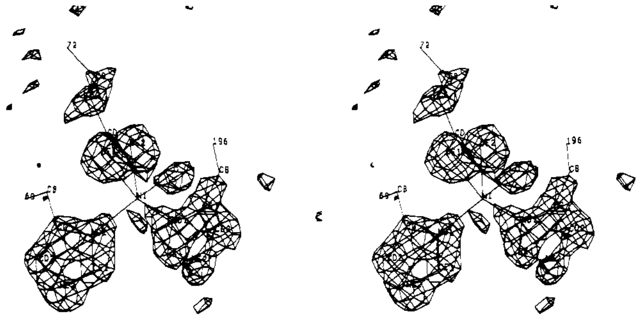

Figure 3. Difference electron density map of the Ni^{2+} enzyme, where the calculated structure factors were determined from the difference between the refined NiCPA coordinates, minus the side chain atoms of His-69, His-196, Glu-72 and all water molecules within 6 Å of the metal, and the observed amplitudes for NiCPA. It is clear from these figures that although the Ni^{2+} and water have moved to give a more nearly octahedral geometry, the sixth ligand position is empty.

of Agarwal.[5] The number of refinement cycles was 19 for the Ni^{2+} enzyme and 13 for the Co^{2+} enzyme and included three cycles of geometric regularization evenly spaced throughout the refinement for each metallo derivative. The final regularized models yielded standard deviations of 0.02 Å for bond lengths and 4° for bond angles and R values of 0.14 for the Ni^{2+} enzyme and 0.15 for the Co^{2+} enzyme. Unconstrained bond lengths are probably accurate to within 0.2 Å. (Table I).

A few detailed lengths are as follows. The protein structures of all three metalloenzymes are the same within experimental error except as noted. For example, root mean square shifts of all atoms of His-69, His-196, and Glu-72 from the Zn^{2+} enzyme to the Ni^{2+} enzyme are 0.15 Å and from the Zn^{2+} enzyme to the Co^{2+} enzyme are 0.10 Å. Only the Ni^{2+} and its bound H_2O have moved about 0.5 Å, to make an octahedral-minus-one geometry; there is no sixth ligand about Ni^{2+} within the limits of accuracy of some 5–10% in occupancy. Temperature factors of all atoms in the metal and its coordination sphere are similar in all three metalloenzymes, except that the metal-bound water has temperature factors (and related occupancies) of 15 (0.7), 8 (1.0), and 12 (1.0) Å^2 for the Zn^{2+}, Ni^{2+}, and Co^{2+} enzymes, respectively.

Table I. Metal Bonds for Zn-, Ni-, and CoCPA[a]

	His-69	His-196	Glu-72		
	ND1	ND1	OE2	OE1	H_2O
Zn	2.17	2.03	2.30	2.19	2.19
	(3.0)	(3.0)	(6.9)	(5.3)	(15.4)
Ni	2.15	2.08	2.49	2.11	2.07
	(3.0)	(3.8)	(3.7)	(3.0)	(8.4)
Co	2.13	2.06	2.26	2.24	2.00
	(3.0)	(3.0)	(3.0)	(3.0)	(12.0)

[a] Numbers in parentheses are temperature factors.

For the Co^{2+} enzyme, spectral and magnetic properties have led to an ambiguous assignment of between 4 and 5 ligands.[6] This result is consistent with our results for the Co^{2+} enzyme, which has a geometry about the metal essentially identical with that of the Zn^{2+} enzyme and differs only slightly from the Zn^{2+} enzyme in the occupancy and/or the range of slight disorder of the metal-bound water molecule.

These results call for a reevaluation of the magnetic susceptibility and spectroscopic results of the Ni^{2+} enzyme.[2] Also the essentially identical positions of atoms of the protein structures, including ligands to the metal site, among these three derivatives make it unlikely that the differences in peptidase and esterase activities can be explained in terms of the entatic state hypothesis.[7]

The activity of carboxypeptidase A in this crystal form[3] has been established previously.[8] While it would be desirable to test the activity of the Co^{2+} and Ni^{2+} enzymes in the crystalline state, the requirement of a substantial quantity of good single crystals has delayed this test to a future study.

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Registry No. Carboxypeptidase A, 11075-17-5; L-histidine, 71-00-1; L-glutamic acid, 56-86-0.

