Enantiomeric Selectivity in the Reactions of Natural Amino Acids with 1-Fluoro-2,4-dinitrobenzene in the Presence of Cyclodextrins

Mônica Barra and Rita H. de Rossi*

Instituto de Investigaciones en Físico-Química de Córdoba (INFIQC), Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Suc. 16, C.C. 61, 5016 Córdoba, Argentina

Received February 22, 1989

The reactions of 1-fluoro-2,4-dinitrobenzene with both enantiomers of the α-amino acids alanine (**2a**), methionine (**2b**), valine (**2c**), leucine (**2d**), tyrosine (**2e**), phenylalanine (**2f**), and tryptophan (**2g**) were studied in the presence of β-cyclodextrin (β-CD). The effect of γ-cyclodextrin (γ-CD) was determined in the reactions with **2a** and **2g**. At pH > 10, all the reactions, except those with L-phenylalanine and D-tryptophan, were catalyzed by β-CD. The catalytic factor, defined as the ratio between the slope and the intercept of linear plots of the observed pseudo-first-order rate constant vs β-CD concentration, decreases in the series as the size of the amino acid increases, so it is 50 for L-alanine and 3 for L-tryptophan. As the catalytic factor decreases, the difference in the values for the D and L enantiomers increases. The effect of γ-CD in the reaction with **2a** is about the same as that of β-CD, but in the reaction with **2g**, the catalytic factor increases from 0 and 3 to 150 ± 75 and 140 ± 35 for the D and L enantiomers, respectively. It is suggested that the catalysis and the chiral discrimination are due to the reaction between the two complexed reactants and the formation of a ternary complex where the substrate and the amino acid are included in the same cavity of the host.

A great variety of reactions have been studied in the presence of cyclodextrins which are cyclic oligomers of α-D-glucose containing six to eight D-glucopyranosyl units.[1] The host-guest interaction of these compounds with organic or inorganic guests has found a lot of applications in fundamental[2] as well as in applied studies.[3]

The cyclodextrin cavity is chiral, and so it is able to include each of the enantiomers of an optically active compound with different affinities, and also the orientation of the included compound within the cavity may be different.[4] This behavior has found application in the analytical separation of enantiomers by liquid chromatography.[5] On the other hand, some enantiomeric selectivity has been found in the hydrolysis of optically active esters.[6]

We found that the reactions of 1-fluoro-2,4-dinitrobenzene with amines such as piperidine and *n*-butylamine are catalyzed by β-cyclodextrin (β-CD), and we have ascribed this catalysis to a favorable interaction of the complexed substrate with the complexed amine.[7] These results led us to think that if the amines were optically active, it would be possible that each enantiomer complexed with cyclodextrin reacted at a different rate. Therefore, the reaction of an electrophile with a racemic mixture of the amine would lead to a kinetic enrichment of the mixture in one of the enantiomers.

We report here our results regarding the reaction of 1-fluoro-2,4-dinitrobenzene (**1**) with α-amino acids **2** in the presence of cyclodextrins.

Results

The reaction of **1** with amino acids is a very well-known reaction since it is used to determine the structure of polypeptides.[8] Nevertheless, kinetic studies are scarce in the literature.[9]

Table I. Observed Pseudo-First-Order Rate Constants for the Reaction of 1-Fluoro-2,4-dinitrobenzene with α-Amino Acids[a]

amino acid[b]	pH	$10^4 k_A$,[c] s^{-1}
D-alanine (1.1×10^{-2})	8.718	0.323
D-alanine (1.02×10^{-3})	10.73	1.17[f]
L-alanine (1.0×10^{-2})	8.718	0.274
L-alanine (1.00×10^{-3})	10.73	1.06[f]
D-methionine (1.05×10^{-3})[d]	10.300	1.16[f]
L-methionine (1.05×10^{-3})[d]	10.298	1.15
L-methionine (1.02×10^{-3})[d]	10.417	1.15
D-valine (1.01×10^{-3})	10.724	2.57[f]
L-valine (1.01×10^{-3})	10.723	2.39[f]
D-leucine (1.0×10^{-2})	8.710	0.769
D-leucine (1.0×10^{-3})	10.482	1.38
D-leucine (1.0×10^{-2})	10.733	17.5
L-leucine (1.1×10^{-2})	8.710	0.872
L-leucine (1.05×10^{-3})	10.482	1.51
L-leucine (1.0×10^{-2})	10.734	17.4
D-tyrosine (2.01×10^{-3})	9.093	1.45
D-tyrosine (1.01×10^{-3})[e]	11.001	5.42[g]
L-tyrosine (1.97×10^{-3})	9.090	1.41
L-tyrosine (1.02×10^{-3})[e]	11.000	5.90[g]
D-phenylalanine (1.0×10^{-3})	10.283	2.13
L-phenylalanine (1.0×10^{-3})	10.283	2.16
D-tryptophan (1.0×10^{-2})	8.450	3.37
D-tryptophan (1.0×10^{-3})	10.573	7.35
D-tryptophan (1.0×10^{-3})	10.582	7.48
L-tryptophan 1.0×10^{-3})	8.449	3.48
L-tryptophan (1.0×10^{-2})	10.571	7.34
L-tryptophan (1.0×10^{-3})	10.581	6.96

[a] Solvent DMSO–water (30% v/v); μ = 0.2 M (NaCl); T = 20 °C; substrate concentration = (4–4.5) × 10^{-5} M unless otherwise indicated. [b] Values within parentheses indicate the total concentration of the amino acid. [c] The second-order rate constants have not been calculated because we do not know the pK_a of the amino acids in the solvent used so that it is not possible to calculate the fraction of the free amine. [d] Substrate concentration = 1.4 × 10^{-5} M. [e] Substrate concentration = (1–1.5) × 10^{-4} M. [f] Obtained from the product $k_{obsd}f_A$. A fraction lower than 0.1 corresponds to 2,4-dinitrophenol. [g] Obtained from the product $k_{obsd}f_A$. At this pH there was also formation of *O*-(2,4-dinitrophenyl)tyrosine.

