concentrations of test substance. Angiotensin I liberated during the course of the reaction was measured by RIA with use of a commercial kit (Clinical Assays Travenol) according to the method of Haber.[49] An angiotensinase inhibitor, phenylmethanesulfonyl fluoride, was added to the reaction medium. For the monkey, dog, hog, and rat, plasma renin activity (PRA) was stimulated by furosemide. The results expressed as IC_{50} values (molar concentration of test product causing 50% inhibition of PRA) were determined by nonlinear regression (logit against log concentration) with five to seven concentrations of test substance. The reproductibility of the IC_{50} values was within ±5.8%, evaluated by using a weighted equation. Pepstatin was tested in each experiment in at least three concentrations near the IC_{50} value.

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Structure–Activity Study of 6-Substituted 2-Pyranones as Inactivators of α-Chymotrypsin

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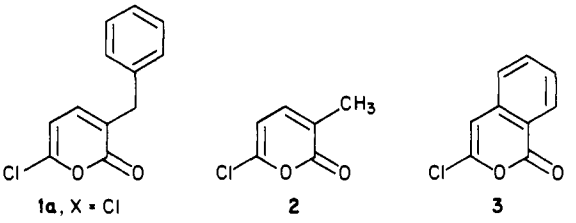
A series of 2-pyranones, bearing halogens or electron-withdrawing groups at the 6-position and alkyl, aryl, or aralkyl groups at positions 3, 4, and 5, were synthesized to investigate their binding to and inactivation of chymotrypsin. Both binding and inactivation by 2-pyranones are sensitive to substitutions on positions 3, 4, 5, and 6. Binding was poorest with alkyl substituents on position 3 and best with phenyl substitution, with benzyl or benzyl-like substitution falling in between. The sequence of binding of 6-substituted pyrones is Cl > Br > H > CF_3. 6-Chloro-2-pyranones bearing 4-phenyl or 3-(2-naphthylmethyl) substituents effected rapid inactivation of chymotrypsin, while those having 3-benzyl or 3-(1-naphthylmethyl) substituents gave slow inactivation and those with 3-phenyl or 3-alkyl substituents gave no inactivation. Only the 6-halopyrones demonstrated inactivation, with chloro-substituted ones acting faster than bromo-substituted ones.

A few 6-chloro-2-pyranones (**1a**, **2**),[1] including the benzopyranones of Powers[2] (**3**), have been reported to inactivate chymotrypsin. While the original premise in designing these inactivators was that the pyrones might act as mechanism-based (suicide) inactivators[1a]—they would then acylate an active-site nucleophile—their inhibition was found to be temporary, and recent evidence suggests that it is due to slow turnover of a stable acyl enzyme.[1b] Nevertheless, the 2-pyrone nucleus offers interesting possibilities as the basis for the development of mechanism-based inactivators, targeted toward serine proteases, and a structure–activity study of the system would be useful. However, little work addressing the binding requirements or the ring activation needed for substrate activity thus far has appeared.



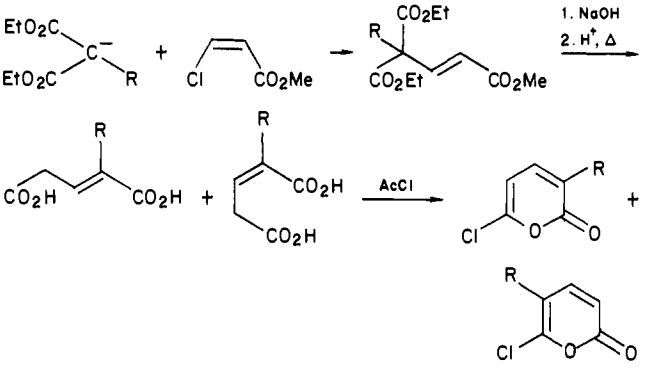
1a, X = Cl **2** **3**

In this report, we describe the preparation of a variety of 2-pyranones, substituted with alkyl, aryl, and aralkyl groups at carbons 3, 4, and 5 and bearing a hydrogen or halogen at carbon 6. We find that all of these substituents have a major effect on the binding affinity and inactivation potency of these pyrones toward chymotrypsin.

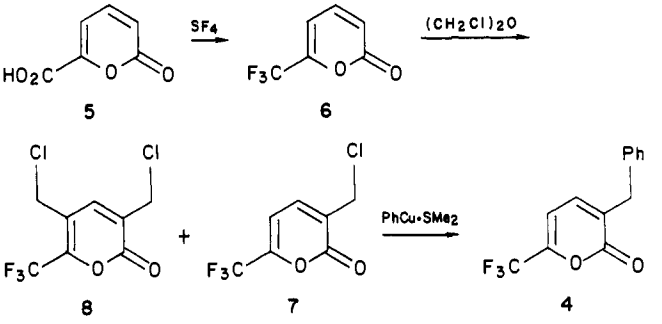
Results and Discussion

The structures of the substituted 2-pyrones prepared for this study are summarized in Table I.