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Solubility Properties in Polymers and Biological Media. 4. Correlation of Octanol/Water Partition Coefficients with Solvatochromic Parameters

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Hansch and Leo[1] have established that hydrogen-bonding forces and solute molecular volumes are the major properties that in-

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Table I. Correlation of the Hansch–Leo Partition Coefficients with the Solvatochromic Parameters

no.	solute	$\overline{V}/100$	π^*[a]	β	log *P*	calcd	diff
1	*n*-hexane	1.306	−0.08	0.00	3.90	3.79	−0.11
2	tetramethylsilane	1.361	0.00[b]	0.00	3.85	3.86	+0.01
3	cyclohexane	1.080	0.00	0.00	3.44	3.11	−0.33
4	neopentane	1.176	0.00[b]	0.00	3.11	3.37	+0.26
5	*n*-pentane	1.152	−0.08[b]	0.00	3.39	3.38	−0.01
6	benzotrifluoride	1.229	0.64[b]	0.00[d]	3.01	2.90	−0.11
7	cyclopentane	0.934	0.00	0.00	3.00	2.75	−0.25
8	*n*-butane	1.001[b]	−0.08	0.00	2.89	2.98	+0.09
9	tetrachloroethylene	1.016	0.08[a]	0.00	2.88	2.87	−0.01
10	carbon tetrachloride	0.968	0.08[a]	0.00	2.83	2.74	−0.09
11	tri-*n*-propylamine	1.895	0.14[b]	0.69[b]	2.79	2.82	+0.03
12	*n*-butyl chloride	1.044	0.39	0.00	2.64	2.64	0.00
13	fluorotrichloromethane	0.82[a]	0.12[a,b]	0.00	2.53	2.31	−0.22
14	1,1,1-trichloroethane	0.989	0.29[a]	0.00	2.49	2.59	+0.10
15	propane	0.859[b]	−0.08[b]	0.00	2.36	2.60	+0.24
16	trichloroethylene	0.897	0.43	0.00	2.29	2.31	+0.02
17	*n*-propyl chloride	0.880	0.39	0.00	2.04	2.21	+0.14
18	di-*n*-propyl ether	1.359	0.27	0.46	2.03	2.04	+0.01
19	chloroform	0.805	0.38[a]	0.00	1.94	2.02	+0.08
20	amphetamine	1.438	0.14[b]	0.62[b,c]	1.76	1.84	+0.06
21	1,2-dichloroethane	0.787	0.81	0.00	1.48	1.56	+0.08
22	triethylamine	1.401	0.14	0.71	1.45	1.44	+0.01
23	2-hexanone	1.235	0.65[b]	0.50[b]	1.38	1.21	−0.17
24	*N*-methylpiperidine	1.216	0.15[b]	0.70[b]	1.30	0.92	−0.38
25	ethyl propionate	1.146	0.47	0.46	1.21	1.33	+0.12
26	methylene chloride	0.624	0.62[a]	0.00	1.15	1.31	+0.16
27	2-pentanone	1.065	0.67[b]	0.50	0.91	0.74	−0.17
28	diethyl ether	1.046	0.27	0.47	0.89	1.18	+0.27
29	butyraldehyde	0.883	0.50[b]	0.38	0.88	0.83	−0.05
30	cyclohexanone	1.036	0.76	0.53	0.81	0.48	−0.33
31	ethyl acetate	0.978	0.55	0.45	0.73	0.79	+0.06
32	dimethylethylamine	1.240	0.14[b]	0.70[b]	0.70	1.04	+0.34
33	propionaldehyde	0.720	0.50[b]	0.38	0.59	0.39	−0.20
34	*n*-propylamine	0.822	0.15[b]	0.60[b]	0.48	0.26	−0.22
35	tetrahydrofuran	0.811	0.58	0.55	0.46	−0.02	−0.48
36	*N,N*-diethylacetamide	1.265	0.86[b]	0.78	0.34	0.14	−0.20
37	2-butanone	0.895	0.67	0.48	0.29	0.36	+0.07
38	hexamethylphosphoramide	1.750	0.87	1.05	0.28	0.51	+0.23
39	methyl acetate	0.798	0.60	0.42	0.18	0.37	+0.19
40	trimethylamine	0.860	0.14[b]	0.65[b]	0.16	0.20	+0.04
41	propionitrile	0.704	0.71	0.37	0.10	0.18	+0.08
42	dimethyl ether	0.735[b]	0.27[b]	0.47[b]	0.10	0.35	+0.25
43	acetone	0.734	0.71	0.48	−0.24	−0.11	+0.15
44	acetonitrile	0.521	0.75	0.35	−0.34	−0.27	+0.07
45	*N,N*-dimethylacetamide	0.924	0.88	0.76	−0.77	−0.72	+0.05
46	dimethylformamide	0.774	0.88	0.69	−1.01	−0.88	+0.13
47	dimethyl sulfoxide	0.710	1.00	0.76	−1.35	−1.40	−0.05