(1) For recent reviews, see: Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1977. Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66. Sirlin, C. *Bull. Soc. Chim. Fr.* **1984**, II-5. Tabushi, I.; Kuroda, Y. In *Advances in Catalysis*; Academic Press: New York, 1983; Vol. 32, p 417.

(2) (a) Breslow, R.; Trainor, G.; Ueno, A. *J. Am. Chem. Soc.* **1983**, *105*, 2739. (b) Cram, D. J.; Lam, P. Y.; Ho, S. P. *J. Am. Chem. Soc.* **1986**, *108*, 839.

(3) Ward, T. J.; Armstrong, D. W. *J. Liq. Chromatogr.* **1986**, *9*, 407.

(4) Hamilton, J. A.; Chen, L. *J. Am. Chem. Soc.* **1988**, *110*, 5833.

(5) Armstrong, D. W. *J. Liq. Chromatogr.* **1984**, *7*(S-2), 353.

(6) Fornasier, R.; Reniero, F.; Schrimin, P.; Tonellato, U. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1121.

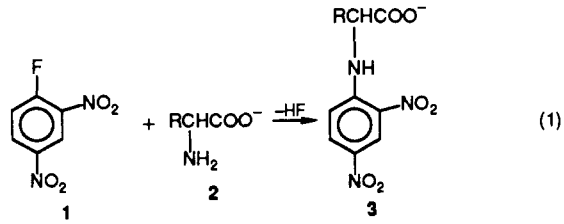
(7) Barra, M.; de Rossi, R. H.; de Vargas, E. B. *J. Org. Chem.* **1987**, *52*, 5004.

(8) Fraenkel-Conrat, H.; Harris, J. I.; Levy, A. L. *Methods Biochem. Anal.* **1955**, *2*, 359.

(9) (a) Bunnett, J. F.; Hermann, D. H. *Biochemistry* **1970**, *9*, 816. (b) Herraez Zarza, M. A.; Sanchez Jimenez, M. *An. Quim., Ser. A* **1982**, *78*, 102. (c) Bunton, C. A.; Wright, J. L. *Tetrahedron* **1975**, *31*, 3013.

0022-3263/89/1954-5020$01.50/0

The reaction of **1** with **2** leads to the corresponding *N*-(2,4-dinitrophenyl)amino acid (eq 1).

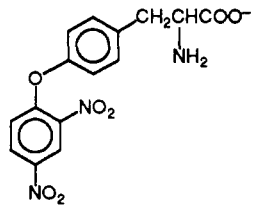


1 2 3 (1)

R (amino acid): CH_3 (alanine, **2a**), $CH_3SCH_2CH_2$ (methionine, **2b**), $(CH_3)_2CH_2$ (valine, **2c**), $(CH_3)_2CHCH_2$ (leucine, **2d**), *p*-$OHC_6H_4CH_2$ (tyrosine, **2e**), $C_6H_5CH_2$ (phenylalanine, **2f**), indol-3-ylmethyl (tryptophan, **2g**)

Most of the reactions were studied at a pH where the predominant species is the anion. In a few cases, 2,4-dinitrophenol was also formed, so the rate of aminolysis was calculated from the observed pseudo-first-order rate constant (k_{obsd}) and the fraction of **3** obtained (f_A). The values of the aminolysis rate constants are summarized in Table I.

In the reaction with tyrosine (**2e**), there was also formation of **4**, which is the product coming from the reaction of the phenolic oxygen of **2e** acting as the nucleophile.



4

All the reactions are overall second order: first order with respect to the substrate and first order with respect to the nucleophile. This indicates that the addition of the nucleophile to the aromatic ring is the rate-determining step.[10]

The thermodynamic activation parameters were determined for L-alanine and for the racemic mixture; these values are $\Delta H^{\ddagger} = 13.3 \pm 0.5$ kcal/mol and $\Delta S^{\ddagger} = 17 \pm 4$ eu for D,L-alanine and $\Delta H^{\ddagger} = 12.4 \pm 0.6$ kcal/mol and $\Delta S^{\ddagger} = 20 \pm 1$ eu for L-alanine. The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are, within experimental error, independent of the stereoisomerism of the nucleophile, as expected for a reaction that does not involve bond breaking at the chiral center.

Effect of β-Cyclodextrin. The pseudo-first-order rate constants for the reactions of **1** with the D and L isomers of the amino acids **2** were determined as a function of β-cyclodextrin concentration (Table II).[11] All the reactions were studied at pH ≈ 10–11, where the ratio $RCH(NH_2)COO^-/RCH(NH_3^+)COO^-$ is higher than 1. Besides the reactions with alanine, leucine and tryptophan were studied at pH ≈ 8.7, where the predominant form of the amino acid is the zwitterion.

