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Steric Enhancement of Imidazole Basicity in *cis*-Urocanic Acid Derivatives: Models for the Action of Chymotrypsin

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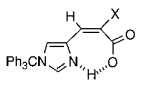
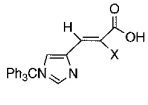
To test the hypothesis that substrate-induced steric compression between His 57 and Asp 102 at the active site of chymotrypsin can increase the basicity of His 57, we have synthesized the *cis*- and *trans*-isomers of 2-bromo-3-(*N*-tritylimidazole)-2-propenoic acid and 2-chloro-3-(*N*-tritylimidazole)-2-propenoic acid and compared selected properties with those of *cis*- and *trans*-urocanic acids. The *cis*-isomers display low field ^{1}H NMR signals at 17 ppm in dimethylsulfoxide, similar to *cis*-urocanic acid; whereas the *trans*-isomers do not show strong hydrogen bonds. Increasing the size of the C2 substituent (H < Cl < Br) in the *cis*-isomers increases the pK_a of the imidazolium group from 6.78 for H to 7.81 and 9.10 for Cl and Br, respectively; whereas the pK_as of the *trans* isomers are all 6.0 ± 0.1. The results indicate that the *cis*-urocanic acid derivatives with large substituents at C2 act as proton sponges in water, and they support the concept that steric compression in the catalytic triad of chymotrypsin can increase the basicity of His 57.

A new concept for the mechanism of general acid–base catalysis in serine proteases such as chymotrypsin has recently been proposed (*1*). The mechanism explains the function of the low barrier hydrogen bond (LBHB) between His 57 and Asp 102 (*2*, *3*). Chemical, spectroscopic, and X-ray crystallographic evidence indicate that a substrate-induced conformational change introduces steric compression between N$^{\delta 1}$ of His 57 and the β-carboxyl group of Asp 102. Strain is relieved by proton transfer from Ser 195 to N$^{\varepsilon 2}$ of His 57 and LBHB formation between N$^{\delta 1}$ of His 57 and Asp 102. Relief of strain by the LBHB can occur only when His 57 is protonated, so that this mechanism increases the basicity of His 57 and its effectiveness as a base in abstracating a proton from Ser 195. To test the hypothesis that steric compression can increase the basicity of imidazole, we have synthesized derivatives **1–4** of *cis*- and *trans*-urocanic acid, with chloro or bromo substituents at C2 of the side chain, and studied their properties.



1 X = Br
2 X = Cl



3 X = Br
4 X = Cl

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EXPERIMENTAL

Synthetic Protocols

N-Tritylurocanic acid, dibutylammonium salt (**6**). Urocanic acid 5 (10.0 g, 72.4 mmol) and trityl chloride (44.4 g, 159.3 mmol, 2.2 eq) were dissolved in 250 ml of *N*,*N*-dimethylformamide (DMF). Et_3N (54.0 ml, 39.2 g, 388 mmol, 5.4 eq) was added and the reaction was stirred for 14 h at room temperature. Methanol (250 ml) was added and the reaction was stirred for 24 h at 65°C. The solution was cooled to room temperature, diluted with 150 ml of ethyl acetate, and washed with 4×125 ml of 10% citric acid. The organic layer was condensed to 80 ml of a brown oil. Dibutylamine (13.0 ml, 10.0 g, 77.1 mmol, 1.1 eq) and 50 ml of ethylacetate was added. A white precipitate formed over 24 h. The solid was isolated and washed repeatedly with hexanes until pure by TLC (R_f = 0.8, 70% hexane/ethyl acetate) and dried under vacuum to give 36.7 g (quantitative yield) of white solid **6.** ^1H NMR (300 MHz, $CDCl_3$) 9.17 (br. s, 1H), 7.44 (d, J = 1.0 Hz, 1H), 7.35–7.31 (m, 9H), 7.28 (half of alkene doublet: other half obscured by 9H multiplet, 0.5H), 7.15–7.12 (m, 6H), 6.91 (d, J = 1.0 Hz, 1H), 6.49 (d, J = 15.8 Hz, 1H), 2.80 (t, J = 7.4 Hz, 4H), 1.67 (quintet, J = 7.4 Hz, 4H), 1.32 (sextet, J = 7.4 Hz, 4H), 0.88 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$, missing 1 quaternary C) 146.9, 143.9, 142.1, 129.7, 128.7, 128.1, 127.9, 127.7, 127.2, 126.9, 81.9, 47.6, 20.2, 13.7. LSIMS calculated for $C_{33}N_3O_2H_{39}$ M = 509 not observed; Ph_3C = 243.1, $C_6N_2O_2H_5$ = 136.0, $H_2N + Bu_2$ = 130.2.