[a] Values for polychlorinated aliphatic solutes are for $(\pi^* + d\delta)$, $d = -0.40$, $\delta = 0.5$; Chawla, B.; Pollack, S. K.; Lebrilla, C. B.; Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* 1981, *103*, 6924. [b] Estimated from corresponding parameters for closely related compounds. [c] The very minor hydrogen bond basicity of the aromatic ring has been neglected because of the uncertainty in the estimated β value. [d] Cf. reference in footnote *a*.

fluence distributions of many types of solutes between *n*-octanol and water. Octanol/water partition coefficients, log *P*, are considered to model blood/lipid partition and have been widely and successfully used in quantitative structure/activity relationships in the fields of pharmacology and toxicology.[1,2] Here, we report a quantitative treatment of log *P* values that supports the Hansch–Leo concepts and provides further insights into the relative importance of molecular factors that determine solute partition coefficients.

The solvatochromic parameters π^* and β,[3] derived to measure dipolar and hydrogen bond acceptor strengths of pure bulk solvents, are also known to apply to good approximation to the corresponding properties of molecular *solutes* (within well-established limitations regarding protonic solvents that self-associate by hydrogen bonding). Thus, π^* values for *select solvents* have been quantitatively correlated with molecular dipole moments,[4] and Fuchs[5] has recently related enthalpies of solution of many solutes in many solvents to both solute and solvent π^* values. Arnett[6] has shown that enthalpies of formation of hydrogen-bonded complexes of phenol with a series of hydrogen bond acceptor (HBA) bases, a basicity property related to β,[3] are nearly the same in dilute CCl_4 solution as in the pure liquid bases. Also, formation constants of phenol (and other HBD's) with HBA bases in dilute CCl_4 solutions were used extensively in the derivation of the β scale.[3]

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Solubility properties (SP) of a protonic solute in a series of nonprotonic solvents have been correlated in the form of eq 1 using

$$\mathrm{SP} = \mathrm{SP}_0 + \text{dipolar term} + \text{hydrogen bonding term} + \text{cavity term} \quad (1)$$

the *solvent* π^*, β, and δ_H values, respectively. δ_H, the Hildebrand solubility parameter,[8] is the *solvent* parameter that gives the magnitude of the cavity term.[9] Equation 1 can also be used to correlate solubility properties, e.g., log P values, for a series of nonprotonic solutes in protonic solvents using the *solute* π^*, β, and $\bar{V}/100$ values.[10] $\bar{V}$, the mean molar liquid volume is the *solute* property that determines the magnitude of the cavity term. For nonprotonic solutes in protonic solvents, we suggest that eq 2

$$\mathrm{SP} = \mathrm{SP}_0 + A\delta_{\mathrm{H},1}\bar{V}_2/100 + B\pi^*{}_1\pi^*{}_2 + C\alpha_1\beta_2 \quad (2)$$

applies, where subscript 1 refers to the solvent and subscript 2 to the solute, and A, B, and C, are constants for the endoergic cavity term, the exoergic dipolar term, and the exoergic hydrogen-bonding term, respectively.

Equation 2 gives, for nonprotonic solutes, the following predictions: since π^* of H_2O (1.09) is higher than π^* of octanol (ca. 0.4), higher solute π^* should favor solution in water; since α of H_2O (1.17) is higher than α of octanol (ca. 0.6), higher solute β should favor solution in water; since δ_H of water (23.4)[11] is higher than δ_H of octanol (10.2), higher solute $\bar{V}$ should favor solution in octanol. Values of log P for 47 widely varying nonprotonic solutes (cf. Table I) confirm these predictions by adherence to correlation eq 3. The data correlated, to our knowledge, include

$$\log P = 0.24(\pm 0.18) + 2.66(\pm 0.12)\bar{V}/100 - 0.96(\pm 0.11)\pi^* - 3.38(\pm 0.12)\beta \quad (3)$$

$$n = 47,\ r = 0.991,\ \mathrm{sd} = 0.18$$

all nonprotonic aliphatic solutes[12] for which the Hansch–Leo parameters and the solvatochromic parameters are known (excluding dioxan and other compounds having multiple oxygen atom base centers[13]).