At high pH, where the anionic form of the amino acid is predominant, the observed rate constant increases with the concentration of β-CD. Under these conditions, there was formation of (2,4-dinitrophenyl)cycloheptaamylose besides the aminolysis and hydrolysis products, as was previously observed in the study of the hydrolysis of **1**.[12] The pseudo-first-order rate constant (k_A) for the aminolysis

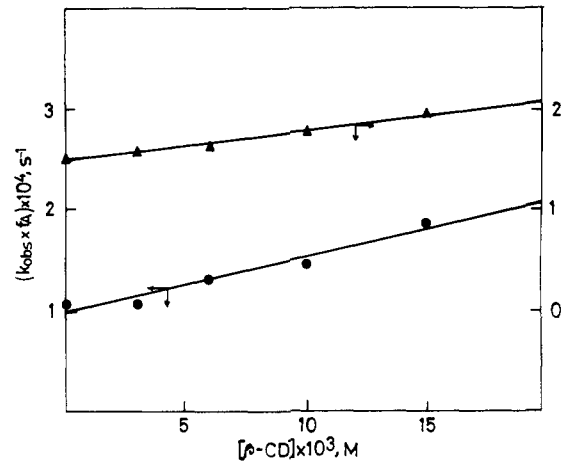


Figure 1. $k_{obsd}f_A$ vs β-cyclodextrin concentration for the reaction of **1** with α-amino acids (●, L-alanine; ▲, L-leucine).

reaction was obtained from the product of the observed pseudo-first-order rate constant and the fraction of the aminolysis product, $k_A = (k_{obsd}f_A)$. The values of k_A so obtained increase linearly with the concentration of β-CD for all the amino acids except for L-phenylalanine and D-tryptophan, which do not change with β-CD concentration (Figure 1 is representative).

In the reactions carried out with **2a**, **2d**, and **2g** at low pH, where the concentration of the anionic form of the amino acids is very low, the only product formed was **3** and the value of $k_A = k_{obsd}$ increased with the concentration of β-CD in all cases except for L-alanine, which did not depend on the β-CD concentration.

In order to determine whether the observed changes were due to a medium effect, we measured the kinetics of the reaction of **1** with L- and D-alanine as a function of soluble starch (a linear analogue of CD) at pH ≈ 10.7. The only products formed were **3** and 2,4-dinitrophenol, and there was not a significant change of the observed rate constants with the concentration of starch. These results indicate that the effect of β-CD involves some type of interaction different from that found with a linear polysaccharide.

On the other hand, the reaction between 1-chloro-2,4,6-trinitrobenzene (a substrate that does not form an inclusion complex with β-CD)[12] and L-alanine at pH ≈ 10.7 is not affected by β-CD. This result indicates that the observed change in rate in the reaction of **1** with **2a** must involve the complexed substrate.

In the reactions of **1** with **2e** at pH ≈ 9, the only product formed was **3**, and the observed rate constant increased with the concentration of β-CD. At pH ≈ 11, the products were **3** and **4**. Under these conditions, the value of $k_A = k_{obsd}f_A$ for the reactions with the L isomer decreases slightly as β-CD increases whereas it increases under the same conditions when the D isomer is the nucleophile, although these changes are barley outside experimental error. Besides, at this pH and with both isomers, the fraction of **4** decreases as β-CD increases.

We also studied the reactions of **1** with **2a** and **2g** in the presence of γ-cyclodextrin (γ-CD) (the member with eight glucose units) under the same conditions as those used with β-CD (Table III). For the reactions with the D and L isomers of **2a**, the catalysis was slightly lower than that observed with β-CD, whereas for the reactions with the D and L isomers of **2g**, it was significantly higher.

Discussion

Since the amino acids are amphoterics, it is important

(10) Bernasconi, C. F. *MTP Int. Rev. Sci.: Org. Chem., Ser. One* **1973**, *3*, 33.
(11) Supplementary material.
(12) de Rossi, R. H.; Barra, M.; de Vargas, E. B. *J. Org. Chem.* **1986**, *51*, 2157.

Table III. Observed Rate Constants for the Reactions of 1-Fluoro-2,4-dinitrobenzene with α-Amino Acids in the Presence of γ-Cyclodextrin[a]

	pH	$10^3[\gamma\text{-CD}]_0$,[b] M	10^4k_{obsd} s^{-1}	f_A[c]	$10^4k_{obsd}f_A$, s^{-1}	f_{CD}[d]
			D-Alanine			
1	10.761		1.23	1	1.23	
2	10.758	3.04	1.45	0.963	1.37	0.037
3	10.761	6.00	1.84	0.745	1.40	0.255
4	10.759	10.0	2.40	0.668	1.60	0.332
5	10.759	15.0	2.63	0.664	1.75	0.336
			L-Alanine			
6	10.757		1.08	1	1.08	
7	10.754	3.02	1.47	0.854	1.26	0.146
8	10.756	6.07	1.43	0.933	1.33	0.067
9	10.754	10.0	1.98	0.808	1.60	0.192
10	10.757	15.0	2.62	0.654	1.71	0.346
			D-Tryptophan			
11	10.595		6.85	1	6.85	
12	10.595	3.04	18.4	0.917	12.9	0.083
13	10.594	6.03	17.2	0.994	17.1	0.006
14	10.592	10.1	22.4	0.961	21.5	0.039
15	10.595	15.1	27.1	0.953	25.8	0.047
			L-Tryptophan			
16	10.595		7.00	1	7.00	
17	10.591	3.01	11.4	0.934	10.7	0.066
18	10.597	6.01	14.5	0.927	13.4	0.073
19	10.593	9.94	21.9	0.910	19.9	0.090
20	10.591	15.0	26.7	0.897	24.0	0.103

