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Synthesis and Structure–Activity Study of Protease Inhibitors. II.^{1,2)} Amino- and Guanidino-Substituted Naphthoates and Tetrahydronaphthoates

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Various amino- and guanidino-substituted naphthoates and tetrahydronaphthoates were synthesized and evaluated for inhibitory activities against trypsin, plasmin, kallikrein, thrombin and Cl esterase. Among these compounds, phenyl 4-guanidino-1-naphthoate (IIIj) and phenyl 6-guanidino-1-naphthoate (IIIi) exhibited potent and selective trypsin inhibition (IC_{50} : 4×10^{-7} and 5×10^{-8} M, respectively) and phenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vb) and *p*-ethoxycarbonylphenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vg) had selective inhibitory activities against thrombin (IC_{50} : 4×10^{-5} and 1×10^{-5} M, respectively).

Keywords—enzyme inhibition; trypsin; plasmin; kallikrein; thrombin; Cl esterase; guanidinonaphthoate; guanidinetetrahydronaphthoate; aminonaphthoate; aminotetrahydronaphthoate

Serine-proteases play important roles in the homeostasis of the living body, but anomalous activation is known to be involved in the pathogenesis of various diseases. Because of these facts, the development of synthetic serine-protease inhibitors has been a goal of many investigators.^{3a-e)} Phenyl *p*-guanidinobenzoate^{1,3a)} and *p*-nitrophenyl *p*-guanidinobenzoate^{3b)} are well-known as potent serine-protease inhibitors and trypsin titrants. Moreover, Tamura *et al.*^{3c)} reported that *N,N*-dimethylamino *p*-(*p*'-guanidinobenzoyloxy)benzylcarbonyloxy glycolate has strong inhibitory activity against trypsin and Muramatsu *et al.*^{3d)} reported that *trans*-4-(guanidinomethyl)cyclohexanecarboxylic acid esters inhibit the esterolytic activity of chymotrypsin.

We have also been interested in synthetic serine-protease inhibitors, and we have synthesized various guanidino ester derivatives and evaluated their effects on serine-proteases. In the previous paper,¹⁾ we reported that aryl esters (Ia, b) of α -ethyl-*p*-guanidinocinnamic acid inhibited Cl esterase potently and selectively. As a continuation of this work, we have synthesized various aminonaphthoates (IIa–h), guanidinonaphthoates (IIIa–l), aminotetrahydronaphthoates (IVa–g) and guanidinetetrahydronaphthoates (Va–g) and examined their inhibitory activities against trypsin, plasmin, kallikrein, thrombin, and Cl esterase. It was found that phenyl 4-guanidino-1-naphthoate (IIIj) and phenyl 6-guanidino-1-naphthoate (IIIi) exhibited potent and selective trypsin inhibition (IC_{50} : 4×10^{-7} and 5×10^{-8} M, respectively) and phenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vb) and *p*-ethoxycarbonylphenyl 7-guanidino-1,2,3,4-tetrahydronaphthoate (Vg) had selective inhibitory activities against thrombin (IC_{50} : 4×10^{-5} and 1×10^{-5} M, respectively).

In this paper, we describe the synthesis and serine-protease inhibitory activities of these compounds, and discuss the structure-activity relationships.

