to contain a syn oriented His-Asp couple,[23] and if additional anti and syn models are found to have ΔpK_a values of less than and greater than about 1 pK_a unit, respectively.

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Note Added in Proof. We thank Professor R. S. Brown for informing us of an anti model, structurally similar to **1**, which gives $\Delta pK_a = 1.2$.[24] This model will be discussed in detail in a future paper.

(23) A preference for a different orientation might be expected when the histidine residue is acting solely as a general acid. However, thermolysin is the only enzyme where the His-Asp couple is proposed to function in this manner, and it also uses the N-3(H)-syn lone pair combination (ref 5).

(24) Skorey, K. I.; Somayaji, V.; Brown, R. S. *J. Am. Chem. Soc.*, in press.

Stereoelectronic Effects and the Active Site of the Serine Proteases

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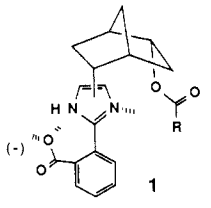
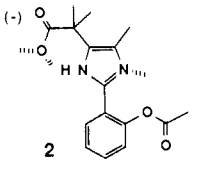
Modelling the active site of the serine proteases is a popular undertaking, and a number of systems have been developed to show how binding forces can lead to large rate enhancements.[1] In cases where both the carboxyl and imidazole groups of the catalytic triad (Chart I) are incorporated into the model, some questions arise concerning stereoelectronic effects at the carboxyl oxygen. Specifically, in structures such as **1**[2] or **2**[3] the less basic anti lone



1



2

pair of the carboxylate is directed toward the imidazole nucleus. Gandour[4] has pointed out that carboxylates at the sites of the serine proteases (and other enzymes) feature the more basic syn lone pairs directed toward the substrate (Chart II). We recently introduced a series of compounds in which carboxyl groups can be oriented with respect to other structural elements in the same molecule.[5] Here we apply these advantages to elaborate new models for the serine proteases.

Chart I

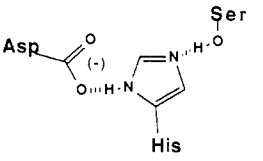
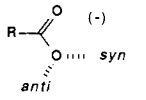


Chart II



Scheme I

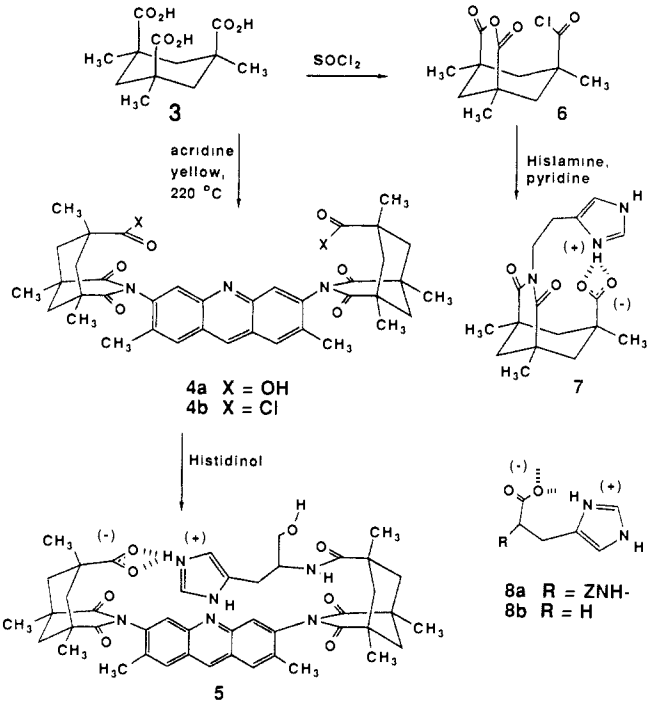


Table I. Acid Dissociation Constants

entry	structure	pK_a COOH	pK_a $NH^{(+)}$	solvent
1	**5**	4.7	7.2	$EtOH/H_2O$
2	**8a**(Z-His)	4.0	6.9	$EtOH/H_2O$
3	**7**	5.7	7.3	$EtOH/H_2O$
4	**9**	6.0		$EtOH/H_2O$
5	**7**	4.8	7.7	H_2O
6	10	5.0		H_2O
7	**11**	3.3	7	H_2O
8	**2**	3.2	7.05	H_2O
9	**8b**	3.8	7.4	H_2O

The systems are rapidly and efficiently assembled from the condensation of Kemp's triacid[6] **3** with appropriate amines. By using acridine yellow in triglyme (Scheme I) a diacid structure (**4a**) is obtained. The carboxyl OH groups converge on a molecular cleft in which a distance of ca. 8 Å separates opposing oxygen atoms.[5] The corresponding diacid chloride[7] **4b** was used to acylate histidinol. Hydrolysis of the intermediate gave th emonofunctionalized derivative[8] **5**. The second structure **7** is

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(8) All new compounds were characterized by high resolution spectroscopy and/or elemental analyses. For **5** mp > 300 °C; **7** mp = 270d (microanalysis invariably indicated the presence of solvent molecules); **10** mp = 272-275 °C. Titrations were performed as described in the following: Gordon, W. E. *J. Phys. Chem.* **1979**, *83*, 1365-1377. Gordon, W. E. *Anal. Chem.* **1987**, *54*, 1595-1601.