[a] Solvent DMSO–water (30%, v/v); μ = 0.2 M (NaCl); T = 20 °C; substrate concentration = (4–4.5) × 10^{-5} M; [amino acid]$_0$ = 1 × 10^{-3} M. The error in all rate constants is less than 10%. [b] γ-Cyclodextrin concentration. [c] Represents the fraction of the aminolysis product. [d] Represents the fraction of (2,4-dinitrophenyl)cyclooctaamylose.

to consider the various proton transfer equilibria involved (eq 2).

$$\underset{\mathbf{2H}}{RCH(NH_3^+)COOH} \underset{H^+}{\rightleftharpoons} \underset{\mathbf{2N}}{RCH(NH_2)COOH} \rightleftharpoons \underset{\mathbf{2Z}}{RCH(NH_3^+)COO^-} \underset{H^+}{\rightleftharpoons} \underset{\mathbf{2A}}{RCH(NH_2)COO^-} \quad (2)$$

It is known that in water solution the ratio **2Z**/**2N** is 10^5, namely, the concentration of **2N** under all conditions is very small compared to the others.[13] This ratio decreases to 10–100 in pure DMSO,[14] and this change was attributed to the fact that DMSO, as a good hydrogen-bond acceptor, stabilizes the ammonium group similarly as water does but it cannot stabilize the carboxylate group through hydrogen bonding.[15] This behavior can be exemplifed by comparing the transfer of $MeNH_3^+$ and PhCOOH from water to DMSO solution (eq 3 and 4).

$$MeNH_3^+\ pK_a\text{: } 10.64(\text{water})^{16} \rightarrow 11.0(\text{DMSO})^{17} \quad (3)$$

$$PhCOOH\ pK_a\text{: } 4.2(\text{water})^{16} \rightarrow 11.0(\text{DMSO})^{18} \quad (4)$$

We conclude that in 30% DMSO (which is the solvent used in our studies) the ratio **2Z**/**2N** must be $>10^2$; thus, under all our reaction conditions, the species to be considered are the zwitterion **2Z** and the anion **2A**. Besides, if any **2N** is present, its reactivity as a nucleophile must be lower than that of **2A** because the carboxylic group decreases the basicity of the amino group while the carboxylate group increases it.[19]

Both **2Z** and **2A** may form inclusion complexes with CD (eq 5 and 6), and if the values of the corresponding equilibrium constants K_5 and K_6 are different, a change in pH will be observed when CD is added to a solution containing **2A** and **2Z**.[20] This effect was observed when β-CD was added to a buffer solution of amines such as *n*-butylamine or piperidine.[7]

$$\mathbf{2Z} + \beta\text{-CD} \overset{K_5}{\rightleftharpoons} \mathbf{2Z}\cdot\beta\text{-CD} \quad (5)$$

$$\mathbf{2A} + \beta\text{-CD} \overset{K_6}{\rightleftharpoons} \mathbf{2A}\cdot\beta\text{-CD} \quad (6)$$

Since β-CD behaves as an acid of $pK_a \approx 12$,[21] the pH of the solution should decrease when β-CD is added, especially at pH's close to the pK_a value.[22] However, the overall change depends also on the relative values of the equilibrium constants for eq 5 and 6. The association equilibrium constant value for the inclusion complex between D-tryptophan and β-CD is 13 M^{-1} in water at pH = 9.8,[23] and we determined that in water K_5 and K_6 are smaller than 10 M^{-1} for all the amino acids studied.[24] Therefore, we can calculate that the changes in pH, when β-CD is added to solutions containing **2A** and **2Z**, should be mainly due to the cyclodextrin ionization. Since we have no data for the equilibrium constants in DMSO–

(13) *Chem. Abstr.* **1916**, *10*, 2167. Adams, E. Q. *J. Am. Chem. Soc.* **1916**, *38*, 1503.
(14) Hughes, D. L.; Bergan, J. J.; Grabowski, E. J. J. *J. Org. Chem.* **1986**, *51*, 2579.
(15) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1.
(16) Jencks, W. P.; Regenstein, J. *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, 1976; p J-150.
(17) Mucci, A.; Domain, R.; Benoit, R. L. *Can. J. Chem.* **1980**, *58*, 953.
(18) Ritchie, C. D.; Uschold, R. E. *J. Am. Chem. Soc.* **1968**, *90*, 2821.
(19) Edward, J. T.; Farrell, P. G.; Hallé, J.-C.; Kirchnerova, J.; Schaal, R.; Terrier, F. *J. Org. Chem.* **1979**, *44*, 615.
(20) Connors, K. A.; Lin, S.-F.; Wong, A. B. *J. Pharm. Sci.* **1982**, *71*, 217.
(21) Van Etten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3253.
(22) Excepting any inclusion phenomenon, the addition of 0.015 M β-CD to an aqueous solution of 10^{-3} M tryptophan at pH = 10.18 should produce a decrease of 0.15 pH units whereas at pH = 8.50 the decrease should be only 0.04 pH units. To estimate these pH changes, we used pK_a = 9.44 for tryptophan (*CRC Handbook of Chemistry and Physics*, 60th ed.; Weast, R. C., Ed.; CRC Press, Inc.: Boca Raton, FL, 1980; p D-161).
(23) Tabushi, I.; Kuroda, Y.; Mizutani, T. *J. Am. Chem. Soc.* **1986**, *108*, 4514.
(24) We used the competition method for this purpose as described in ref 7.