Scheme I



Scheme II



5 **6** **8** **7** **4**

Chemistry. Substituted Glutaconic Acids. The synthesis of substituted 6-halo-2-pyranones is dependent upon the availability of similarly substituted glutaconic acid precursors.[3] It was necessary to modify the classical synthesis of these glutaconic acids to allow a wider range of pyrone substitutions (Scheme I): The appropriately substituted diethyl malonate sodium salt was heated at 80 °C with methyl *cis*-2-chloroacrylate[4] in THF; the re-

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(3) Conrad, M.; Guthzeit, M. *Ann.* **1883**, *222*, 261.

(4) (a) Kurtz, A. N.; Billups, W. E.; Greenlee, R. B.; Hamil, H. F.; Pace, W. T. *J. Org. Chem.* **1965**, *30*, 3141. (b) House, H. O.; Roelofs, W. L.; Trost, B. M. *J. Org. Chem.* **1966**, *31*, 646.

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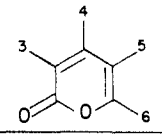
Table I. Summary of the Inhibition and Inactivation Properties of the Substituted 2-Pyranones toward Chymotrypsin[a]



compd	substituent 3	4	5	6	K_i, μM	k_{inact}, min^{-1}
1a	CH_2Ph			Cl	76	0.0027
1b	CH_2Ph			Br	112	0.0014
1c	CH_2Ph			H	160	NI
4	CH_2Ph			CF_3	927	NI
19a			CH_2Ph	Cl	50	0.00254
19b			CH_2Ph	Br	49	0.0197[b]
19c			CH_2Ph	H	295	NI
9	Et			Cl	785	NI
10			Et	Cl	5.6	0.00551
11	Bu			Cl	1980	NI
12			Bu	Cl	3.9	0.00217[c]
16a	Ph			Cl	0.27	NI
16b	Ph			H	160	NI
18a		Ph		Cl	382	0.769
18b		Ph		H	495	NI
17a			Ph	Cl	1.3	NI
17b			Ph	H	75	NI
13a	CH_2(2-Np)			Cl	625	0.0912
13b	CH_2(2-Np)			Br	d	0.0239
13c	CH_2(2-Np)			H	267	NI
15a	CH_2(1-Np)			Cl	93	0.00087
15b	CH_2(1-Np)			H	324	NI
14			CH_2(2-Np)	Cl	63	0.047

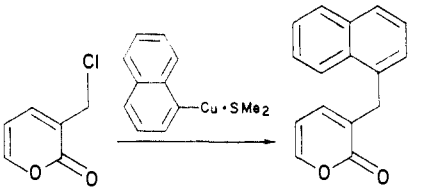
[a] The inhibitory and inactivating properties of the substituted pyrones toward chymotrypsin were determined as follows: Enzyme, exposed to different concentrations of the lactone, was periodically diluted into a chromogenic assay mixture and the rate of inactivation determined. The inactivation rate constant (k_{inact}) is the first-order rate constant describing the maximum rate of inactivation, attained at saturation. Where no time-dependent inactivation was apparent, NI is indicated in the column for k_{inact}. A constant for competitive inhibition (K_i) was determined by standard methods.[12] The rates of spontaneous hydrolysis (k_h) were all less than 0.0003 min^{-1}, except for lactone 18a, which has a k_h of 0.00144 min^{-1}. [b] Inhibitor showed a 10-min lag time. [c] Only 15% of the enzyme was inactivated; the balance of the enzyme was unaffected. [d] The compound was too insoluble to obtain reliable data.

sulting triester was saponified, acidified, and decarboxylated to give the correspondingly substituted glutaconic acid, often as a mixture of (synthetically equivalent) isomers.

Ring Closure. The classical method of preparing 6-chloro-2-pyranones from glutaconic acids by means of acetyl chloride could not be used in some cases; it proved more efficient to prepare these pyrones using thionyl chloride[5] or phosphorus pentachloride.[6] Where 6-bromo-2-pyranones were desired, phosphorus tribromide was used to effect the cyclization. All the 6-halopyrones were obtained as mixtures of 3- and 5-substituted isomers; these were found to be thermally interchangeable, with the equilibrium generally favoring the 3-substituted pyrone.[7] The bromopyrones appeared more sensitive to isomerization than the chloropyrones.[7]

In one case (**4**), a 6-CF_3 group was desired. This was prepared by reaction of 2-pyranone-6-carboxylic acid (**5**)[8] with sulfur tetrafluoride and hydrogen fluoride in methylene chloride at 100 °C (Scheme II). This gave a 65% yield of 6-(trifluoromethyl)-2-pyranone (**6**). Chloromethylation by the method of Shusherina[9] with bis(chloromethyl) ether and sulfuric acid at 75 °C gave an inseparable mixture of mono- and bis(chloromethyl)pyranones **7** and **8**. However, when the mixture was treated with phenylcopper–dimethyl sulfide in THF at 35 °C, only **7** reacted, giving the desired pyrone **4** and recovered **8**.