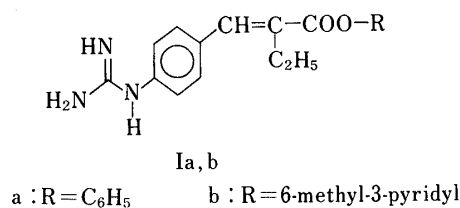


Chart 1

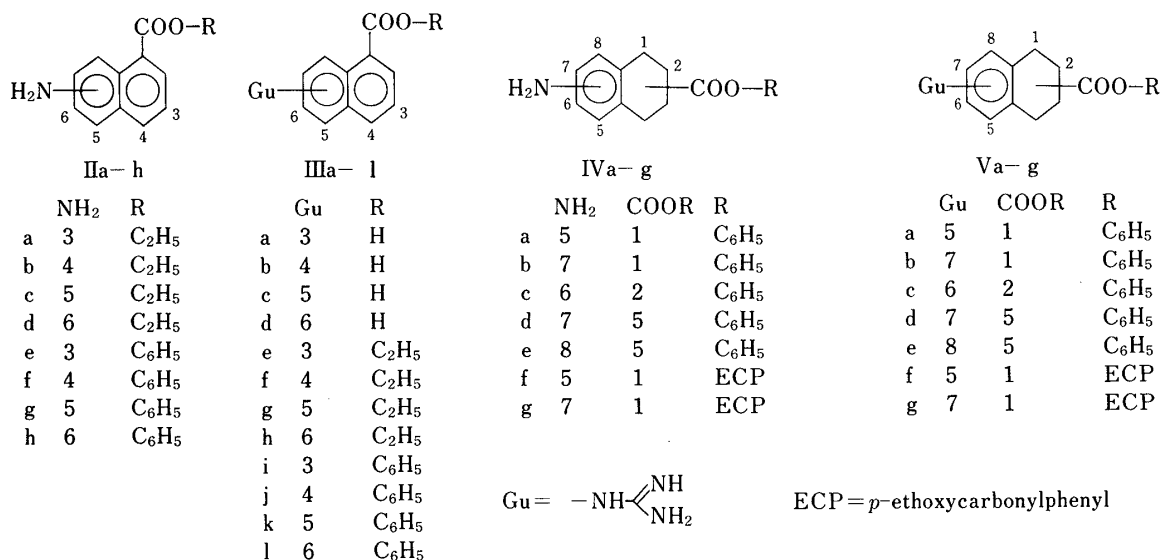


Chart 2

Synthesis

As shown in Chart 2, we synthesized various ethyl and phenyl esters of amino- and guanidino-substituted naphthoic and tetrahydronaphthoic acids as well as free guanidino-naphthoic acids and *p*-ethoxycarbonylphenyl esters of amino- and guanidino-tetrahydronaphthoic acids. In principle, as illustrated in Chart 3, nitro acids were converted to esters, followed by reduction with hydrogen and palladium on a carbon (Pd-C) catalyst to obtain amino-esters and these were further converted to guanidino-esters by reaction with cyanamide.

Nitro-acids (VIb, d, VIIa-f)^{4b-f)} were prepared by nitration of the corresponding naphthoic acid (IX) and tetrahydronaphthoic acids (X-XII), except for VIa, c, ^{4a)} which were obtained as a mixture by hydrolysis of 3-nitro-1,8-naphthalic anhydride (VIII) followed by decarboxylation. Separation of the mixtures of nitro-acids to obtain each isomer was performed either with or without esterification. Thus, VIb and VIc were separated directly from the mixture by recrystallization, while the mixtures of VIa, c and of VIId-f were each converted to esters, followed by chromatographic separation of the components on a silica-gel column, and VIIa and VIIb were obtained by conversion of the mixture to *p*-methoxycarbonylphenyl esters (XIIIa, b), which were separated by recrystallization and then hydrolyzed.

Esterification was achieved either with EtOH/H₂SO₄ to obtain ethyl nitro-esters (VIe-g) or by means of acid chloride formation with PCl₅ to prepare phenyl or *p*-ethoxycarbonylphenyl nitro-esters (VIh-j, VIIg-n). Esterification of 8-nitro-1-naphthoic acid (VIId) by these procedures was unsuccessful. An attempt to prepare a corresponding amino-ester from phenyl 6-nitro-1,2,3,4-tetrahydro-5-naphthoate (VIIj) by reduction with hydrogen on