Ethyl N-tritylurocanate (**7**). **6** (2.0 g, 3.93 mmol, 1 eq) was dissolved in 100 ml of $CHCl_3$. 2,4,6-Trichlorobenzoyl chloride (1.9 ml, 2.92 g, 12.0 mmol, 3 eq) and Et_3N (1.2 ml, 0.87 g, 8.6 mmol, 2.2 eq) were added and the reaction was stirred for 10 min. Ethanol (1.0 ml, 0.80 g, 17.4 mmol, 4.4 eq) and 4-dimethylaminopyridine (1.27 g, 10.4 mmol, 2.6 eq) were added and the reaction was stirred for 12 h. The $CHCl_3$ was reduced to about 10 ml *in vacuo* and the crude reaction mixture was chromatographed (SiO_2, 70% hexane/ethyl acetate) to give, after removal of solvent, 1.08 g (67% yield) of white solid. ^1H NMR (300 MHz, $CDCl_3$) 7.51 (d, J = 15.6 Hz, 1H), 7.47 (s, 1H), 7.37–7.32 (m, 9H), 7.16–7.11 (m, 6H), 6.53 (d, J = 15.6 Hz, 1 H), 4.21 (q, J = 7.4 Hz, 2H), 1.29 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 167.4, 141.9, 140.3, 137.2, 136.1, 129.7, 128.3–128.2 (large signal, 2 trityl C's), 123.8, 116.3, 75.7, 60.1, 14.3. LRMS calculated for $C_{27}N_2O_2H_{24}$ M = 408; found M = 408.

Ethyl 2,3-dibromo-3-(*N-tritylimidazole*) *propionate* (**8**). **7** (425 mg, 1.0 mmol) was dissolved in 30 ml CCl_4 and cooled to 0°C in an ice bath. Bromine (Br_2, 70 μl, 217 mg, 1.4 mmol, 1.3 eq) was added and the reaction was stirred for 1 h at 0°C. It was then warmed to room temperature and stirred for 1 h. The solvent was removed *in vacuo* to give 600 μl of pale yellow solid (quantitative yield). ^1H NMR (200 MHz, $CDCl_3$) 7.62 (br s, 1H), 7.40–7.34 (m, 9H), 7.16–7.11 (m, 6H), 6.92 (d, J = 1.5 Hz, 1H), 5.38 (d, J = 11.5 Hz, 1H), 5.14 (d, J = 11.5 Hz, 1H), 4.32 (q, J = 6.8 Hz, 2H), 1.34 (t, J = 6.8 Hz, 3H). LSIMS and HRMS and LRMS: dibromide decomposed rather than fragmenting in all cases. MS of bromoalkenes for which this compound is a precursor are provided.