Scheme III



15b

The 6-protio-2-pyranones were easily prepared from the corresponding 6-chloro-2-pyranones by a zinc/acetic acid reduction, except **15b**, which was made by an alternate route due to the low yield of **15a** (Scheme III).

Chymotrypsin Inhibition and Inactivation Studies. The potential of these pyrones as time-dependent inactivators of chymotrypsin was assayed by exposing the enzyme to the pyrone and periodically assaying the surviving activity by dilution of an aliquot into a buffer medium containing a chromogenic substrate. This assay was repeated, using increasing concentrations of the pyrone, until a maximum rate of inactivation was obtained, the first-order rate constant describing this rate being k_{inact}. We have described a related application of this Kitz and Wilson[10] inactivation kinetics to chymotrypsin.[11] In all cases, the competitive inhibition constant (K_i) was determined by standard methods.[12] In the case of the compounds that show time-dependent inactivation, assays were conducted over a minimum period of time, to minimize the effect of the inactivation process. The spontaneous hydrolysis rates, measured as described previously,[11] are all very slow (cf. Table I, footnote *a*). These data are summarized in Table I.

The benzylpyrone **1a** was chosen as a reference compound. This pyrone resembles phenylalanine, a preferred substrate of chymotrypsin, and was prepared and assayed previously by Abeles.[1a] We noted some difference between our rate of inactivation with **1a** ($t_{1/2}$ = 256 min) and that reported by Abeles (80 min).[1a] For completeness, the 5-substituted pyranones were assayed as well, although it is believed that they inactivate chymotrypsin by a different mechanism.[1]

Binding (K_i). Although chymotrypsin prefers residues with a hydrophobic side chain, there is a tremendous variability in substituents that might be considered hydrophobic. Compared to the 3-benzylpyrone **1a**, alkyl substituents on position 3, as in the ethylpyrone **9** or the *n*-butylpyrone **11**, resulted in very poor binding (Table I). Increasing the hydrophobic binding area of **1a** by enlarging the aromatic region [(naphthylmethyl)pyrones **13a** and **15a**] does not improve binding; in the case of **13a**, the 3-(2-naphthylmethyl)pyrone, it is somewhat worse. How-

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(7) For a closely related isomerization, see: (a) Pirkle, W. H.; Seto, H.; Turner, W. V. *J. Am. Chem. Soc.* **1970**, *92*, 6984. (b) Turner, W. V. Ph.D. Dissertation, University of Illinois, Urbana, IL, 1975.
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(10) Kitz, R. J.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245.
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(12) Seigel, I. H. *Enzyme Kinetics*; Wiley-Interscience: New York, 1975.

ever, removal of the methylene of **1a** to give the rigid phenylpyrone **16a** results in a dramatic increase in binding, which is reflected by the 5-phenylpyrone **17a**. This molecule closely resembles biphenyl in size and shape but presents the lactone carboxyl group and the chlorine substituent. When biphenyl was assayed, it was found not to bind to chymotrypsin. Consequently, we believe that the pyrone carboxyl plays the major role in binding, perhaps in a manner analogous to a peptide. Exactly how the 3-substituent influences the binding process is not clear from this limited set, though the traditional "size and shape" arguments appear inadequate to explain the process. Direct phenyl substitution on the ring introduces the possibility of a π interaction between the two rings; it may be significant that the 3- and 5-phenylpyrones **16a** and **17a** bind relatively well, but binding is over 100-fold worse for the electronically different 4-phenylpyrone **18a**.

The nature of the 6-substituent of the pyrone also strongly affects the binding, which follows the sequence Cl > Br > H > CF_3. This apparently is not solely an electronegativity trend, as **4** (where $\sigma_m(CF_3) = 0.43$)[13] shows binding much worse than **1a** or even **1c** (where $\sigma_m(Cl) = 0.37$).[13]

Inactivation (k_{inact}). The inactivation of chymotrypsin is also highly sensitive to the 3- and 5-substitutions and shows no relationship to the strength of binding. The alkylpyrones **9** and **11** do not inactivate, nor do the strongly binding phenylpyrones **16a** or **17a**. This contrasts with the rapid inactivation ($t_{1/2}$ = 1.1 min) by **18a**, the electronically different 4-phenylpyrone, and **13a** ($t_{1/2}$ = 7.6 min), the 3-(2-naphthylmethyl)pyrone. The substitution on the naphthalene ring is apparently critical, as 3-(1-naphthylmethyl)pyrone **15a** shows barely measurable inactivation. In any case, the naphthalene ring produces low aqueous solubility, which results in a slow physical separation of the pyrone from solution if high proportions of Me_2SO (15%) are not used as a cosolvent.