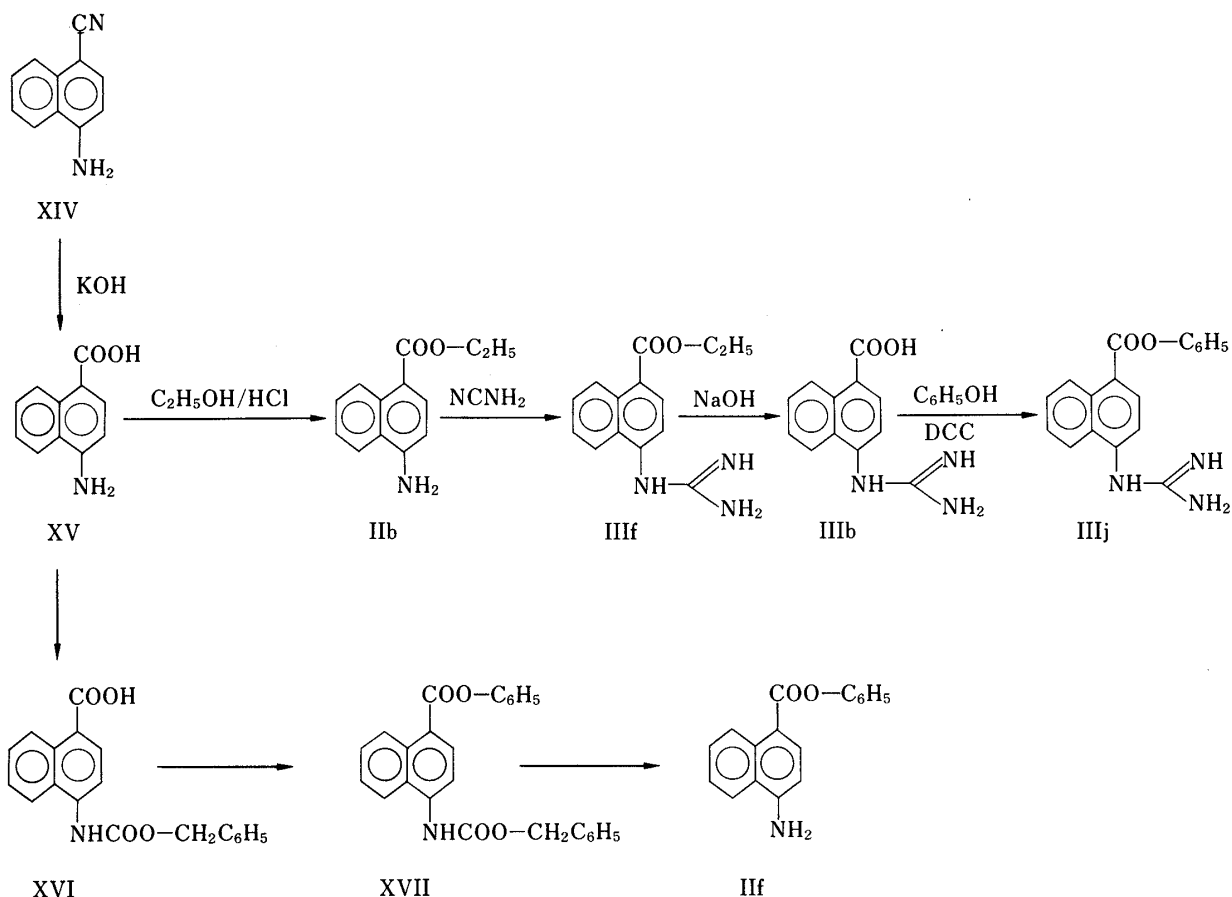


Chart 5

Pd-C also failed.

Free guanidino-acids (IIIa, c, d) were obtained from the corresponding ethyl guanidino-esters (IIIe, g, h) by hydrolysis in aqueous NaOH.

Derivatives of 4-amino and 4-guanidino-1-naphthoic acid were synthesized by a separate route starting from 4-amino-1-naphthalenecarbonitrile (XIV). Compound XIV was hydrolyzed in aqueous KOH to give 4-amino-1-naphthoic acid (XV) which was converted, in turn, to the ethyl amino-ester (IIb) and the ethyl guanidino-ester (IIIc) by reaction with cyanamide, to the free acid (IIIb) by hydrolysis and to the phenyl guanidino-ester (IIIj) by treatment with dicyclohexylcarbodiimide (DCC). The phenyl amino-ester (IIf) was obtained by benzyloxycarbonylation (XVI) of XV with benzyloxycarbonyl chloride, conversion of XVI to the phenyl ester (XVII) *via* the acid chloride and subsequent reduction with hydrogen on Pd-C.

Protease Inhibitory Activities

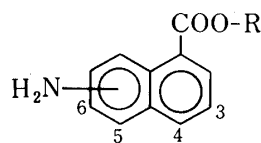
The inhibitory activities against trypsin, plasmin, kallikrein and thrombin were determined as described in the previous paper and expressed similarly in terms of IC_{50} , the molar concentration inhibiting 50% of the activity of enzymes to hydrolyze the designated substrate.

The potency of compounds whose inhibitory activities correspond to IC_{50} of less than $1 \times 10^{-3} \text{ M}$ is expressed as the percent inhibition at a concentration of $1 \times 10^{-3} \text{ M}$.

Results and Discussion

The results are shown in Tables I–IV. It can be concluded that the ester linkage is

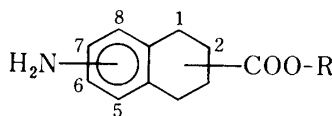
TABLE I. Enzyme Inhibition by Aminonaphthoates



Compd. No.	NH ₂	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IIa	3	C ₂ H ₅	<10 ^{a)}	<10	<10	<10	
IIb	4	C ₂ H ₅	3 × 10 ^{-4 b)}	<10	<10	11	
IIc	5	C ₂ H ₅	11	<10	<10	11	
IId	6	C ₂ H ₅	19	<10	32	<10	
IIe	3	C ₆ H ₅	8 × 10 ⁻⁴	<10	<10	<10	<10
IIf	4	C ₆ H ₅	2 × 10 ⁻⁴	<10	<10	<10	<10
IIg	5	C ₆ H ₅	9 × 10 ⁻⁴	<10	<10	<10	<10
IIh	6	C ₆ H ₅	5 × 10 ⁻⁵	<10	<10	31	<10

a) % inhibition at a concentration of the compound of 1 × 10⁻³ M. b) IC₅₀.

TABLE II. Enzyme Inhibition by Aminotetrahydronaphthoates



Compd. No.	NH ₂	COOR	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IVa	5	1	C ₆ H ₅	38 ^{a)}	<10	<10	22	
IVb	7	1	C ₆ H ₅	1 × 10 ^{-3 b)}	<10	<10	3 × 10 ⁻⁴	
IVc	6	2	C ₆ H ₅	8 × 10 ⁻⁵	<10	<10	24	
IVd	7	5	C ₆ H ₅	4 × 10 ⁻⁴	<10	<10	<10	<10
IVe	8	5	C ₆ H ₅	2 × 10 ⁻⁴	<10	<10	<10	<10
IVf	5	1	ECP ^{c)}	8 × 10 ⁻⁵	<10	11	22	<10
IVg	7	1	ECP	3 × 10 ⁻⁵	29	<10	5 × 10 ⁻⁵	<10

a) % inhibition at a concentration of the compound of 1 × 10⁻³ M.