Table IV. Catalytic Factors for the Reactions of 1-Fluoro-2,4-dinitrobenzene with α-Amino Acids in the Presence of β-Cyclodextrin[a]

amino acid	C^b D isomer	C^b L isomer	hydrophobicity[c] value
alanine[d]	42 ± 12	50 ± 15	0.5
alanine[e]	30 ± 11	0	
alanine[f]	30 ± 4	36 ± 12	
methionine[g]	32 ± 9	33 ± 11	1.3
methionine[h]		36 ± 6	
valine[i]	27 ± 5	46 ± 6	1.5
leucine[j]	13 ± 7	20 ± 3	1.8
leucine[k]	16 ± 2	10 ± 1	
tyrosine[l]	7 ± 1	5 ± 1	2.3
phenylalanine[m]	5 ± 5	0	2.5
tryptophan[n]	0	3 ± 1	3.4
tryptophan[o]	18 ± 3	14 ± 3	
tryptophan[p]	150 ± 75	140 ± 35	

[a] Solvent DMSO–water (30% v/v); μ = 0.2 M (NaCl); T = 20 °C. [b] Catalytic factor defined as the ratio between the slope and intercept of a plot of k_{obsd} vs CD concentration, unless otherwise indicated (data from Table II). [c] Taken from: Nozaki, Y.; Tandorf, C. *J. Biol. Chem.* **1971**, *246*, 2211. [d] pH = 10.30. [e] pH = 8.72. [f] γ-Cyclodextrin instead of β-cyclodextrin was added; pH = 10.76 (data from Table III). [g] pH = 10.30. [i] pH = 10.72. [j] pH = 10.48. [k] pH = 8.71. [l] pH = 9.09. The catalytic factor was calculated as the ratio between the coefficient for the first-order term of the second-order polynomial and the independent term. [m] pH = 10.28. [n] pH = 10.58. [o] pH = 8.45. [p] γ-Cyclodextrin instead of β-cyclodextrin was added; pH = 10.59 (data from Table III).

water (30% v/v), we could not estimate the expected changes in pH for our experimental conditions. However, under conditions where **2A** is the predominant form, the pH of solutions of any of the amino acids studied decreases when β-CD is added in an amount similar to that calculated for an aqueous solution, whereas under conditions where **2Z** is predominant, the pH tends to increase slightly. These results indicate that $K_5 > K_6$ and that the values of K_5 and K_6 are as low as in water, so that there will not be a significant change in the relative amount of the reactive nucleophile when β-CD is added. It follows that the increase in rate observed in the reactions of **1** with **2** in the presence of CD is not due to an increase in the effective concentration of the nucleophile.

In order to compare the effect of CD on the reactions with all the amino acids, we defined the catalytic factor as the ratio between the slope and the intercept of the plots of k_{obsd} vs CD concentration. For the case of tyrosine, the observed rate constant was adjusted to a second-order polynomial and the catalytic factor was calculated as the ratio between the coefficient for the first-order term of the polynomial and the independent term. The values so obtained are collected in Table IV. In the last column of this table, the hydrophobicity values of the amino acids, as reported in the literature,[25] are indicated. We can see that the catalytic factor depends on the size of the amino acid, the size of the host, and the pH.

Comparing the data taken at high pH, where the predominant form of the amino acid is **2A**, we can see that the catalytic factor decreases as the size and the hydrophobicity value[25] of the amino acid increase. Besides, the catalytic factor is the same for both enantiomers of alanine, methionine, and leucine but it is different for valine, phenylalanine, and tryptophan. For the reactions with alanine, leucine, and tryptophan at pH ≈ 8.7, where the zwitterion is predominant, the kinetic discrimination for one of the enantiomers decreases as the hydrophobicity value of the amino acid increases.

(25) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1971**, *246*, 2211.

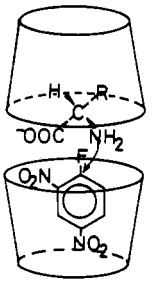


Figure 2.

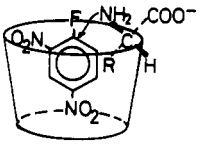


Figure 3.

As discussed above, the observed catalysis is not due to changes in the concentration of the nucleophile. On the other hand, since the reaction of 1-chloro-2,4,6-trinitrobenzene with alanine is not catalyzed by β-CD, the catalyzed pathway in the reactions of **1** must involve the complex between **1** and the cyclodextrin. The decrease in the catalytic factor and the increase in enantioselectivity with the size of the amino acid indicate that the catalysis also involves some kind of specific interaction between the nucleophile and the cyclodextrin. Thus, the reaction of **1** with **2** must involve the complexation of both reactants. One possibility is the reaction between the complexed amino acid and the complexed substrate (Figure 2).