The 6-bromo-2-pyranones **1b** and **13b** inactivate about half as fast as the corresponding chloropyrones **1a** and **13a**; the 6-protiopyrones **1c**, **13c**, **15b**, **16b**, and **18b** do not inactivate and are not substrates of chymotrypsin, consistent with Abeles' initial observations.[1a] Moreover, the 6-(trifluoromethyl)pyrone **4** does not inactivate nor act as a substrate. Apparently the ring activation for initial serine acylation is provided by the halogen, with the more electronegative chloride activating better than bromide. As with the binding, this is not solely an electronegativity effect, since the 6-(trifluoromethyl)pyrone **4** is not a substrate. The possibility that ring activation might involve a π interaction of the 6-substituent with the pyrone carboxyl rather than a σ inductive effect is intriguing and would explain the ineffectiveness of CF_3 as a substituent.

In all cases, enzyme that was inactivated by the 3- and 4-substituted pyrones regained activity with time. This was a rapid process in the case of **18a** ($t_{1/2}$ = 20 min), but slower for the other pyrones. The transient nature of the inactivation suggests that the lactones are acting simply as alternatant substrate inactivators, by forming relatively stable acyl enzymes. This has been confirmed, in the case of pyrone **1a**, in more recent studies by Abeles.[1b]

We noted that choice of the cosolvent used during the inactivation studies, especially if in proportions greater than 10%, can affect the results of the inactivation; in fact, we found that chymotrypsin actually showed time-dependent inactivation in solutions of 20% acetonitrile or acetone in buffer, such that after 24 h the activity remaining in the acetone solution was 27% and in the acetonitrile solution was 0%. To avoid this problem, Me_2SO, which caused no time-dependent inactivation, was used as a cosolvent for poorly soluble inhibitors.

Conclusion

Our studies on the binding of various substituted 2-pyranones to chymotrypsin and, in some cases, the resultant inactivation, indicate that these interactions are complex, being sensitive to both the nature and position of the hydrocarbon substituents at positions 3, 4, and 5 and the nature of the group at position 6.

Binding was poorest with alkyl substituents on position 3, and best with phenyl substitution, with benzyl or benzyl-like substitution falling in between. The sequence of binding affinity of 6-substituted pyrones is Cl > Br > H > CF_3. 4-Phenyl- or 3-(2-naphthylmethyl) substitution of the 6-chloro-2-pyrone gave compounds that resulted in rapid inactivation, while 3-benzyl or 3-(1-naphthylmethyl) substitution gave pyrones with slow inactivation and 3-phenyl or 3-alkyl substitution gave no inactivation. Only 6-halopyrones demonstrated inactivation, with chloro being faster than bromo.

Experimental Section

Chemistry. Proton magnetic resonance spectra were obtained on a Varian EM-390, XL-200, or an HR-220 spectrometer. Low-resolution mass spectra were run on a Varian MAT CH-5, and high-resolution mass spectra were run on a Varian 731 mass spectrometer, both by electron impact at 70 eV. Elemental analyses were performed by the Microanalytical Service of the University of Illinois School. Infrared spectra were run on either a Beckman IR-12 or a Nicolet 7199 FT IR spectrometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

Unless specified differently, the standard isolation procedure for the 6-halo- and 6-protio-2-pyranones is as follows: quenching the reaction mixture with water, extraction with methylene chloride, washing the extracts with 10% aqueous sodium bicarbonate solution, followed by drying over magnesium sulfate. The crude mixture was concentrated in vacuo and flash column chromatographed by the method of Still,[14] using methylene chloride.

3-Benzyl-6-chloro-2-pyranone (1a), 5-benzyl-6-chloro-2-pyranone (19a), and 3-benzyl-2-pyranone (1c) were prepared by the method of Abeles;[1a] spectra and analyses corresponded to those reported.

3-Ethyl-6-chloro-2-pyranone (9) and 5-ethyl-6-chloro-2-pyranone (10) were prepared under conditions reported for **9**.[15] A mixture of 2-ethylglutaconic acids (2.22 g, 14 mmol) prepared by the method of Guthzeit and Dressel[16] and acetyl chloride (20 mL) were combined in a sealed tube and heated at 100 °C for 12 h; the acetyl chloride was removed in vacuo. After product isolation, the faster running pyrone was sublimed at 50–70 °C (0.10 mm) to give **9** (0.4125 g, 18.6%) as an oil at room temperature: NMR ($CDCl_3$) δ 7.1 (1 H, d, J = 6 Hz, CH), 6.2 (1 H, d, J = 6 Hz, CH), 2.5 (2 H, q, J = 6 Hz, CH_2), 1.2 (3 H, t, J = 6 Hz, CH_3). Anal. ($C_7H_7ClO_2$) C, H.

The slower running pyrone was sublimed at 50–70 °C (0.10 mm) to give **10** (0.133 g, 6%): NMR ($CDCl_3$) δ 7.3 (1 H, d, J = 9 Hz, CH), 6.2 (1 H, d, J = 9 Hz, CH), 2.45 (2 H, q, J = 6 Hz, CH_2), 1.1 (3 H, t, J = 6 Hz, CH_3). Anal. ($C_7H_7ClO_2$) C, H.