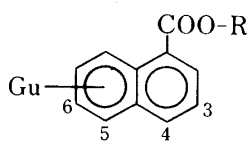
b) IC₅₀. c) ECP = *p*-ethoxycarbonylphenyl.

necessary for compounds to show protease inhibitory activities; free guanidinonaphthoic acids studied were practically devoid of activity. In comparison with amino derivatives, guanidino esters, in both the naphthoate and tetrahydronaphthoate series, were more potent in terms of both the activity and the range of protease inhibitory effects. More precisely, the inhibitory effects against trypsin were limited in the case of amino-esters of both series, whereas the inhibitory effectiveness of guanidino-esters was higher against trypsin and extended to proteases other than trypsin.

In a series of tetrahydronaphthoates, the introduction of an ethoxycarbonyl group in the *p*-position of the ester phenyl moiety increased the inhibitory action in terms of both the activity and the range.

Comparison of a series of congeners, *e.g.* phenyl 5-guanidino-1-naphthoate (IIIk), phenyl 5-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Va) and *p*-ethoxycarbonylphenyl 5-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vf), revealed that IIIk and Vf showed practically the same

TABLE III. Enzyme Inhibition by Guanidinonaphthoates



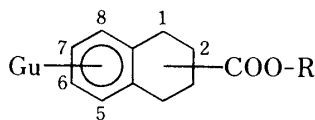
Compd. No.	Gu ^{a)}	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IIIa	3	H	<10 ^{b)}	<10	<10	<10	<10
IIIb	4	H	19	<10	19	<10	<10
IIIc	5	H	47	<10	<10	14	<10
IIId	6	H	16	<10	<10	<10	<10
IIIe	3	C ₂ H ₅	42	<10	<10	16	41
IIIf	4	C ₂ H ₅	42	47	<10	13	7 × 10 ⁻⁴
IIIg	5	C ₂ H ₅	1 × 10 ^{-3 c)}	<10	<10	<10	22
IIIh	6	C ₂ H ₅	2 × 10 ⁻⁴	48	<10	<10	2 × 10 ⁻⁴
IIIi	3	C ₆ H ₅	31	31	1 × 10 ⁻⁴	8 × 10 ⁻⁴	42
IIIj	4	C ₆ H ₅	4 × 10 ⁻⁷	5 × 10 ⁻⁴	28	1 × 10 ⁻⁴	7 × 10 ⁻⁴
IIIk	5	C ₆ H ₅	6 × 10 ⁻⁵	30	18	15	4 × 10 ⁻⁴
IIIl	6	C ₆ H ₅	5 × 10 ⁻⁸	1 × 10 ⁻⁵	48	7 × 10 ⁻⁶	4 × 10 ⁻⁵

a) Gu = -NH- $\begin{array}{c} \text{NH} \\ \diagup \quad \diagdown \\ \text{NH}_2 \end{array}$.

b) % inhibition at a concentration of the compound of 1 × 10⁻³ M.

c) IC₅₀.

TABLE IV. Enzyme Inhibition by Guanidinotetrahydronaphthoates



Compd. No.	Gu ^{a)}	COOR	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
Va	5	1	C ₆ H ₅	<10 ^{b)}	<10	18	<10	18
Vb	7	1	C ₆ H ₅	7 × 10 ^{-4 c)}	19	43	4 × 10 ⁻⁵	4 × 10 ⁻⁴
Vc	6	2	C ₆ H ₅	40	1 × 10 ⁻³	26	4 × 10 ⁻⁴	3 × 10 ⁻⁴
Vd	7	5	C ₆ H ₅	7 × 10 ⁻⁴	<10	2 × 10 ⁻⁴	2 × 10 ⁻⁴	30
Ve	8	5	C ₆ H ₅	3 × 10 ⁻⁴	<10	<10	39	24
Vf	5	1	ECP ^{d)}	3 × 10 ⁻⁴	20	7 × 10 ⁻⁴	38	5 × 10 ⁻⁴
Vg	7	1	ECP	7 × 10 ⁻⁵	7 × 10 ⁻⁵	2 × 10 ⁻⁴	1 × 10 ⁻⁵	6 × 10 ⁻⁴

a) Gu = -NH- $\begin{array}{c} \text{NH} \\ \diagup \quad \diagdown \\ \text{NH}_2 \end{array}$.

b) % inhibition at a concentration of the compound of 1 × 10⁻³ M.

c) IC₅₀. d) ECP = *p*-ethoxycarbonylphenyl.

inhibitory effectiveness whereas Va was almost ineffective.