It is significant that the intercept in eq 3 is close to the theoretical value of 0.00[14] and that the standard deviation is well within the range of experimental error. Equation 3 shows that the major effects are the opposing influences of solute molecular volume and HBA basicity, with solute dipolarity exerting second-order influences. Unless correctly unraveled, the log P vlues can appear complex. For example, 2-butanone, hexamethylphosphoramide, methyl acetate, and trimethylamine (Table I, 37–40) have quite similar log P_H values, although $\bar{V}/100$, π^*, and β values range from 0.80 to 1.75, from 0.14 to 0.87, and from 0.42 to 1.05, respectively. In demonstrating how the specific molecular properties influence partition, eq 3 provides guidance in the design of pharmacologically active molecules.

It is also of interest to compare eq 3 with our preliminary correlation equation[12b] for cyclohexane/water partitioning of nonprotonic aliphatic solutes:

$$\log P(C_6H_{12}/H_2O) = -0.05 + 3.69\bar{V}/100 - 1.15\pi^* - 5.64\beta \quad (4)$$

$$n = 17,\ r = 0.998,\ \mathrm{sd} = 0.07$$

The relevant solvent parameters for cyclohexane are $\pi^* = \alpha = 0$ and $\delta_H = 8.2$. In accord with eq 2 and the greater differences between the solvent parameters for cyclohexane/water compared with ocatanol/water, the coefficients of $\bar{V}/100$, π^*, and β are larger in eq 4 than eq 3.

Equations 3 and 4 are particularly significant from the standpoint of the leading hydrogen bond term, since they, for the first time, confirm the general applicability of the β scale of HBA strengths *in aqueous and hydroxylic environments*. We have earlier shown that the β scale has marked differences from the familiar and frequently invoked pK_a scale or the gas-phase basicity scale.[3,15,16] Thus, our methodologies for measuring[3] and for correlating and predicting β values based on molecular structural parameters[12b,17] are directly applicable to most biologically important bases in biological media.

Acknowledgment. We are pleased to acknowledge the assistance of Prof. C. Hansch in providing comments and log P values.

Registry No. 1-Octanol, 111-87-5.

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[12] (a) In the full paper, aromatic bases will be considered, as well as partition functions for other solvent/water systems. (b) Work in progress.

[13] If one uses $\beta = 0.74$ for dioxan (0.37 for each site), log P calculated (−0.52) by eq 3 is only slightly more negative than the experimental value (−0.27). Both $(RO)_3PO$ and RNO_2 compounds appear by negative derivations of ca. 0.5 to behave as more complicated multiple-site bases. The calculated values of all cyclic compounds of Table I are too negative by 0.4 ± 0.1, suggesting that $\bar{V}$ for these is too small by ca. 13 cm^3/mol.

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[16] In ref 3 we have shown that the relationship between aqueous pK_A and β is given to good approximation by the equation: $\Delta \mathrm{p}K_a$(relative to NH_4^+) $= -19.4 + 12.8\beta + 11.5\zeta$, where ζ is a coordinate covalency parameter having the values −0.20 for P═O bases, 0.00 for S═O and C═O bases, 0.20 for single-bonded oxygen bases, 0.60 for pyridine bases, and 1.00 for single-bonded sp^3-hybridized nitrogen bases.

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Photodimerization of Lewis Acid Complexes of Cinnamate Esters in Solution and the Solid State

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Photochemical cyclodimerization of cinnamic acid derivatives is highly inefficient in dilute solution due to very rapid cis–trans photoisomerization.[1] Efficient photodimerization of *trans*-cinnamic acid derivatives is observed in the solid state[2] (subject to topological control) and in solution, when high local concentrations are achieved either by omission of solvent[1] or by linking two or more cinnamate residues together as a diester so that intramolecular photodimerization can occur.[3] We recently reported that

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