3-*n*-Butyl-6-chloro-2-pyranone (11) and 5-*n*-Butyl-6-chloro-2-pyranone (12). To the anion from diethyl 2-*n*-butylmalonate (21.63 g, 0.10 mol) and pentane-washed sodium hydride (50% in oil, 4.8 g, 0.10 mol) in THF (120 mL) was added methyl *cis*-2-chloroacrylate (12.05 g, 0.10 mol). The resulting

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(14) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(15) (a) Thole, F. B.; Thorpe, J. F. *J. Chem. Soc.* **1911**, *99*, 2208. (b) *Ibid.* **1911**, *99*, 2187.

(16) Guthzeit, M.; Dressel, O. *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 3182.

mixture was heated at 80 °C for 12 h. After cooling, the reaction was quenched with water and extracted with ether. The ether extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and then concentrated in vacuo. The resulting triester was usually clean enough to saponify directly with sodium hydroxide (19 g, 0.475 mol) in water (150 mL) by refluxing the mixture for 8 h. The mixture was then cooled, adjusted to pH 1 with concentrated HCl, and then refluxed for 1 h further. After cooling, the mixture was extracted with ether. The extracts were dried over sodium sulfate and the solvent removed in vacuo to give an otherwise clean isomeric mixture of synthetically equivalent 2-*n*-butylglutaconic acids (17.56 g, 94%) that could not be separated by vapor-phase techniques or by chromatography: IR ($CHCl_3$) 3050 (br), 1705, 1655, 1274 cm^{-1}. Anal. ($C_9H_{14}O_4$) C, H.

The mixture of 2-*n*-butylglutaconic acids was treated with acetyl chloride as for the preparations of **9** and **10**, except they were not sublimed; **12** was easily converted thermally to **11**. Obtained **11** (45.1%), which was distilled at 96 °C (0.05 mm): NMR ($CDCl_3$) δ 7.0 (1 H, dt, J = 7 Hz, 0.5 Hz, CH), 6.15 (1 H, d, J = 7 Hz, CH), 2.4 (2 H, td, J = 8 Hz, 0.5 Hz, CH_2), 1.1–1.17 (4 H, m, CH_2), 0.9 (3 H, m, CH_3). Anal. ($C_9H_{11}O_2Cl$) C, H.

The pyrone **12** was obtained as an oil (14.1%): NMR ($CDCl_3$) δ 7.1 (1 H, d, J = 9 Hz, CH), 6.2 (1 H, d, J = 9 Hz, CH), 2.4 (2, m, CH_2), 1.2–1.7 (4 H, m, CH_2), 0.9 (3 H, m, CH_3). Anal. ($C_9H_{11}O_2Cl$) C, H.

3-(2-Naphthylmethyl)-6-chloro-2-pyranone (13a) and 5-(2-Naphthylmethyl)-2-pyranone (14). In the same manner as for **9** and **10**, the anion of diethyl 2-(2-naphthylmethyl)malonate[17] was added to methyl *cis*-2-chloroacrylate; saponification and decarboxylation of the resulting triester gave 2-(2-naphthylmethyl)glutaconic acid (46%) as white crystals, mp 201 °C. Anal. ($C_{16}H_{14}O_4$) C, H.

A mixture of 2-(2-naphthylmethyl) glutaconic acid (1.00 g, 3.70 mmol) and thionyl chloride (1.32 g, 11.1 mmol) was dissolved in 10 mL of benzene. To this was added one drop of DMF, and the mixture was heated at 60 °C for 3.5 h. It was then dried with a stream of nitrogen and the residue vacuum sublimed at 185 °C (0.15 mm) to give a crude mixture of the chloropyrones. These were separated by flash column chromatography, using methylene chloride on silica gel. First to elute was **13a** (0.297 g, 27.9%): mp 83 °C; NMR ($CDCl_3$) δ 7.5–7.8 (4 H, m, CH), 7.1–7.4 (4 H, m, CH), 6.75 (1 H, dt, J = 7 Hz, 1.5 Hz, CH), 5.95 (1 H, d, J = 7 Hz, CH), 3.80 (2 H, s, CH_2). Anal. ($C_{16}H_{11}O_2Cl$) C, H.

Eluting next was **14** (0.077 g, 7.7%): oil; NMR ($CDCl_3$) δ 7.2–7.85 (7 H, m, CH), 7.1 (1 H, d, J = 10 Hz, CH), 6.1 (1 H, d, J = 10 Hz, CH), 3.83 (2, s, CH_2). Anal. ($C_{16}H_{11}O_2Cl$) C, H.

2-Phenylglutaconic Acid. This compound was prepared from diethyl phenylmalonate and methyl *cis*-2-chloroacrylate in the same manner as the (naphthylmethyl)glutaconic acids: mp 168–169 °C; NMR (Me_2SO-d_6) δ 11–13 (2 H, br s, acid H), 6.95–7.50 (6 H, m, Ph, CH), 3.05 (2 H, d, J = 8 Hz, CH_2). Anal. ($C_{11}H_{10}O_4$) C, H.