Ethyl 2,3-dichloro-3-(*N-tritylimidazole*) *propionate* (**11, 12**). To condense Cl_2, a 15″ syringe needle was attached to a lecture bottle and inserted into an NMR tube in a dry ice/CH_3CN bath. The chlorine gas was bubbled into the tube until 100 μl of gas had been collected. The NMR tube was then rapidly inverted into 5 ml of precooled $CHCl_3$. Meanwhile, **7** (42 mg, 0.10 mmol) was dissolved in $CHCl_3$ and cooled to −45°C in a dry ice/CH_3CN bath. A stock $Cl_2/CHCl_3$ solution (480 μl, 1.1 eq) was added and the reaction was stirred for 2 h at −45°C. It was warmed to room temperature and solvent was removed *in vacuo.* A TLC (70% hexane/EtOAc) of the crude reaction product revealed two spots. Chromatography (SiO_2, 70% hexane/EtOAc) gave 8 mg of pure material ($R_f = 0.7$), 14 mg of pure material ($R_f = 0.6$), and 10 mg of a mixture of the two compounds (65% combined yield). The two products were assigned as the *syn-* and *anti*-diastereomers on the basis of elimination experiments in refluxing $CHCl_3$. The higher R_f material gave a mixture of products while the lower R_f material gave only the *trans*-urocanate derivative. Thus, the products result from *anti-* and *syn*-addition of Cl_2, respectively. In general, the diastereomeric mixture was carried on without separation. *anti*-Addition product: ^{1}H NMR (200 MHz, $CDCl_3$) 7.45 (d, $J = 1.5$ Hz, 1H), 7.37–7.32 (m, 9H), 7.15–7.10 (m, 6H), 6.91 (d, $J = 1.5$ Hz, 1H), 5.20 (d, $J = 10.3$ Hz, 1H), 4.89 (d, $J = 10.3$ Hz, 1H), 4.31 (q, $J = 6.8$ Hz, 2H), 1.37 (t, $J = 6.8$ Hz, 3H). *Syn*-addition product: ^{1}H NMR (200 MHz, $CDCl_3$) 7.40 (d, $J = 1.5$ Hz, 1H), 7.38–7.31 (m, 9H), 7.15–7.08 (m, 6H), 6.92 (br. s), 5.45 (d, $J = 6.5$ Hz, 1H), 5.00 (d, $J = 6.5$ Hz, 1H), 4.18 (q, $J = 7.5$ Hz, 2H), 1.24 (t, $J = 7.5$ Hz, 3H). Mixture of *syn-* and *anti*-dichlorides: LSIMS calculated for $C_{27}N_2O_2Cl_2H_{24}$ M = 478; found M + 1 = 479.1.

Ethyl 2-bromo-3-(*N-tritylimidazole*)*-2-propenate* (**9**). E_2 reaction conditions: **8** (115 mg, 0.20 mmol) was dissolved in 5.0 ml CH_2Cl_2 and cooled to −78°C in a dry ice/acetone bath. DBU (61 μl, 103 mg 0.68 mmol, 3.3 eq) was added and the reaction was stirred at −78°C for 10 min, until TLC (70% hexane/ethyl acetate) indicated no starting material remained. The reaction was warmed to room temperature and solvent was removed *in vacuo* to give 187 mg of brown oily solid which was a 20:1 mixture of isomers, as determined by NMR. Chromatography (SiO_2, 70% hexane/EtOAc) gave 75 mg of white solid (76% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 7.55 (br. s, 1H), 7.41 (d, $J = 1.1$ Hz, 1H), 7.35–7.33 (m, 9H-trityl, ^{1}H-alkene), 7.15–7.12 (m, 6H), 4.16 (q, $J = 7.2$ Hz, 2H), 1.20 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 164.0, 142.0, 138.9, 135.6, 134.8, 129.7, 128.2, 124.6, 107.5, 75.7, 61.9, 13.9. HRMS calculated for $C_{27}N_2O_2BRH_{23}$ M = 486.094, 488.092; found M = 486.094, 488.090.

Ethyl 2-bromo-3-(*N-tritylimidazole*)*-2-propenate* (**10**). β-Elimination reaction conditions: **8** (100 mg, 0.18 mmol) was dissolved in 2.5 ml $CHCl_3$. Et_3N (25 μl, 18.2 mg. 0.18 mmol, 1 eq) was added and the reaction was refluxed for 14 h. The solvent was removed *in vacuo* to give 100 mg of brown solid. Chromatography (SiO_2, 70% hexane/EtOAc) gave 25 mg of *cis* bromoalkene **9** and 32 mg of *trans* bromoalkene **10** (65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 8.28 (s, 1H), 7.90 (d, $J = 0.9$ Hz, 1H), 7.49 (d, $J = 0.9$ Hz, 1H), 7.34–7.30 (m, 9H), 7.15–7.10 (m, 6H), 4.26 (q, $J = 7.3$ Hz, 2H), 1.30 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.3, 141.8, 139.3, 135.8, 135.4, 129.5, 128.2, 128.0, 125.6, 110.1, 76.0, 62.3, 14.1. HRMS calculated for

$C_{27}N_2O_2BrH_{23}$ M − 486.094, 488.090; found M − 486.094, 488.090. X-Ray crystal structure was also obtained.