The reaction between two complexed molecules may be the reason for the second-order dependence of the observed rate constant (k_{obsd}) with β-CD concentration when tyrosine is the nucleophile at pH ≈ 9. However, due to the low equilibrium constants, the amount of complexed amino acid is lower than 10% at the highest β-CD concentration and so this pathway should be very efficient to be observed. In fact, as was shown before, this is the major pathway of reaction when the nucleophile is an amine such as piperidine.[7] However, with all the other amino acids, the plots of k_{obsd} vs β-CD concentration for the reactions at pH ≈ 10–11 are linear. Under these conditions, a considerable fraction of β-CD is ionized, so, as we suggested in a previous work,[7] electrostatic repulsion could prevent the approximation of the two included reactants.

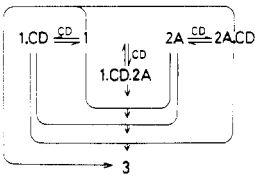
Another possible pathway is the formation of a ternary complex, where the substrate and the nucleophile are included in the same cavity of the cyclodextrin (Figure 3).

Ternary complexes are known in other systems and have been shown to facilitate bimolecular reactions.[26] Such complexes could be stabilized through hydrogen bonds between the carboxylate and the hydroxyl groups in the rim of CD, so the catalysis observed could be attributed to an increase in the time of permanence of the nucleophile close to the reacting center of the electrophile.[27] The cavity size of γ-CD (12 Å) is larger than that of β-CD (10.8 Å), and both hosts catalyze equally well the reactions with alanine (the smallest amino acid studied), but the former is a better catalyst than the latter in the reactions with tryptophan (the biggest one). These results are in good agreement with the formation of a ternary complex since γ-CD can fit **1** and **2** better than β-CD does. The increase

(26) (a) Kasatani, K.; Ohashi, M.; Kawasaki, M.; Sato, H. *Chem. Lett.* **1987**, 1633. (b) Schneider, H. J.; Sangwan, N. K. *J. Chem. Soc., Chem. Commun.* **1986**, 1787.

(27) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128.

Scheme I



in enantioselectivity with the size of the amino acid is also consistent with the involvement of a ternary complex. It was recently demonstrated that one of the requirements for chiral recognition is a tight fit of the included moiety within the β-CD cavity.[5] If a ternary complex is formed, a closer fitting will be expected with the largest amino acids, which is the case in the reactions with tryptophan. Besides, it has been observed that the association equilibrium constant value for a 2:1 inclusion complex increases 10^2–10^3 times with respect to the value for the 1:1 complex.[28] Considering that the association equilibrium constant for the complex between 1-fluoro-2,4-dinitrobenzene and β-cyclodextrin is $K_{CD} = 2 \times 10^3$ M^{-1},[12] the amount of a ternary complex would be significant.

Thus, we conclude that the overall mechanism of reaction can be described as shown in Scheme I.

In this scheme **1**·CD and **2A**·CD represent the inclusion complexes of the substrate and the anionic form of the amino acid, respectively, and **1**·CD·**2A** represents the ternary complex. The reaction may take place through all the pathways indicated, and the relative contribution of each of them depends on the different factors discussed above. However, it is not possible, with the data presented here, to determine all the elemental rate constants.

The changes in the catalytic factor and enantioselectivity with pH may be due in part to the change in the fraction of β-CD ionized. As we mentioned above, the reaction between the two complexed reactants at high pH could be limited by electrostatic repulsion, and so the contribution of this catalytic pathway will decrease as the pH increases. On the other hand, from the observed changes in pH we inferred that the zwitterionic form of the amino acid (which cannot act as a nucleophile) has more affinity for β-CD than the anionic one, in accordance with their relative hydrophobicities. A decrease in pH, which involves an increase in the concentration of the zwitterionic form, produces a significant relative increase in the concentration of **1**·CD·**2Z** (which cannot lead to product), and so the contribution of the catalytic pathway involving a ternary complex will decrease as pH decreases.

Conclusions

The rate of nucleophilic reactions between optically active α-amino acids **2** and the electrophile **1** is in most cases catalyzed by CD. The amount of catalysis depends on the pH, the size of the amino acid, and the size of the host. Comparing the data at high pH, where the anionic form of the amino acid is predominant, the kinetic discrimination for one of the enantiomers increases as the hydrophobicity value of the amino acid increases. The reverse order is observed at a pH where the zwitterionic form of the amino acid is predominant. At this pH the cyclodextrin is not ionized, while at high pH a significant fraction is negatively charged. We conclude that there are two catalytic pathways, one that involves the association between the complexed substrate and the complexed amino acid, and another in which both the reactant and the nucleophile are included in the cavity of one cyclodextrin molecule. We suggest that the contribution of the first catalytic pathway increases as pH decreases because of the decrease in the fraction of the β-CD ionized, whereas the second catalytic pathway decreases as pH decreases because of an increase in the concentration of the zwitterionic form of the amino acid. This species can also be included in the cavity of the cyclodextrin together with the substrate, but this ternary complex cannot lead to product.

The fact that some enantioselectivity is observed indicates that under the appropiate experimental conditions a racemic mixture of an α-amino acid could be resolved by reaction with 1-fluoro-2,4-dinitrobenzene.

Experimental Section

Aqueous solutions were made up from water purified in a Millipore apparatus. DMSO was distilled under vacuum and stored over molecular sieves (4 Å).