3-Phenyl-6-chloro-2-pyranone (16a) and 5-Phenyl-6-chloro-2-pyranone (17a). These were formed in the same manner as for **9** and **10**, using 2-phenylglutaconic acid and acetyl chloride.

16a: mp 92 °C; 18%; NMR ($CDCl_3$) δ 7.3–7.7 (6 H, m, Ph, CH), 6.23 (1 H, d, J = 8 Hz, CH). Anal. ($C_{11}H_7ClO_2$) C, H.

17a: mp 85 °C; 2.6%; NMR ($CDCl_3$) δ 7.38 (1 H, d, J = 9 Hz, CH), 7.35 (5 H, m, Ph), 6.3 (1 H, d, J = 9 Hz, CH). Anal. ($C_{11}H_7ClO_2$) C, H.

4-Phenyl-6-chloro-2-pyranone (18a). 3-Phenylglutaconic acid[15b] (1.94 g, 9.42 mmol) and powdered phosphorus pentachloride (3.92 g, 18.8 mmol) were combined and swirled until the mixture liquified. It was then heated on a steam bath for 15 min, followed by cooling. After standard product isolation, **18a** was obtained (1.11 g, 56%), which was recrystallized from methylene chloride/hexane to give crystals: mp 61–62 °C; NMR ($CDCl_3$) δ 7.5 (5 H, s, Ph), 6.5 (1 H, d, J = 1.5 Hz, CH), 6.3 (1 H, d, J = 1.5 Hz, CH). Anal. ($C_{11}H_7O_2Cl$) C, H.

3-(2-Naphthylmethyl)-6-bromo-2-pyranone (13b). This compound was prepared in a similar manner as was the corresponding chloropyrone, **13a**, except phosphorus tribromide was substituted for the thionyl chloride. essentially all the 5-substituted isomer was converted to **13b** by warming above 75 °C; consequently, only **13b** was obtained (38.8%): mp 99 °C; NMR ($CDCl_3$) δ 7.6–7.85 (4 H, m, CH), 7.15–7.60 (3 H, m, CH), 6.73 (1 H, d, J = 7.5 Hz, CH), 6.15 (1 H, d, J = 7.5 Hz, CH), 3.85 (2 H, s, CH_2). Anal. ($C_{16}H_{11}O_2Br$) C, H.

3-(1-Naphthylmethyl)-6-chloro-2-pyranone (15a). The 2-(1-naphthylmethyl)glutaconic acid was prepared in a similiar manner as was the precursor to **13a**, using diethyl 2-(1-naphthylmethyl)malonate.[18] Use of thionyl chloride to effect the ring closure gave a low yield (8.2%) of **15a**: NMR ($CDCl_3$) δ 7.6–7.9 (3 H, m, Np), 7.30–7.55 (4 H, m, Np, CH), 6.5 (1 H, d, J = 7 Hz, CH), 5.9 (1 H, d, J = 7 Hz, CH), 4.2 (2 H, s, CH_2). Anal. ($C_{16}H_{11}O_2Cl$) C, H.

3-Benzyl-6-bromo-2-pyranone (1b) and 5-Benzyl-6-bromo-2-pyranone (19b). These compounds were prepared in a manner identical with that for **13b**; however, since no sublimation was performed, **19b** was also obtained.

1b: 12.6%; mp 93 °C; NMR ($CDCl_3$) δ 7.0–7.3 (5 H, m, Ph), 6.75 (1 H, d, J = 7 Hz, CH), 6.2 (1 H, d, J = 7 Hz, CH), 3.65 (2 H, s, CH_2). Anal. ($C_{12}H_9O_2Br$) C, H.

19b: 8%; oil; NMR ($CDCl_3$) δ 7.0–7.35 (5 H, m, Ph), 7.1 (1 H, d, J = 10 Hz, CH), 6.13 (1 H, d, J = 10 Hz, CH), 3.75 (2 H, s, CH_2). Anal. ($C_{12}H_9O_2Br$) C, H.

3-(2-Naphthylmethyl)-2-pyranone (13c), 3-Phenyl-2-pyranone (16b), 5-Phenyl-2-pyranone (17b), and 4-Phenyl-2-pyranone (18b). These compounds were prepared by a zinc/acetic acid reduction[1] of the corresponding chloropyrone (except for **17b**, which was coproduced with **16b**).

13c: 20.4%; NMR ($CDCl_3$) δ 7.55–7.90 (4 H, m, Np), 7.2–7.5 (4 H, m, Np, CH), 6.85 (1 H, dd, J = 8.5 Hz, 0.5 Hz, CH), 6.05 (1 H, dd, J = 8.5 Hz, 1 Hz, CH), 3.90 (2 H, s, CH_2). Anal. ($C_{16}H_{12}O_2 \cdot 0.5H_2O$) C, H.