Among the compounds studied, the 1,6-substituted compound (IIIh) in the amino-naphthoates was a weak but selective inhibitor of trypsin, and the 1,4- and 1,6-substituted compounds (IIIj, l) in the guanidino-naphthoates showed particularly strong inhibition of trypsin with IC₅₀ values of 4 × 10⁻⁷ and 5 × 10⁻⁸ M, respectively, together with moderate inhibition on other proteases including Cl esterase and thrombin, while the 1,7-substituted compounds (IVb, g) in the amino-tetrahydronaphthoates showed moderate inhibitions of

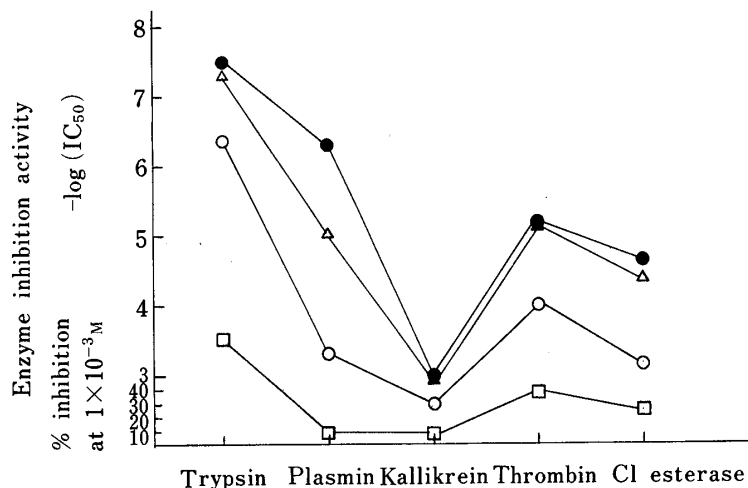
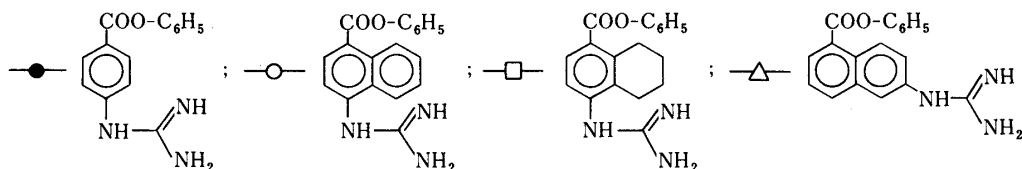


Fig. 1. Enzyme Inhibition Spectrum of Guanidino Phenyl Esters



both trypsin and thrombin. Among guanidino-tetrahydronaphthoates, the 1,7-substituted compounds (Vb, g) showed particularly potent inhibition of thrombin, the inhibition spectrum of *p*-ethoxycarbonylphenyl compound (Vg) being particularly broad.

Figure 1 compares the spectrum of protease inhibitory activities of 4 representative phenyl-esters which can be regarded as analogues of phenyl *p*-guanidinobenzoate reported in the previous paper. It seems likely that the inhibitory effectiveness may be determined by many factors, such as the distance between the guanidino group and the ester linkage, the electron atmosphere of the molecules and, particularly, the steric configuration of the carrier moiety of the guanidino and ester groups.

Experimental

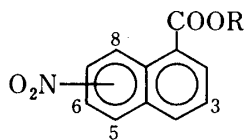
Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. The infrared (IR) spectra were determined on a Shimadzu IR-430, proton-nuclear magnetic resonance ($^1\text{H-NMR}$) on a JEOL FX-60Q or Varian T-60 and carbon-13 nuclear magnetic resonance ($^{13}\text{C-NMR}$) on a JEOL FX-60Q (with tetramethylsilane as an internal standard). The physical data for the compounds are summarized in Tables V–X.

3-Nitro-1-naphthoic Acid (VIa) and 6-Nitro-1-naphthoic Acid (VIc) (Method A)—3-Nitro-1,8-naphthalic anhydride (50 g) and then a solution of 49.1 g of mercuric oxide in 120 ml of water and 40 ml of acetic acid were added to a solution of 27 g of NaOH in 1000 ml of water with stirring. The reaction mixture was refluxed for 98 h, then cooled, and the precipitates were collected and washed with water. The precipitates were suspended in 420 ml of conc. HCl and the mixture was refluxed for 3 h, then cooled. The precipitates were collected and recrystallized from acetic acid to give 25.4 g (57%) of VIa^{4a)} as pale yellow needles. The filtrate was concentrated to dryness *in vacuo* to give a mixture of VIa and VIc. The mixture was used for the next reaction without purification.

5-Nitro-1-naphthoic Acid (VIb) and 8-Nitro-1-naphthoic Acid (VIc) (Method B)—1-Naphthoic acid (10 g) was added to 28 ml of conc. HNO_3 ($d=1.38$) under ice cooling and the mixture was warmed for 4 h in a boiling water bath. The mixture was then poured into ice water and the precipitates were collected, washed with water, and dissolved in 100 ml of boiling 8% Na_2CO_3 solution. After filtration, the solution was acidified with 10% HCl. The precipitates formed were collected and recrystallized from EtOH to give 4.6 g (37%) of VIb^{4b)} as pale yellow needles. The filtrate was concentrated and the residue was recrystallized from EtOH to give 5.5 g (44%) of VIc^{4c)} as pale yellow prisms.