The β-cyclodextrin, α-amino acids, and soluble starch were purchased from Sigma and used as received. The γ-cyclodextrin (Aldrich) was a generous gift from Dr. F. Menger. 1-Fluoro-2,4-dinitrobenzene was distilled under vacuum.

The pH measurements were carried out on a Corning 101 digital pH meter at 20 °C. Standard buffers prepared according to procedures described in the literature[29] were used to calibrate the pH meter.

***N*-(2,4-Dinitrophenyl)amino Acids.** The *N*-(2,4-dinitrophenyl) derivatives of alanine, methionine, valine, leucine, phenylalanine, and tryptophan were prepared by a modified published method:[30] 1-fluoro-2,4-dinitrobenzene (1 mmol) dissolved in ethanol (saturated solution) was added dropwise to a saturated aqueous solution of the corresponding α-L-amino acid (1.1 mmol) containing 2.2 mmol of $NaHCO_3$. The mixture was stirred for 2 h in the dark and then acidified with HCl. Ethanol was removed under vacuum, and the mixture was allowed to cool slowly for 12 h. The product was filtered out, washed with cold water, then recrystallized, and dried under vacuum. The melting points were in good agreement with the literature data.[30] The ^1H NMR data obtained with a Varian T-60 spectrometer for the *N*-(2,4-dinitrophenyl)tryptophan are the following: δ_H (DMSO-d_6) 3.4 (2 H, d), 4.9 (1 H, m), 7.1 (6 H, m), 8.1 (1 H, m), 8.8 (2 H, m), and 10.8 (1 H, br absorption, RCOOH).

The *N*- and *O*-(2,4-dinitrophenyl) derivatives of tyrosine were obtained as follows: 1-fluoro-2,4-dinitrobenzene (1 mmol) dissolved in ethanol (saturated solution) was added dropwise to 250 mL of an aqueous solution of L-tyrosine (1.1 mmol, borax buffer, pH = 9.2). The mixture was stirred for 3 h in the dark, then acidified with HCl, and allowed to cool slowly for 12 h. The products were filtered out and separated by column chromatography on silica gel by elution with ethanol and methanol: 0.1596 g (67%) of *N*-(2,4-dinitrophenyl)tyrosine, mp 164 °C dec (lit.[31] mp 158–9 °C), and 0.0484 g (20%) of *O*-(2,4-dinitrophenyl)tyrosine, mp 177 °C dec (lit.[31] 201 °C dec). The mass spectral data obtained with a Finnigan Model 3300 mass spectrometer are the following. For the N derivative (70 ev): *m/e* (relative intensity) 74 (25), 107 (23), 167 (4), 240 (3), and 302 (5). For the O derivative (20 eV): 74 (56), 107 (39), 167 (10), 273 (56), 302 (16), and 384 (4).

Kinetic Procedures. The reactions were initiated by adding the substrate dissolved in DMSO to a solution containing all the other constituents. The total DMSO concentration was 30% (v/v). The reactions were run at 20 °C, at constant ionic strength (μ = 0.2 M), NaCl being used as compensating electrolyte, and at constant pH. The buffer was the same amino acid used as nucleophile.

Thermodynamic activation parameters were determined from the temperature dependence of the second-order rate constant

(28) Schiller, R. L.; Lincoln, S. F.; Coates, J. H. *J. Inclusion Phenom.* **1987**, *5*, 59.

(29) *Analytical Chemistry: The Working Tools*; Strouts, C. R. N., Gilfillan, J. H., Wilson, H. N., Eds.; Oxford University Press: London, 1958; Vol. II, Chapter 2, p 229.

(30) Rao, K. R.; Sober, H. A. *J. Am. Chem. Soc.* **1954**, *76*, 1328.

(31) Aonuma, S.; Mimura, T.; Morita, K.; Honda, H. *Yakugaku Zasshi* **1956**, *76*, 936.

for the reaction of 1 and alanine.[32] The second-order rate constants were obtained from the slope of the linear plots of the observed pseudo-first-order rate constants (measured at 10, 20, 25, and 30 °C) vs the concentration of the anionic form of the amino acid. Buffer solutions were prepared by adding NaOH to solutions of alanine, in the amount required to have equal concentrations of the anionic and zwitterionic forms of the amino acid.

In order to determine the effect of β- or γ-cyclodextrin, a series of solutions containing the same amount of amino acid and variable concentrations of β- or γ-cyclodextrin were prepared. The pH values were adjusted to that of the solution without cyclodextrin by adding a drop of diluted acid or base.

The observed rate constants (k_{obsd}) were determined by following the appearance of the aminolysis product. The change in optical density at the maximum absorption of the product was recorded during a kinetic run on a Beckman 24 spectrophotometer or on a Shimadzu 260 recording spectrophotometer, both with a thermostated cell compartment. All the reactions were run under pseudo-first-order conditions and were followed up to 80–90% conversion.

The yield of the aminolysis (f_A) and hydrolysis (f_H) products were determined as indicated before.[33] The yield of (2,4-dinitrophenyl)cycloheptaamylose (f_{CD}) was calculated as $f_{CD} = 1 - (f_A + f_H)$. In the reactions with tyrosine, the fraction $f = 1 - (f_A + f_H)$ corresponds to the sum of the yield of (2,4-dinitrophenyl)cycloheptaamylose and that of 4. The yield of the former was independently determined through a series of reactions carried out under the same experimental conditions but in the absence of tyrosine. Thus, the yield of *O*-(2,4-dinitrophenyl)tyrosine (f_O) was calculated as $f_O = 1 - (f_H + f_A + f_{CD})$.

