between the individual π bonds is consistent with their chemical reactivity. The answer to the critical question of whether stabilization is made possible from such a π-orbital arrangement on the concave underside is decidely negative. That homoconjugative interaction is entirely prohibited is evidenced further by the complete lack of anchimeric assistance to ionization in *exo*-2,3-dihydrotriquinacen-2-ol tosylate[16] and the electronic properties of 2,3-dihydrotriquinacen-2-one.[17]

The increased nonbonded distance separating the allylic carbons in *cis,cis,cis*-1,4,7-cyclononatriene will result in a somewhat more favorable canting of the π orbitals in the molecular interior. It seems reasonable to assume therefore that **2** will be capable of better through space interaction than **3** and that the photoelectron spectroscopic results[18] provide a quantitative measure of these effects. In other words, through space interaction between the three π-bond segments increases with increased molecular puckering. For bridged bicyclic dienes of the Dewar benzene, norbornadiene, bicyclo[2.2.2]octadiene, etc., type, the same is true. In this series, through space interaction falls off as the length of the saturated bridge increases from 0 to 4.[18] This comparable behavior points up convincingly the somewhat analogous orbital alignments which characterize these two groups of molecules.

Acknowledgment. This research was supported by the National Science Foundation.

Registry No.—**3**, 6053-74-3.

Supplementary Material Available. A complete listing of intramolecular bond distances and bond angles (4 pages). Ordering information is given on any current masthead page.

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Quantitative Structure–Activity Relationships of D- and L-*N*-Acyl-α-aminoamide Ligands Binding to Chymotrypsin. On the Problem of Combined Treatment of Stereoisomers[1a]

Masafumi Yoshimoto[1b] and Corwin Hansch*

Department of Chemistry, Pomona College, Claremont, California 91711

Received November 18, 1975

The binding of D- and L-*N*-acyl-α-aminoamides [RCH(NHCOR′)CONH$_2$] to chymotrypsin is correlated with the molar refractivity (MR) of R and NHCOR′. The group with the largest MR value appears to bind in the so-called hydrophobic cleft (ρ_2 area); the smaller group binds in ρ_1 space. Working from this premise, the K_m values for 23 L amides and the K_i values for 22 D amides can be correlated in a single equation. This appears to be the first instance where the structure–activity relationship of optical isomers has been correlated in a single equation.

One of the challenges facing those interested in the formulation of quantitative structure–activity relationships (QSAR) is the problem of including different stereoisomers in a single equation. Sometimes there is little difference in the biological activity of, say, D and L isomers and sometimes there is vast difference between high activity and complete inactivity. An excellent system with which this problem can be explored is α-chymotrypsin and the various ligands with which it interacts. The structure and mechanism of action of this enzyme are probably better understood than those of any other enzyme.

Over the years many studies of substituent effects on ligand interactions with enzymes have been made but attempts to formulate linear free-energy relationships of the Hammett type correlating structure with activity have been limited to sets with small numbers of congeners. Until recently, these correlation studies have tried to rationalize substituent effects using a single electronic parameter such as σ or σ$^-$. With the development[2] of the hydrophobic parameter π, analogous to σ, it has been shown that taking hydrophobic effects as well as steric effects (E_s) of substituents into account enables one to formulate much more comprehensive QSAR for chymotrypsin.[3] Various groups have begun to test the use of hydrophobic parameters in the formulation of structure–activity relationships with chymotrypsin.[4–10]

The parameter π is defined as $\pi_X = \log P_X - \log P_H$ where P_X is the octanol/water partition coefficient of a derivative and P_H that of the parent compound. Considerable evidence has accumulated to indicate that π models the hydrophobic interaction of substituents with lipophilic portions of enzymes[11] as well as other macromolecules.[12]

While much attention has been focused on the interaction

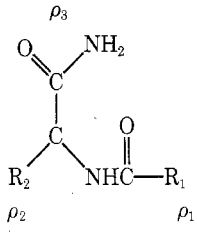
of ligands with the hydrophobic portions of enzymes, little discussion has developed about how ligands might interact with the nonhydrophobic parts of enzymes; that is, in terms of substituent constants one should a priori expect two types of constants to be necessary to describe the interaction of ligand substituents with biomacromolecules. π would be expected to model cases where desolvation is the main driving force. We have found[3,13-15] that molar refractivity (MR) appears to account for a second type of nonspecific interaction where desolvation does not play the dominant role.