5-Nitro-1,2,3,4-tetrahydro-1-naphthoic Acid (VIIa) and 7-Nitro-1,2,3,4-tetrahydro-1-naphthoic Acid (VIIb) (Method C)—1,2,3,4-Tetrahydro-1-naphthoic acid (30 g) was added to 300 ml of conc. HNO_3 ($d=1.38$) at room

TABLE V. Physical Data



Compd. No.	NO ₂	COOR	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd	Found	
								C	H	N
VIa	3	1	H	260—261 ^{a)}	A	1700				
VIb	5	1	H	232—234.5 ^{b)}	B	1690				
VIc	8	1	H	208—209 ^{c)}	B	1685				
VIe	3	1	C ₂ H ₅	87—88 ^{d)}	H	1720	C ₁₃ H ₁₁ NO ₄	63.67 (63.64)	4.52 (4.43)	5.71 (5.66)
VI f	5	1	C ₂ H ₅	94—94.5 ^{e)}	H	1725	C ₁₃ H ₁₁ NO ₄	63.67 (63.55)	4.52 (4.44)	5.71 (5.71)
VIg	6	1	C ₂ H ₅	113—114 ^{f)}	H, F	1720	C ₁₃ H ₁₁ NO ₄	63.67 (63.62)	4.52 (4.43)	5.71 (5.69)
VIh	3	1	C ₆ H ₅	168—169	E	1733	C ₁₇ H ₁₁ NO ₄	69.62 (69.60)	3.78 (3.74)	4.78 (4.78)
VIi	5	1	C ₆ H ₅	143—144	E	1720	C ₁₇ H ₁₁ NO ₄	69.62 (69.55)	3.78 (3.72)	4.78 (4.77)
VIj	6	1	C ₆ H ₅	135—135.5	F	1722	C ₁₇ H ₁₁ NO ₄	69.62 (69.52)	3.78 (3.72)	4.78 (4.79)

a) Lit.,^{4a)} 270—271.5°C. b) Lit.,^{4b)} 234—235°C. c) Lit.,^{4c)} 215°C.
d) Lit.,^{4a)} 87.5—88.5°C. e) Lit.,^{4d)} 92—93°C. f) Lit.,^{4a)} 111.5—112°C.

temperature with stirring and the mixture was stirred for 10 h at room temperature, then poured into water. The precipitates were collected, washed with water and air-dried at room temperature to give 23.9 g (63%) of a mixture of VIIa, b. A suspension of the mixture of VIIa, b in 200 ml of EtOAc was treated with 25 g of PCl₅. The reaction mixture was stirred for 3 h at room temperature, then concentrated *in vacuo*. The residue was washed with hexane and dissolved in 300 ml of EtOAc. To this solution, 16.5 g of *p*-methoxycarbonylphenol and 13 g of triethylamine were added with stirring. The reaction mixture was stirred for 3 h at room temperature, then washed with 10% HCl, 5% NaOH, and satd. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Recrystallization from EtOAc gave 12.6 g of *p*-methoxycarbonylphenyl 7-nitro-1,2,3,4-tetrahydro-1-naphthoate (XIIIb) as a colorless powder, mp 178—179.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1745 (C=O), 1712 (C=O). Anal. Calcd for C₁₉H₁₇NO₆: C, 64.22; H, 4.82; N, 3.94. Found C, 64.13; H, 4.74; N, 3.90.

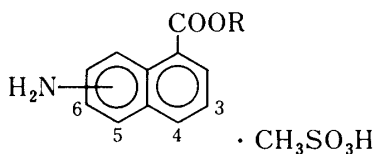
The filtrate was concentrated to dryness *in vacuo* and the residue was recrystallized from EtOH to give 21.4 g of *p*-methoxycarbonylphenyl 5-nitro-1,2,3,4-tetrahydro-1-naphthoate (XIIIa) as a colorless powder, mp 101—101.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1745 (C=O), 1714 (C=O). Anal. Calcd for C₁₉H₁₇NO₆: C, 64.22; H, 4.82; N, 3.94. Found C, 64.11; H, 4.73; N, 3.86.

XIIIa (10 g) was added to a solution of 40 ml of 20% NaOH and 10 ml of EtOH, and the mixture was stirred for 1 h at room temperature, then acidified with 10% HCl. The precipitates were collected and washed with water. Recrystallization from EtOAc gave 5 g of VIIa^{4e)} as pale yellow prisms. VIIb^{4e)} was obtained from XIIIb by the same procedure.