Acknowledgment. This research was supported in part by the Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina (CONICET), and the Consejo Provincial de Investigaciones Cientificas y Tecnológicas de Córdoba, Argentina (CONICOR). M.B. is a grateful recipient of a fellowship from the CONICET.

Registry No. β-CD, 7585-39-9; γ-CD, 17465-86-0; D-Ala, 338-69-2; L-Ala, 56-41-7; D-Met, 348-67-4; L-Met, 63-68-3; D-Val, 640-68-6; L-Val, 72-18-4; D-Leu, 328-38-1; L-Leu, 61-90-5; D-Tyr, 556-02-5; L-Tyr, 60-18-4; D-Phe, 673-06-3; L-Phe, 63-91-2; D-Trp, 153-94-6; L-Trp, 73-22-3; *N*-2,4-DNP-L-Trp, 1655-51-2; *N*-2,4-DNP-L-Tyr, 10457-30-4; *O*-2,4-DNP-L-Tyr, 10567-73-4; 1-fluoro-2,4-dinitrobenzene, 70-34-8.

Supplementary Material Available: Table II containing the observed rate constants for the reactions of 1 with 2 at different β-cyclodextrin concentrations (6 pages). Ordering information is given on any current masthead page.

(32) Bunnett, J. F. *Techniques of Chemistry*; Lewis, E. S., Ed.; John Wiley & Sons: New York, 1974; Vol. 6, Chapter VIII, p 402.
(33) de Vargas, E. B.; de Rossi, R. H. *J. Org. Chem.* 1984, *49*, 3979.

Experimental and Theoretical Study on the Reactivity of Imidazo-1,2,6-thiadiazine 2,2-Dioxide Derivatives toward Electrophiles

Angela Herrero,[†] Carmen Ochoa,*[,†] Manfred Stud,[†] Feliciana Florencio,[‡] Irmina Hernández-Fuentes,[§] Cristina Abradelo,[§] and José Luis G. De Paz[∥]

Instituto de Química Médica, CSIC, 28006 Madrid, Spain, UEI de Cristalografía, Instituto de Química Física "Rocasolano", 28006 Madrid, Spain, Departamento de Química Física, Universidad Complutense, 28040 Madrid, Spain, and Departamento de Química Física, Universidad Autónoma de Madrid, 28049 Madrid, Spain

Received June 7, 1989

The differences found in the reactivity toward electrophiles of two dimethyl isomers, **1a** and **2a**, derived from 4-amino-1*H*,5*H*-imidazo[4,5-*c*]-1,2,6-thiadiazine 2,2-dioxide (**1e**) are reported. While 1,7-dimethyl isomer **2a** reacts with electrophiles, giving 6-substituted derivatives, 1,5-dimethyl isomer **1a** does not react. In order to find whether there are some geometrical or thermodynamic reasons for this behavior, experimental studies, including X-ray analysis and dipole moment measurements, as well as STO-3G* theoretical calculations have been carried out. Theoretical and experimental findings are in good agreement. No thermodynamic aspects seem to be responsible for the different behaviors, and so the difference must be due to kinetic factors.

In the course of the preparation of several imidazo-[4,5-*c*]-1,2,6-thidiazine 2,2-dioxides, we observed noticeable differences in the reactivity of dimethyl isomers **1a** and **2a** toward electrophiles. Nitration, which could be achieved by using HNO_3/H_2O mixtures, was the first reaction studied. In these conditions, the existence of nitryl cation (NO_2^+) is very unfavorable; because of this, and because of the complexity of nitration equilibria involving water,[1] a more simple aromatic electrophilic substitution, using an aromatic diazonium ion, was studied. In both cases, the behavior of **1a** and **2a** was similar: nitration and diazonium coupling reactions of 1,7-dimethyl derivative **2a** yielded the corresponding 6-substituted derivatives **2b** and **2c**, respectively, while the 1,5-dimethyl isomer **1a** did not react.

Due to these interesting features and in order to complete former studies[2] of these derivatives, the X-ray analysis of **1a** was carried out and measurements of dipole moments were made.

In an attempt to find if some thermodynamic aspects could explain the different reactivities, a theoretical study using ab initio calculations, at the STO-3G* level,[3] of six closely related structures (**1a**, **1d**, **1e**, **2a**, **2d**, and **2e**) was performed by using our VM/CMS version of the Gaussian

[†] Instituto de Química Médica.
[‡] Instituto de Química Física "Rocasolano".
[§] Universidad Complutense.
[∥] Universidad Autónoma de Madrid.

(1) Hoggett, J. G.; Moodie, R. B.; Penton, J. R.; Schofield, K. *Nitration and Aromatic Reactivity*; Cambridge University Press: Cambridge, 1971; pp 9–12.
(2) Smith-Verdier, P.; Florencio, F.; García-Blanco, S. *Acta Crystallogr.* 1977, *B33*, 2812–2816.
(3) Collins, J. B.; Schleyer, P. R.; Binkley, J. S.; Pople, J. A. *J. Chem. Phys.* 1976, *64*, 5142–5151.

0022-3263/89/1954-5025$01.50/0