Pauling and Pressman appear to have been the first to suggest[17] the use of MR for the correlation of substituent effects on the binding of haptens by antibodies. More recently Agin et al.[18] have discussed the theory behind the use of this parameter in correlating ligand interaction. Both groups started with the London equation and the idea that dispersion forces are most important in the attachment of enzyme and ligand. They have shown, with certain assumptions, that MR of the substituent should be correlated with the logarithm of the binding constant. Agin et al. believe that one should not neglect the ionization potential of the ligand, while Pauling and Pressman neglect this quantity. Because ionization potentials of organic molecules have a small range for compounds consisting mainly of second-row elements, we have followed the lead of Pauling and Pressman.

Franks[19] has recently presented evidence for a second type of "hydrophobic bonding" in which groups with their surrounding flickering clusters of water are held together in solution without desolvation playing the major role. It may be that high correlation with MR reflects this type of interaction. It should be mentioned that Franks restricts his second type of hydrophobic interaction to apolar groups. We are employing MR to model substituent effects of polar as well as apolar groups. With apolar substituents such as halogens and alkyl groups, π and MR are so collinear that, in an operational sense, they yield the same information in correlation analysis. When π and MR are orthogonal and the QSAR is related to MR, all that can be said at present is that the interaction does not depend primarily on desolvation.

In the present report we are concerned with obtaining a better general understanding of the forces which bind enzyme and substrate or inhibitor. We have employed data from Niemann's extensive studies on D and L amides of the type

Chart I



The L form of the amide binding to ρ_1, ρ_2, and ρ_3 space of the enzyme is shown in Chart I. This use of ρ corresponds to that proposed by Hein and Niemann.[20] The H on the α carbon in Chart I is projecting below the plane of the page. In general, L amides function as substrates, while D amides act as inhibitors of chymotrypsin hydrolysis. We have formulated eq 1-3 from Niemann's data and the physicochemical constants of Table I to correlate the binding of inhibitors (K_i) and substrates (K_m) to chymotrypsin.

Experimental Section

The constants π and MR of Table I are from our recent compilation.[21] In order to test the characteristics of our correlation equations, three new amides were synthesized and their K_m values determined under the conditions employed by Niemann by means of a Radiometer titration.

α-*N*-Nicotinyl-L-4-nitrophenylalanine Ethyl Ester. To a mixture of L-4-nitrophenylalanine (5.6 g, 27 mmol) and 100% ethanol (100 ml), hydrogen chloride gas was introduced until solution resulted (20 min) and a precipitate occurred (1 h). The mixture was then refluxed for 2 h, after which the alcohol was evaporated under reduced pressure. The residue was washed with ether to give crude nitrophenylalanine ethyl ester hydrochloride (5.9 g, 21 mmol) of mp 212-214 °C. Careful treatment of the hydrochloride with potassium carbonate solution converted it to the oily free base. To this material was added nicotinyl azide (3.7 g, 25 mmol) and ethyl acetate (20 ml) and the mixture was allowed to stand overnight. This solution was extracted with 5% HCl. This phase was neutralized with $NaHCO_3$ and extracted with ethyl acetate. After drying the extract over $MgSO_4$, the solvent was removed under reduced pressure. The residue was washed with ether and recrystallized from acetone–ether to give needles (3.8 g), mp 135-137 °C.

Anal. Calcd for $C_{17}H_{17}N_3O_5$: C, 59.47; H, 4.99. Found: C, 59.83; H, 5.04.