16b: 45%; mp 102–103 °C; NMR ($CDCl_3$) δ 7.6 (1 H, d, J = 7 Hz, CH), 7.55 (1 H, d, J = 5 Hz, CH), 7.2–7.5 (5 H, m, Ph), 6.2 (1 H, dd, J = 7 Hz, 5 Hz). Anal. ($C_{11}H_8O_2$) C, H.

17b: 18.3%; mp 59–58.1 °C; NMR ($CDCl_3$) δ 7.6 (1 H, s, CH), 7.5 (1 H, dd, J = 9 Hz, 3 Hz, CH), 7.3 (5 H, s, Ph), 6.3 (1 H, dd, J = 9 Hz, 1.5 Hz, CH). Anal. ($C_{11}H_8O_2$) C, H.

18b: 75.6%; mp 72–72.5 °C; NMR (acetone-d_6) δ 7.5–7.8 (3 H, m, Ph), 7.3–7.5 (3 H, m, Ph, CH), 6.70 (1 H, dd, J = 5.8 Hz, 2 Hz), 6.50 (1 H, dd, J = 2 Hz, 0.8 Hz). Anal. ($C_{11}H_8O_2$) C, H.

3-(1-Naphthylmethyl)-2-pyranone (15b). The Grignard from 1-naphthyl chloride (1.58 g, 7.62 mmol) and magnesium (0.186 g, 7.62 mmol) was prepared in 10 mL of dry THF and cooled to 0 °C under nitrogen. With rapid stirring, CuBr-SMe_2 (1.57 g, 7.62 mmol) was added. After 10 min, 3-(chloromethyl)-2-pyranone[9] (0.92 g, 6.34 mmol) in 10 mL of dry THF was added. After 2 h at 0 °C, the mixture was warmed to room temperature overnight. After standard product isolation, **15b** (41%) was obtained. Recrystallization from ether/hexane gave crystals: mp 118–118.5 °C; NMR ($CDCl_3$) δ 7.65–7.95 (3 H, m, Np), 7.05–7.60 (6 H, m, Np, CH, CH), 6.2 (1 H, dd, J = 6.75 Hz, 0.5 Hz, CH), 4.0 (2 H, s, CH_2). Anal. ($C_{16}H_{12}O_2$) C, H.

6-(Trifluoromethyl)-2-pyranone (6). 2-Pyranone-6-carboxylic acid[8] (25 g, 0.178 mol) was suspended in 50 mL of methylene chloride in a stainless-steel bomb. The bomb was sealed and cooled to −78 °C. HF (100 mL) was distilled in, followed by sulfur tetrafluoride (40 g, 0.37 mol). The bomb was rocked and heated at 120 °C for 4 h, after which it was cooled and vented. More methylene chloride was added and the solution was washed with saturated sodium bicarbonate until neutral; it was then dried over magnesium sulfate. The solvent was removed in vacuo and the residue distilled at 60 °C (2.5 mm) to give **6** (20.86 g, 71.5%): NMR ($CDCl_3$) δ 7.48 (1 H, dd, J = 10 Hz, 7 Hz, CH), 6.70 (1 H, d, J = 7 Hz, CH), 6.50 (1 H, d, J = 10 Hz, CH). Anal. ($C_6H_3F_3O_2$) C, H.

3-(Chloromethyl)-6-(trifluoromethyl)-2-pyranone (7) and 3,5-Bis(chloromethyl)-6-(trifluoromethyl)-2-pyranone (8). A mixture of **6** (10.0 g, 61 mmol), bis(chloromethyl) ether (28 g, 0.224 mol), and concentrated sulfuric acid (20 mL) was stirred

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at 85 °C under a reflux condenser. After 15 h, the reaction mixture was cooled, diluted with water, and extracted with chloroform. The chloroform extracts were washed with 10% sodium bicarbonate, treated with Norit to remove tar, and then dried over magnesium sulfate. After concentration in vacuo, the crude product was flash column chromatographed with 30% pentane in methylene chloride on silica gel. The major component (single spot by TLC) was a mixture of 7 and 8; we were unable to find a satisfactory method (chromatographic or gas phase) to resolve the two components, so the compounds were both partially characterized and employed in the next step as a mixture: 4.5 g; 7:8 = 1:0.875.

7: NMR ($CDCl_3$) δ 7.55 (1 H, d, J = 7 Hz, CH), 6.66 (1 H, d, J = 7 Hz, CH), 4.4 (2 H, s, CH_2); HRMS ($C_7H_4ClF_3O_2$).

8: NMR ($CDCl_3$) δ 7.6 (1 H, s, CH), 4.45 (4 H, s, CH_2); HRMS ($C_8H_5Cl_2F_3O_2$).