Ethyl 2-chloro-3-(N-tritylimidazole)-2-propenate (**13, 14**). A diastereomeric mixture of dichlorides **11** and **12** (370 mg, 0.77 mmol) was dissolved in 20 ml CH_2Cl_2 and cooled to −78°C in a dry ice/acetone bath. DBU (231 μl, 235 mg, 1.55 mmol, 2.0 eq) was added and the reaction was stirred for 2 h at −78°C. The reaction was warmed to room temperature and solvent was removed *in vacuo.* Chromatography (70% hexane/EtOAc) gave 65 mg (2% yield) of **13** and 200 mg (59% yield) of **14.** (**13**) ^{1}H NMR (300 MHz, $CDCl_3$) 7.74 (dd, J = 1.5, 0.8 Hz, 1H), 7.44 (d, J = 1.5 Hz, 1H), 7.37 − 7.32 (m, 9H), 7.25 (d, J = 0.8 Hz, 1H), 7.17 − 7.13 (m, 6H), 4.17 (q, J = 7.0 Hz, 2H), 1.20 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.2, 142.0, 138.8, 134.5, 132.8, 129.8, 129.3, 128.4, 125.4, 118.7, 75.8, 61.7, 14.0. (**14**) ^{1}H NMR (300 MHz, $CDCl_3$) 8.00 (s, 1H), 7.76 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 1.5 Hz, 1H), 7.37–7.32 (m, 9H), 7.17–7.14 (m, 6H), 4.28 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 163.0, 141.7, 139.3, 135.0, 131.8, 129.5, 128.0, 127.9, 125.8, 119.3, 75.9, 62.0, 14.1. HRMS of *cis*/*trans* mixture: Calculated for $C_{27}N_2O_2ClH_{23}$ M = 442.1448; Found 442.1433.

Ester Hydrolysis

Esters of the four *cis*- and *trans*-urocanates were all synthesized by same procedure, one example of which is given here.

2-Bromo-3-(N-tritylimidazole)-2-propenoic acid (**1**). **9** (20 mg, 0.04 mmol) was dissolved in 1.4 ml THF. LiOH · H_2O (5 mg, 0.12 mmol, 2.9 eq) was dissolved in 600 μl of H_2O and added to the THF solution. The reaction was stirred for 2 days at room temperature until no more starting material was present (TLC, 70% hexane/EtOAc). The THF was removed *in vacuo* and the aqueous solution was acidified to pH 7.0 using 3 N HCl. White precipitate formed and was filtered and washed with water. Drying for 24 h on a drying pistol with P_2O_5 (toluene) gave 18 mg (95% yield) of product. ^{1}H NMR (500 MHz, d_6-DMSO) 16.82 (s, 1H, COOH), 7.77 (s, 1H), 7.64 (s, 1H), 7.37 (m, 10H), 7.09 (m, 6H). ^{13}C NMR (125.8 MHz, d_6-DMSO, 1C overlapped) 173.6, 152.4, 149.2, 140.1 (large signal, trityl), 139.4 (large signal, trityl), 139.2 (large signal, trityl), 136.2, 117.2, 90.2, 86.7. HRMS calculated for $C_{25}N_2O_2BrH_{19}$ M = 459.3492 not observed: M-CO_2 = 414.0698 (calc 414.0687, M-Br-Co_2 = 334.1470 (calc 334.1471), Trityl group = 243.1121 (calc 243.1171).

(**2**) ^{1}H NMR (300 MHz, d_6-DMSO) 17.2 (s, 1H, COOH), 7.60 (s, 1H), 7.37 (m, 10H), 7.07 (m, 6H). ^{13}C NMR (125.8 MHz, D_2O) 161.6, 141.2, 138.2, 132.2, 129.1, 128.4, 128.3, 127.5, 125.8, 79.1, 75.9.

(**3**) ^{1}H NMR (500 MHz, d_6-DMSO) 7.85 (s, 1H, COOH), 7.71 (s, 1H), 7.48 (s, 1H), 7.41 (m, 10H), 7.10 (m, 6H).