α-*N*-Nicotinyl-L-4-nitrophenylalaninamide. The above ester (3 g) was dissolved in methanol (15 ml) and liquid ammonia (15 ml). This solution was allowed to stand at room temperature for 2 days after which the solvent was evaporated. The solid residue was recrystallized from ethanol to give fine needles (2.4 g), mp 200-201 °C.

Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49. Found: C, 57.93; H, 4.03.

α-*N*-Nicotinyl-L-alanine Ethyl Ester. A mixture of ethyl alaninate (from 3.07 g of the hydrochloride) and nicotinyl azide (3.70 g) in ethyl acetate (20 ml) was allowed to stand overnight and then extracted with 5% HCl. The aqueous phase was neutralized with $NaHCO_3$ and extracted with ethyl acetate. The extracts were dried and solvent removed under reduced pressure. The solid residue was washed with hexane and recrystallized from acetone–pentane to afford needles (3.55 g), mp 92-93 °C.

Anal. Calcd for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35. Found: C, 60.11; H, 6.01.

α-*N*-Nicotinyl Alaninamide. The above ethyl ester (0.75 g) was dissolved in 15 ml of methanol and 15 ml of liquid ammonia. After standing overnight, the solvent was removed under reduced pressure and the solid residue recrystallized from ethanol–acetone to give needles (0.50 g), mp 230-232 °C.

Anal. Calcd for $C_9H_{11}N_3O_2$: C, 55.95; H, 5.74. Found: C, 56.20; H, 5.71.

α-*N*-Benzoyl-4-nitrophenylalanine Ethyl Ester. To a mixture of benzoic acid (12.2 g) and pyridine (30 ml) was added benzenesulfonyl chloride (6.4 ml) and then L-4-nitrophenylalanine ethyl ester (13.7 g). After standing for 1 day, the excess pyridine was vacuum evaporated and the residue was partitioned between water and ethyl acetate. The water phase was extracted with ethyl acetate and the combined extracts were washed with 5% HCl, NaCl solution, $NaHCO_3$ solution, and again with NaCl solution. The ethyl acetate solution was dried over potassium carbonate and evaporated to dryness. Three recrystallizations of the residue from aqueous ethanol gave needles (10 g), mp 117-119 °C.

Anal. Calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30. Found: C, 63.93; H, 5.67.

α-*N*-Benzoyl-4-aminophenylalanine Ethyl Ester. α-*N*-Benzoyl-4-nitrophenylalanine ethyl ester (2.5 g) was dissolved in 60 ml of ethanol and hydrogenated in the presence of PtO_2 at 33-52 psi for 25 min. The catalyst was removed by filtration and the solvent evaporated. Recrystallization of the residue from aqueous ethanol gave colorless needles (2.0 g), mp 140-143 °C.

Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45. Found: C, 68.92; H, 6.50.

α-*N*-Benzoyl L-4-Methanesulfonylamidophenylalaninamide. α-*N*-Benzoyl L-4-aminophenylalanine ethyl ester (0.5 g) was dissolved in 100 ml of pyridine and placed in an ice bath. To this solution methanesulfonyl chloride (1 ml) was added dropwise. After standing for 1 day, the pyridine was removed by vacuum evaporation and the residue partitioned between ethyl acetate and 5% HCl. The organic phase was washed with NaCl solution, $NaHCO_3$ solution, and then NaCl solution. After drying over K_2CO_3, the solvent was evaporated and the oily residue dissolved in 10 ml of methanol and 15 ml of liquid ammonia. After standing overnight, it was evaporated to dryness and the solid residue recrystallized from aqueous methanol to afford needles (0.5 g), mp 258-260 °C.

Anal. Calcd for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30; N, 11.63. Found: C, 56.90; H, 5.28; N, 11.24.