6-Nitro-1,2,3,4-tetrahydro-2-naphthoic Acid (VIIf), 6-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIId), 7-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIe) and 8-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIIf) (Method D)—VIIf was prepared by nitration of 1,2,3,4-tetrahydro-2-naphthoic acid with conc. HNO₃ (*d* = 1.38) at 40°C and VIId, e, f were prepared by nitration of 1,2,3,4-tetrahydro-5-naphthoic acid with fum. HNO₃ (*d* = 1.52) at 0°C according to method C. The mixture of VIId, e, f, was further subjected to esterification without purification. Analytical samples of VIe, f were prepared by hydrolysis of their phenyl esters (VIIf, I) with 10% NaOH.

Phenyl 3-Nitro-1-naphthoate (VIh) (Method E)—A suspension of 13.8 g of VIa in 100 ml of EtOAc was treated with 17.3 g of PCl₅. The mixture was stirred overnight at room temperature, then concentrated to dryness *in vacuo*. The residue was washed with hexane to give the acid chloride. A solution of the acid chloride in 50 ml of EtOAc was added to a solution of 6 g of phenol and 8.4 g of triethylamine in 200 ml of EtOAc. The whole was stirred overnight at

TABLE VI. Physical Data



Compd. No.	NH ₂	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
							Calcd	(Found)	
							C	H	N
IIa	3	C ₂ H ₅	155—156 ^{b)}	I	1719	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.92)	5.50 (5.50)	4.50 (4.43)
IIb	4	C ₂ H ₅	217—220 (d) ^{c)}	K	1718	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.51)	5.50 (5.48)	4.50 (4.48)
IIc	5	C ₂ H ₅	213—214.5 (d) ^{d)}	I	1706	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.89)	5.50 (5.50)	4.50 (4.40)
IIe	6	C ₂ H ₅	204—205	I	1688	^{a)}	60.16 (60.08)	4.77 (4.77)	3.90 (3.80)
IIe	3	C ₆ H ₅	200—200.5	I	1740	C ₁₇ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (59.67)	4.77 (4.81)	3.90 (4.02)
IIg	4	C ₆ H ₅	233—236	O	1738	C ₁₇ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.23)	4.77 (4.75)	3.90 (3.83)
IIh	5	C ₆ H ₅	243—(d)	I	1730	C ₁₇ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.12)	4.77 (4.72)	3.90 (4.01)
IIh	6	C ₆ H ₅	240 <	I	1730	C ₁₇ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.12)	4.77 (4.72)	3.90 (4.01)

a) The structure of IIe was confirmed by the following spectral data: IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3450, 3360, 3100—2500 (NH), 1688 (COO), ¹H-NMR (DMSO-*d*₆): 1.40 (3H, t, *J* = 7.1 Hz, CH₃—CH₂—), 2.48 (3H, s, CH₃—SO₃—), 4.43 (2H, q, *J* = 7.1 Hz, CH₃—CH₂—), 7.45—9.01 (6H, m, aromatic H), 9.29 (3H, br, NH₃⁺).

b) Lit.,^{6a)} free base bp 170—175 °C (0.5 mmHg).

c) Lit.,^{6b)} free base 80 °C. d) Lit.,^{6c)} free base 92 °C.

room temperature, then washed with 10% HCl, 5% NaOH and satd. NaCl, dried over anhyd. MgSO₄ and concentrated to dryness *in vacuo*. Recrystallization from EtOH-acetone gave 17 g (92%) of VIh as pale yellow needles.

Phenyl 6-Nitro-1-naphthoate (VIj) (Method F)—A mixture of VIh, j was prepared from VIa, c by reaction with phenol according to method E and the mixture was chromatographed on a silica gel column to separate VIh and VIj, the former being obtained from the first eluate with benzene : hexane = 1 : 1 and the latter from the second eluate.

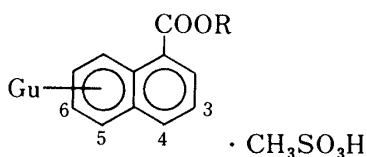
Phenyl 6-Nitro-1,2,3,4-tetrahydro-5-naphthoate (VIIj), Phenyl 7-Nitro-1,2,3,4-tetrahydro-1-naphthoate (VIIk) and Phenyl 8-Nitro-1,2,3,4-tetrahydro 5-naphthoate (VIII) (Method G)—A mixture of VIIj, k, l was prepared from VIId, e, f and phenol by method F. The isomers, VIII, VIIk and VIIj, were obtained from the first, second and third eluates, respectively, with benzene : hexane = 1 : 1.

Ethyl 5-Nitro-1-naphthoate (VIi) (Method H)—A mixture of 15 g of 5-nitro-1-naphthoic acid and 3 ml of conc. H₂SO₄ in 150 ml of anhyd. EtOH was refluxed overnight, then concentrated to dryness *in vacuo* and the residue was dissolved in EtOAc. This solution was washed with satd. NaHCO₃ and satd. NaCl, dried over anhyd. MgSO₄ and concentrated to dryness *in vacuo*. Recrystallization of the residue from EtOH gave 14.8 g (88%) of VIi as colorless needles.