(**4**) ^{1}H NMR (200 MHz, D_2O) 7.50 (br. s, 2H), 7.40 (s, 1H), 6.95 (m, 15H). ^{1}H NMR (500 MHz, d_6-DMSO) 7.50 (s, 1H), 7.42 (m, 10H), 7.12 (m, 6H). (C_2H and COOH exchanged out—not detected). ^{13}C NMR (125.8 MHz, d_6-DMSO) 166.0, 150.0, 147.8, 147.8, 143.1, 137.7, 137.5, 136.5, 134.3, 132.8, 83.7.

Evaluation of pK_as. The imidazolium pK_as of *trans*-urocanic acid and **1–4** were evaluated from potentiometric titration data obtained at 25°C by use of an iterative

computer program written by W. W. Cleland. For **1,** subtraction of pH increases caused by NaOH addition to H_2O was required. For **2–5,** this subtraction was unnecessary as the pK_a-values were below the region where NaOH interfered.

RESULTS AND DISCUSSION

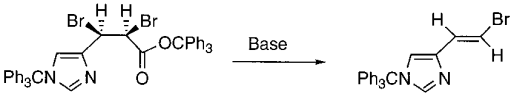
Synthesis of cis- and trans-2-halo-3-(N-tritylimidazole)-2-propenoic acids **1–4.** The synthesis of compounds **1–4** is shown in Scheme 1. *N*-Triphenylmethyl (trityl) protection of commercially available *trans*-urocanic acid **5** following the procedure described by Mutter and Hersperger gave **6** in quantitative yield (*4*).[1] Yamaguchi esterification *via* formation of the mixed anhydride using 2,4,6-trichlorobenzoyl chloride gave ethyl ester **7** in 70% yield (*5*).[2] Bromination of **7** afforded **8** (100%). Elimination of HBr at low temperature (−78°C) with DBU afforded an 80% yield of bromoalkenes **9** and **10,** with **9** formed as the major product (>20:1).[3] At higher temperatures (refluxing $CHCl_3$) with a weaker base (Et_3N) (*6*), the β-elimination product **10** was the major product while E_2 elimination to give **9** as the minor product (3:2; 65% combined yield). Esters **9** and **10** were separated and independently subjected to basic hydrolysis to give products **1** and **3** (95 and 100%; Scheme 1c).

The chlorides were obtained from **7** in a similar fashion (Scheme 1b). Chlorination of 7 gave a mixture of *syn*- and *anti*-addition products **11** and **12** (65%). Reaction of this mixture with DBU at −78°C gave a 1:3 mixture of **13** and **14** in 80% yield. E_2 elimination of HCl from **11** produced **13,** while E_2 elimination from **12** and β-elimination from **11** both produced **14.** Esters **13** and **14** were separated and hydrolyzed to afford **2** and **4** (both 80%; Scheme 1c).

Low field signals in the ^1H *NMR spectra of* **1–4.** The ^1H NMR spectra of **1–4** were obtained in DMSO-d^4. The chemical shift of the carboxylic acid proton in the spectra of both the chloro- and the bromo-substituted derivatives of *cis*-urocanic acid is about 17 ppm (**1,** 16.8 ppm; **2,** 17.2 ppm in DMSO). Low field signals have been used as a diagnostic of LBHBs (*7*), and such a signal was originally reported

[1] *N*-Trityl protection was required as bromination of dimethyl-protected **3** occurred exclusively on the imidazole ring.

[2] Esterifications using dimethyl sulfate or methyl iodide were unsuccessful, presumably due to the low solubility of **4** in appropriate solvents. The trityl ester was not suitable because exposure of the derived dibromide to base caused cleavage of the trityl group followed by loss of CO_2:



[3] X-ray crystal structures of **7, 8** and **12** were obtained to verify product assignments.

[4] In organic solvents such as $CDCl_3$ or d_6-DMSO, the pK_a of the carboxylic acid will be elevated relative to the aqueous pK_a, while the pK_as of positively charged acids such as imidazolium ions will be similar to their values in water. Thus, a low aqueous pK_a for the carboxylic acid should create a system which has been more highly optimized for LBHB formation. For further explanation, see reference [8] (and references cited therein).