Enzyme Experiments. All enzymatic hydrolyses were conducted

Table I. Constants Used for Deriving Equations 1–3

No.	Compd	Log 1/K Obsd[a]	Log 1/K Calcd[b]	|Δ log 1/K|	MR-L	MR-S	I-1	I-2	Ref
1	CH_3CO-Gly-NH_2	0.46	0.35	0.11	1.49	0.10	0.0	1.0	16a
2	C_6H_5CO-Gly-NH_2	1.89	1.77	0.12	3.46	0.10	0.0	1.0	16d
3	D-Tyr-NHOH	1.40	1.67	0.27	3.18	0.54	0.0	1.0	16e
4	D-C_2H_5OCO-Tyr-NH_2	1.68	2.03	0.35	3.18	2.12	0.0	1.0	16a
5	D-CF_3CO-Tyr-NH_2	1.70	1.87	0.17	3.18	1.43	0.0	1.0	16a
6	D-CH_3CO-Tyr-NH_2	1.92	1.88	0.04	3.18	1.49	0.0	1.0	16a
7	D-CH_3CO-Phe-NH_2	2.00	1.75	0.25	3.00	1.49	0.0	1.0	16a
8	D-NiCO-Tyr-NH_2	2.05	2.31	0.25	3.23	3.18	0.0	1.0	16a
9	D-NiCO-Phe-NH_2	2.05	2.27	0.22	3.23	3.00	0.0	1.0	16a
10	D-CH_3CO-Tyr-$NHNH_2$	2.12	2.21	0.09	3.18	1.49	1.0	1.0	16c
11	D-CH_3CO-Tyr-NHOH	2.12	1.88	0.24	3.18	1.49	0.0	1.0	16c
12	D-$ClCH_2CO$-Tyr-NH_2	2.19	2.00	0.19	3.18	1.98	0.0	1.0	16a
13	D-Try-NH_2	2.40	2.42	0.02	4.23	0.54	0.0	1.0	16d
14	D-CF_3CO-Try-NH_2	2.40	2.63	0.23	4.23	1.43	0.0	1.0	16d
15	D-CH_3CO-Try-$NHCH_3$	2.74	2.64	0.10	4.23	1.49	0.0	1.0	16c
16	D-CH_3CO-Try-NH_2	2.77	2.64	0.13	4.23	1.49	0.0	1.0	16a
17	D-NiCO-Try-NH_2	2.80	3.04	0.24	4.23	3.23	0.0	1.0	16a
18	D-CH_3CO-Try-$NHNH_2$	3.10	2.96	0.14	4.23	1.49	1.0	1.0	16d
19	D-C_6H_5CO-Try-NH_2	3.15	3.09	0.06	4.23	3.46	0.0	1.0	16d
20	D-4-$CH_3OC_6H_4CO$-Try-NH_2	3.22	3.24	0.02	4.23	4.10	0.0	1.0	16d
21	D-4-$NO_2C_6H_4CO$-Try-NH_2	3.74	3.24	0.50	4.23	4.10	0.0	1.0	16f
22	L-CH_3CO-Tyr-$NHCH_3$	1.21	1.57	0.36	3.18	1.49	0.0	0.0	16b
23	L-CH_3CO-Tyr-NHOH	1.37	1.57	0.20	3.18	1.49	0.0	0.0	16b