3-Benzyl-6-(trifluoromethyl)-2-pyranone (4). The phenyl Grignard formed from phenyl bromide (2.40 g, 15.3 mmol) and magnesium (0.372 g, 15.3 mmol) in THF (50 mL) was cooled to 0 °C under nitrogen. CuBr–SMe_2 (3.14 g, 15.3 mmol) was sifted in and the mixture stirred for 15 min. Then, the mixture of 7 and 8 (4.50 g of a 0.875:1 mixture of 8 to 7; 10.2 mmol of 7) in 10 mL of THF was added as rapidly as possible. The mixture was then slowly warmed to 50 °C for 4 h. The reaction was then quenched with saturated sodium chloride and extracted with ether. The ether extracts were washed with acidic saturated sodium chloride and dried over magnesium sulfate, and the solvent was removed in vacuo. The crude material was chromatographed first with 40% pentane in methylene chloride on silica gel to remove biphenyl and 8; the fractions containing 4 were combined and rechromatographed with 5% ethyl acetate in hexane, giving pure 4 (0.8492 g, 32.8%) as an oil: NMR ($CDCl_3$) δ 7.1–7.3 (5 H, m, Ph), 6.95 (1 H, d, J = 6 Hz, CH), 6.47 (1 H, d, J = 6 Hz, CH), 3.70 (2 H, s, CH_2). Anal. ($C_{13}H_9F_3O_2$) C, H.

Biochemistry. The inactivation assays were performed on a Varian 635 UV–vis double-beam spectrometer or a Hewlett-Packard 8451A diode array single-beam spectrometer. α-Chymotrypsin (three times crystallized and free of autolysis products and low molecular weight contaminants) was obtained from Worthington Biochemical. *N*-Benzoyltyrosine ethyl ester (BTEE) was obtained from Sigma. *N*-Acetyltyrosine *p*-nitroanilide was prepared according to Bundy.[19] *N*-Acetyltyrosine *p*-nitrophenyl ester[20] was obtained from Sigma. The phosphate buffer used in the inactivation studies and competitive assays was 0.1 M KH_2PO_4/K_2HPO4, pH 7.5.

Time-Dependent Loss of Activity Assay. A 5 mM stock solution of the inhibitor was prepared in Me_2SO, and depending upon solubility limits, 25–50 μL of this solution was added to 1.9 μM chymotrypsin (0.950–0.975 mL) in buffer at 25 °C. A 50-μL aliquot was removed from this mixture starting from t = 0, and at regular intervals afterward to determine the activity. The activity was determined by adding the aliquot to 0.95 mL of a 50 mM solution of BTEE in 15% acetonitrile/buffer. For 18a, the cosolvent of BTEE was changed to Me_2SO. The rate of hydrolysis was followed at 256 nm and the percent activity at time t found by dividing the slope of the hydrolysis curve by that of t = 0. The assay was repeated with increasing concentrations of inhibitor until no further increase in rate of inactivation was observed. The exception was 18a, whose rapid inactivation and reactivation of enzyme required the simultaneous substrate–inhibitor assay of Main,[21] as described by Katzenellenbogen.[11]

Competition Assays. Except for 18a, time-dependent inhibition was sufficiently slow to allow standard competitive inhibition assays to be performed. The competitive K_i was determined for each compound, using either *N*-acetyltyrosine *p*-nitrophenyl ester or *N*-acetyltyrosine *p*-nitroanilide in 15% Me_2SO/buffer, depending upon the UV absorbances of the inhibitor. The K_m of the substrate was determined each time a competitive inhibitor was tested, and the assays were run in triplicate.

Hydrolysis Assays. Where feasible, the hydrolysis constants were determined by the loss of the pyrone chromophore (290–315 nm).[1]

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Supplementary Material Available: Full analytical compound characterizations (5 pages). Ordering information is given on any current masthead page.

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Analogues of Substance P. Peptides Containing D-Amino Acid Residues in Various Positions of Substance P and Displaying Agonist or Receptor Selective Antagonist Effects

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Agonist and antagonist analogues of substance P were synthesized by replacing at least two of the amino acid residues with D-Trp, D-Phe, D-Val, or D-Pro residues. The syntheses of these compounds were achieved by solid-phase methodology using the hydroxymethyl resin. The analogues were tested for agonist and antagonist activity on guinea pig ileum and rat spinal cord preparations. Two types of antagonists were obtained. The first type of compounds, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (1), antagonized SP and SP(6–11)-hexapeptide on the ileum but only SP(6–11)-hexapeptide on the spinal cord. The second type of antagonists, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe (17), were inactive on the ileum but were potent antagonists of the hexapeptide on the spinal cord. Two of the antagonists, [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP (3) and [D-Trp^{7,8,9}]-SP (43), were also tested in vivo. Both of these depressed hypotensive responses to SP and SP(6–11)-hexapeptide in rabbits.

Substance P (SP) has been implicated in the transmission of primary afferent nociceptive signals to the dorsal horn of the spinal cord.[1] It has therefore been hypothesized that antagonism of its actions on dorsal horn

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