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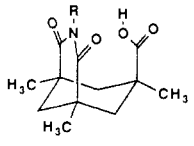
obtained by mere condensation of the anhydride acid chloride[6] **6** with histamine in pyridine.

Both structures permit only the more basic lone pairs of the carboxylates to be directed toward one of the histidine nitrogens. In doing so, they are the first model systems that reproduce this structural feature of the serine proteases. The conformation shown for **5** is taken from an energy-minimized structure obtained by using the AMBER[9] force field. The calculations indicate bifurcated hydrogen bonding (ca. 2.65 Å) between the heavy atoms of the carboxylate and the *distal* nitrogen of imidazolium. Aryl stacking between aromatic nuclei is also likely, as the calculated distance between these surfaces is 3.4 Å. Similar analysis of **7** also suggests bifurcated hydrogen bonding, but now the *proximal* nitrogen of the imidazolium is involved with the carboxylate.

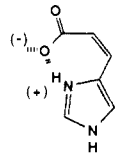
What is significance of this arrangement? Poor solubility of **5** in H_2O prevented its titration in that most biorelevant solvent. The pK_a of the acid in **5** is 4.7 (Table I) and that of the imidazolium function is 7.2 in 50% EtOH/H_2O (w/w). However, the protonated *acridine* nitrogen shows a pK_a of 6.2 in this molecule, and its role in modifying the acidity of the other functions is not easily predicted. The corresponding values for Z-His **8a** in the same solvent system suggest that only a slight enhancement of imidazole basicity is due to the syn lone pairs (entry 1 versus 2).

Systems which permit unambiguous assessment of the stereoelectronic effects rather than inductive effects, steric effects, or entropic effects are difficult to find, but **7** is a reasonable candidate. The acidity of **9**[10] or **10** may be used as the standard for the carboxyl function in this skeleton; their pK_a values are unexceptional. In either EtOH/H_2O or H_2O the interplay of acid and base in **7** can be seen. Thus, the nearby carboxylate enhances the basicity of the imidazole, and the imidazolium nucleus enhances the acidity of the carboxylic acid. Indeed **7** is the strongest base of the series.

Relvant comparison are possible with the cis isomer **11** although the alkene bond permits additional forms of communication between the two functions.[11] The saturated **8b** also shows enhanced



9 R = H
10 R = CH_3



11

basicity.[12] The high basicity of the imidazole of these (entries 7, 8, and 9) is most likely due to the intramolecular hydrogen bond involving the anti lone pair. Thus, even the anti lone pair of a carboxylate can offer significant stabilization of an imidazolium function. The additional effect of 0.3 to 0.7 pK_a units (entry 5 versus 7-9) can be attributed to the syn lone pair. This provides modest support for Gandour's hypothesis[4] concerning the stereoelectronics of carboxyl oxygen at the active sites of enzymes. Structure **5** also incorporates a primary hydroxyl group in the vicinity of the acid-base pair, and its reactivity as an elaborated serine protease model is under investigation.

Acknowledgment. We thank the National Institutes of Health for support and Professor Wm. Gordon for helpful advice.

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The Effects of Pressure on Porphyrin *c*-Cytochrome b_5 Complex Formation

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Cytochrome b_5 forms a reasonably stable 1:1 complex with cytochrome *c* in solution at low ionic strength and at a pH (7.5) that is approximately the average of the isoelectric points of the two proteins.[1] Previous studies of the interaction between these two proteins have employed electronic difference spectroscopy,[1] NMR spectroscopy,[2] specifically modified derivatives of cytochrome *c*,[3] and computer graphics modelling[4] in combination with electrostatics[5] and molecular dynamics calculations.[6] Together, these studies have produced a reasonably detailed model for the mechanism of interaction between these two proteins, many features of which require further experimental evaluation. To obtain complementary hydrodynamic information concerning the cytochrome b_5·cytochrome *c* complex, we have now determined the change in partial specific volume that occurs on formation of this complex by monitoring the quenching of porphyrin cytochrome *c* fluorescence[7] by cytochrome b_5 as a function of pressure under solution conditions known to promote stable 1:1 complex formation at ambient pressure.

Trypsin solubilized cytochrome b_5 was purified from bovine liver microsomes as described previously.[12] Porphyrin cytochrome *c* was prepared from horse heart cytochrome *c* (Type VI, Sigma Chemical Co.) as described previously.[11a-c] The quenching of

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(7) The use of porphyrin *c* in this work is based on the assumption that this derivative of the protein exhibits hydrodynamic and electrostatic properties that are approximately equivalent to those of the native protein. This assumption is clearly imperfect insofar as removal of Fe(III) from cytochrome *c* is expected to change the dipole moment of the protein[8] as well as to affect (in a currently undefined manner) the electrostatic potential surface of the protein. It is also conceivable that the thermal stability of porphyrin *c* may be lower than that of the native cytochrome. Nevertheless, viscosity measurements, circular dichroism spectra, and tryptophan fluorescence studies[9] have provided experimental evidence that the solution structure of porphyrin *c* is virtually unchanged from that of cytochrome *c*. In addition, we note that the distance between the prosthetic groups of the proteins in the porphyrin cytochrome *c*·cytochrome b_5 complex estimated by Forster energy transfer (17-18 Å[10]) is consistent with the distance predicted by the computer graphics model for the complex proposed by Salemme.[4] On the basis of many of these considerations, a substantial literature has developed with the implicit assumption that these two proteins are interchangeable,[11] so the present work has been undertaken, in part with these precedents in mind.

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