24[c]	L-$(CH_3)_3CCO$-Tyr-$NHNH_2$	1.40	2.22	0.82	3.18	2.89	1.0	0.0	16b
25	L-CH_3CO-Tyr-NH_2	1.41	1.57	0.16	3.18	1.49	0.0	0.0	16a
26	L-CH_3CO-Phe-NH_2	1.51	1.44	0.07	3.00	1.49	0.0	0.0	16a
27	L-CH_3CO-Tyr-$NHNH_2$	1.52	1.90	0.38	3.18	1.49	1.0	0.0	16b
28	L-$ClCH_2CO$-Tyr-NH_2	1.57	1.69	0.12	3.18	1.98	0.0	0.0	16a
29	L-CH_3CO-$C_6H_{11}CH_2$-NH_2	1.57	1.54	0.03	3.13	1.49	0.0	0.0	16b
30	L-CF_3CO-Tyr-NH_2	1.58	1.56	0.02	3.18	1.43	0.0	0.0	16a
31	L-CH_3CO-Tyr-Gly-NH_2	1.64	1.57	0.07	3.18	1.49	0.0	0.0	16b
32	L-NiCO-Phe-NH_2	1.72	1.96	0.24	3.23	3.00	0.0	0.0	16a
33	L-NiCO-Tyr-NH_2	1.80	2.00	0.20	3.23	3.18	0.0	0.0	16a
34	L-HCO-Tyr-NH_2	1.92	1.47	0.45	3.18	1.03	0.0	0.0	16b
35	L-HCO-Tyr-$NHNH_2$	2.01	1.79	0.22	3.18	1.03	1.0	0.0	16b
36	L-PiCO-Tyr-NH_2	2.04	2.00	0.04	3.23	3.18	0.0	0.0	16b
37	L-iso-NiCO-Tyr-NH_2	2.05	2.00	0.05	3.23	3.18	0.0	0.0	16c
38	L-NiCO-Tyr-$NHNH_2$	2.10	2.32	0.22	3.23	3.18	1.0	0.0	16b
39	L-Try-NH_2	2.10	2.11	0.01	4.23	0.54	0.0	0.0	16d
40	L-C_2H_5OCO-Tyr-NH_2	2.19	1.72	0.47	3.18	2.12	0.0	0.0	16a
41	L-CH_3CO-Try-$NHCH_3$	2.19	2.33	0.14	4.23	1.49	0.0	0.0	16b
42	L-Cl_2CHCO-Tyr-$NHNH_2$	2.28	2.12	0.16	3.18	2.48	1.0	0.0	16b
43	L-CH_3CO-Try-NH_2	2.30	2.33	0.03	4.23	1.49	0.0	0.0	16a
44	L-NiCO-Try-NH_2	2.60	2.73	0.13	4.23	3.23	0.0	0.0	16a
45	L-C_6H_5CO-Tyr-NH_2	2.60	2.16	0.44	3.46	3.18	0.0	0.0	16b
46	L-C_6H_5CO-Tyr-$NHNH_2$	2.66	2.49	0.17	3.46	3.18	1.0	0.0	16b
47[d]	L-NiCO-Ala-NH_2	1.40	1.40	0.00	3.23	0.57	0.0	0.0	
48[d]	L-C_6H_5CO-4-CH_3SO_2NHPhe-NH_2	1.78	3.14	1.36	4.72	3.46	0.0	0.0	
49[d]	L-NiCO-4-NO_2Phe-NH_2	2.34	2.30	0.04	3.63	3.23	0.0	0.0	

[a] Calculated from results of Niemann et al. (1–46) and the present authors (see ref 16). [b] Calculated using eq 3. [c] This molecule not used in deriving equations. [d] Compounds made in this work. These points not used in deriving eq 1–3.

at 25 °C, pH 7.9 ± 0.02 with 0.10 M sodium chloride. The rate of hydrolysis was determined by means of a Radiometer pH-stat system. Crystalline chymotrypsin was obtained from Worthington Biochemical Corp. The procedure used was that of Niemann. The value of K_m for α-*N*-nicotinyl-L-tyrosinamide was determined as a standard and found to be identical with that obtained by Kartz and Niemann.[22]

Results and Discussion

Equations 1 and 2 correlate K_m for the L isomers and K_i for D isomers and glycine derivatives of the *N*-acyl-α-aminoamides, respectively. In these equations, *n* is the number of data points, *r* is the correlation coefficient, *s* is the standard deviation, and the figures in parentheses are the 95% confidence intervals. MR-L refers to the molar refractivity of the larger of the two α substituents regardless of its stereochemistry and MR-S refers to the smaller. This approach yields considerably better results than using MR-1 and MR-2 for R_1 and R_2 where

$$\log 1/K_m = 0.666(\pm0.27)\text{MR-L} + 0.227(\pm0.12)\text{MR-S} + 0.300(\pm0.27)\text{I-1} - 0.867(\pm0.99) \quad (1)$$

n	*r*	*s*
24	0.826	0.249

$$\log 1/K_i = 0.744(\pm0.18)\text{MR-L} + 0.225(\pm0.10)\text{MR-S} + 0.344(\pm0.37)\text{I-1} - 0.824 \quad (2)$$

n	*r*	*s*
21	0.955	0.234

MR-1 refers to the acylamino or amino substituent and MR-2 refers to the α-R group. The close correspondence between the coefficients in eq 1 and 2 strongly supports the idea that the larger substituent preferentially attaches the ligand by interactions in ρ_2 space. The correlation with eq 1 is not as good as eq 2 with respect to r; it is also somewhat poorer with respect to s. Part of this apparent poorer correlation is due to the smaller range in the values of log $1/K$ for the L isomers compared to the D isomers. Another possible contribution to the lower correlation of eq 1 may be that since K_m is not a pure constant, it may contain information on the hydrolytic step as well as simple binding.

In eq 1 and 2, I-1 is an indicator variable assigned a value of 1 for hydrazides and a value of 0 for all other cases. Its positive coefficient shows that, on the average, hydrazides bind twice as strongly as amides. Substituted amides such as NHOH and $NHCH_3$ do not need special correction.

The coefficients in eq 1 and 2 are so similar that they at once suggest that, to a first approximation, the intermolecular forces holding substrate and inhibitor to the enzyme are the same.

Equation 3 results from merging the two data sets. I-1 in eq 3

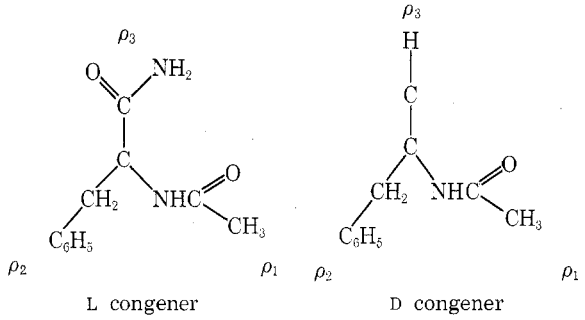
$$\log 1/K = 0.72(\pm0.13)\text{MR-L} + 0.230(\pm0.07)\text{MR-S} + 0.323(\pm0.20)\text{I-1} + 0.311(\pm0.15)\text{I-2} - 1.062(\pm0.45) \quad (3)$$

n	r	s
45	0.928	0.235

is the indicator variable for hydrazides, I-2 takes the value of 1 for D congeners and glycine amides which are inhibitors, while L congeners are assigned a value of 0. The positive coefficient with I-2 reflects the tighter binding of the inhibitors. All of the terms in eq 3 are justified at $\alpha = 0.005$ by the stepwise application of the F test. The overall picture given by eq 3 seems reasonable. Neurath and Hartley[23] first summarized evidence to show that K_m appears to represent a simple binding constant for chymotrypsin. Zerner and Bender[24] have emphasized that K_i and K_m are comparable parameters for the interaction of amides with chymotrypsin. They felt that such an assumption is not justified with esters since they are hydrolyzed so much more rapidly. The good fit of the data to eq 3 supports the idea that K_m and K_i are quite similar equilibrium constants for amides.

Since the D congeners are not hydrolyzed and since the large apolar groups show the same binding characteristics, we can rationalize the results as follows.

Chart II

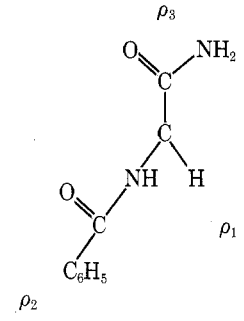


L congener D congener

If hydrolysis occurs in ρ_3 space, the L congener binds properly as a substrate; however, the D congener binds so that the $CONH_2$ is now in ρ_H space and not well positioned for hydrolysis. Hence these ligands act as inhibitors.

The two glycine derivatives (**1**, **2** in Table I) present a special problem since they act as inhibitors despite the fact that they can, in principle, assume proper binding.

Chart III



It would appear that a group at least as large as NH_2 or CH_3 must bind in ρ_1 space to induce hydrolysis. Such binding may cause a conformational change which places the amide function in proper position for hydrolysis. The glycine derivatives lacking such activating groups act only as inhibitors.

Compound **24** of Table I was not used in formulating eq 3. Its very poor fit to eq 3 is probably the result of the very bulky *tert*-butyl group not fitting into ρ_2 space.

In order to test eq 3 we have synthesized three new acylamides (**47–49**) and measured their K_m values under the conditions employed by Niemann. Two of these derivatives (**47** and **49**) are well fit by eq 3 and the third (**48** with 4-$NHSO_2Me$) is poorly fit. The poor fit of **48** is not unexpected since the so-called hydrophobic cleft in chymotrypsin has been shown not to properly accommodate 4-iodophenylalanine; in fact, the size of this pocket has been well established from x-ray crystallographic studies. Steitz et al.[25] state that it is a flattened shape with dimensions of 10–12 Å by 5.5–6.5 Å by 3.5–4.0 Å. They note that 4-iodophenylacetate binds with its I deep in this pocket. Our results with **48** support the limited dimensions of the cleft in chymotrypsin.

The fact that **49** is well fit shows that a substituent as large as 4-NO_2 can be accommodated by this pocket.

The most interesting case is that of **47**. Since the point is well fit by eq 3, "wrong-way binding" is implied with the nicotinyl moiety falling into ρ_2 space. If MR-1 and MR-2 parameters are employed in eq 3, compound **48** is very poorly fit ($\Delta\log 1/C = 0.52$). One of the glycine derivatives is also very poorly fit by MR-1 and MR-2. Because there are only two data points (**47** and **48**) for which MR-1 $\gg$ MR-2, more compounds should be tested to confirm this finding. Since the larger α substituent appears to preempt ρ_2 space, it would appear that **47** undergoes hydrolysis even though "wrong-way binding" takes place. Since compound **47** shows a very slow rate of hydrolysis it appears that both "right" and "wrong-way binding" can occur and that there is enough flexibility in the enzyme to allow some hydrolysis. Somehow the amide group must be reasonably well fit in ρ_3 space.

It was concluded in our earlier survey of the interactions of ligands with chymotrypsin that ρ_1 space was not typically hydrophobic since binding in this area correlated well with MR and not with π for data not highly collinear in MR and π. At that time, ρ_2 space was characterized as hydrophobic although, for the data studied, π and MR were highly collinear. The present study confirms our earlier conclusion that ρ_1 is not hydrophobic. More interesting, however, is the finding that binding in ρ_2 space is not well correlated by π but is well correlated by MR.

If, in eq 3, we substitute the corresponding π constants for MR, a quite poor correlation is obtained ($r = 0.669$). It is evident from the squared correlation matrix of Table II that MR-L and π of MR-L are reasonably noncollinear ($r^2 = 0.29$). Thus it appears that desolvation is not the primary determinate of binding in ρ_2 space.

Our conclusion that the binding pocket around the active

Table II. Squared Correlation Matrix for Variables Pertaining to Equation 3

	MR-L	MR-S	π-MR-L	π-MR-S	I-1	I-2
MR-L	1.00	0.06	0.29	0.04	0.00	0.03
MR-S		1.00	0.07	0.50	0.00	0.01
π-MR-L			1.00	0.48	0.00	0.02
π-MR-S				1.00	0.00	0.02
I-1					1.00	0.02
I-2						1.00

site in chymotrypsin is not typically hydrophobic is supported by the analysis of Dickerson and Geis.[26] The "hydrophobic" pocket in chymotrypsin is circumscribed by the following two peptide sequences:

Gly Ala Ser Gly Val Ser Ser Cys Met
184 185 186 187 188 189 190 191 192

Ilu Val Ser Trp Gly Ser Ser Thr Cys Ser Thr Ser Thr Pro Gly Val
212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227

The vast majority of these residues are hydrophilic, not hydrophobic; thus, correlation with MR can be used to characterize nonhydrophobic enzyme space as π can be used for hydrophobic space.[27]

Equation 3 does establish the fact that it is possible to construct QSAR for stereoisomers by taking into account the type of space into which substituents fall. We believe that the approach used in formulating eq 3 should be generally applicable to problems involving stereoisomers.

Registry No.—α-*N*-Nicotinyl-L-4-nitrophenylalanine ethyl ester, 58816-65-2; L-4-nitrophenylalanine, 949-99-5; L-4-nitrophenylalanine ethyl ester HCl, 58816-66-3; L-4-nitrophenylalanine ethyl ester, 34276-53-4; nicotinyl azide, 4013-72-3; α-*N*-nicotinyl-L-4-nitrophenylalaninamide, 58816-67-4; α-*N*-nicotinyl-L-alanine ethyl ester, 58816-68-5; ethyl alaninate, 3082-75-5; α-*N*-nicotinyl alaninamide, 53503-62-1; α-*N*-benzoyl-4-nitrophenylalanine ethyl ester, 58816-69-6; α-*N*-benzoyl-4-aminophenylalanine ethyl ester, 58816-70-9; α-*N*-benzoyl-L-4-methanesulfonylamidophenylalaninamide, 58816-71-0; methanesulfonyl chloride, 124-63-0.

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(1) (a) This investigation was supported by Public Health Service Research Grant CA-11110 from the National Cancer Institute and the Sankyo Co. of Tokyo, Japan; (b) Visiting Scientist from the Sankyo Co.
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Ring Opening of Aziridine Phosphonates. Correlation of Structure, Nuclear Magnetic Resonance Spectra, and Reactivity[1a]

Alfred Hassner* and James E. Galle

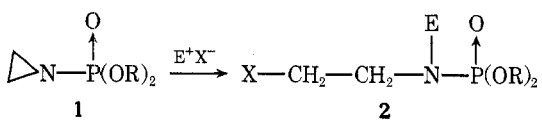
Department of Chemistry, State University of New York at Binghamton,[1b] Binghamton, New York 13901

Received April 1, 1975

The ring opening of several dimethyl *N*-aziridinylphosphonates **3** with Cl_2 and HCl was studied. The reaction was found to be stereospecific and in most cases regiospecific. Conformational preferences in these compounds could be correlated with 1,3 P–H (PNCCH) coupling constants and with reactivity in ring opening.

The importance of aziridines as well as their N-phosphorylated derivatives in biological systems is well documented.[2] It is generally assumed that the cytotoxic behavior of such compounds is due to their ability to undergo ring opening by nucleophilic sites of enzymes.

The ring opening of unsubstituted aziridine phosphonates of type **1** to **2** with electrophilic reagents (E^+X^-) including



1 **2**

carboxylic acids, chlorine, and alkyl halides has been investigated by Russian chemists.[3a] Related *N,N*-dialkylaminoaziridinyl phosphoric amides react similarly.[3b]

In this study we are reporting on the chlorination of several ring substituted aziridine phosphonates **3** in an effort to determine the factors which influence the stereochemistry, regiochemistry, and the rate of ring opening.

Results and Stereochemistry. The reaction of dimethyl *N*-aziridinylphosphonates **3a–i** with chlorine in CCl_4 solution at 0–5 °C leads to dimethyl *N*-chloro-*N*-(β-chloroethyl)phosphoramidates **4a–i** in high yield. These *N*-chloro compounds cannot be purified effectively, but are reduced