Methanesulfonic Acid Salt of Phenyl 5-Amino-1-naphthoate (IIg) (Method I)—Methanesulfonic acid (2.4 g) and 10% Pd-C (2 g) were added to a solution of 6 g of VIi in 60 ml of demethylformamide (DMF). The mixture was hydrogenated with stirring at room temperature under atmospheric pressure, then filtered. Ethyl ether was added to the filtrate and the precipitates were collected. Recrystallization from EtOH gave 6.3 g (88%) of IIg as colorless needles.

Methanesulfonic Acid Salt of Phenyl 5-Guanidino-1-naphthoate (IIIk) (Method J)—A mixture of 3.6 g of IIg and 0.84 g of cyanamide in 30 ml of MeOH was stirred overnight at 50 °C, then filtered. The filtrate was concentrated to half the initial volume, then poured into satd. NaHCO₃ solution. The precipitates were collected and washed with water and acetone. A suspension of the precipitates in DMF was treated with 1.2 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate, and the precipitates were collected. Recrystallization from EtOH gave 1.9 g (46%) of IIIk as colorless prisms.

TABLE VII. Physical Data



Compd. No.	Gu ^{a)}	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}	Formula	Analysis (%)		
							Calcd (Found)		
							C	H	N
IIIa	3	H	232.5—233	M	1715	$\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	47.99 (47.95)	4.65 (4.71)	12.92 (12.84)
IIIb	4	H	260—261.5	M	1705	$\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	47.99 (47.87)	4.65 (4.65)	12.92 (12.91)
IIIc	5	H	264—265	M	1700	$\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	47.99 (47.89)	4.65 (4.62)	12.92 (12.79)
IIId	6	H	224—225	M	1710	$\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	47.99 (47.78)	4.65 (4.73)	12.92 (12.80)
IIIe	3	C_2H_5	186—187	L	1690	$\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	50.98 (50.86)	5.42 (5.44)	11.89 (11.88)
IIIf	4	C_2H_5	175.5—176	L	1710	$\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	50.98 (50.83)	5.42 (5.40)	11.89 (11.86)
IIIg	5	C_2H_5	186.5—187	L	1690	$\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	50.98 (50.81)	5.42 (5.45)	11.89 (11.87)
IIIh	6	C_2H_5	183.5—184	L	1706	$\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	50.98 (50.85)	5.42 (5.41)	11.89 (11.93)
IIIi	3	C_6H_5	206.5—207	J	1722	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	56.85 (56.58)	4.77 (4.81)	10.47 (10.50)
IIIj ^{b)}	4	C_6H_5	219.5—220	N	1722	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{C}_7\text{H}_8\text{O}_3\text{S}$	62.88 (62.44)	4.85 (4.93)	8.80 (8.92)
IIIk	5	C_6H_5	187—187.5	J	1720	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	56.85 (56.35)	4.77 (4.84)	10.47 (10.47)
IIIl	6	C_6H_5	235—237	J	1732	$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_2 \cdot \text{CH}_4\text{O}_3\text{S}$	56.85 (56.60)	4.77 (4.74)	10.47 (10.50)

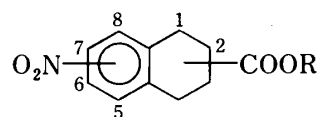
a) $\text{Gu} = -\text{NH} \begin{smallmatrix} \text{NH} \\ \diagup \diagdown \\ \text{NH}_2 \end{smallmatrix}$ b) *p*-Toluenesulfonate.

Methanesulfonic Acid Salt of Ethyl 4-Amino-1-naphthoate (IIb) (Method K)—A mixture of 8.4 g of 4-amino-1-naphthalenecarbonitrile in 100 ml of 50% KOH was refluxed overnight. After cooling, the mixture was diluted with 200 ml of water and filtered. The filtrate was acidified with conc. HCl and the precipitates were collected and air-dried at room temperature. Dry HCl gas was passed into a suspension of the precipitates in 200 ml of EtOH with stirring at room temperature until saturation. The reaction mixture was stirred overnight at room temperature, then the reaction mixture was concentrated to dryness *in vacuo*, and satd. NaHCO_3 and EtOAc were added to the residue. The separated organic layer was washed with 5% NaOH and with satd. NaCl, dried over anhyd. MgSO_4 and concentrated to dryness *in vacuo*. The residue was dissolved in ethyl ether and the solution was filtered. The filtrate was treated with 5.8 g of methanesulfonic acid and the precipitates were collected and recrystallized from EtOH–ethyl ether to give 8.4 g (54%) of IIb as colorless needles.

Methanesulfonic Acid Salt of Ethyl 4-Guanidino-1-naphthoate (IIIf) (Method L)—A mixture of 9.2 g of IIb and 3.6 g of cyanamide in 50 ml of MeOH was stirred overnight at 50°C. The mixture was concentrated to half the initial volume, and the concentrate was poured into satd. NaHCO_3 . The precipitates were collected and washed with water and acetone. A suspension of the precipitates in 15 ml of EtOH was treated with 4.5 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected and recrystallized from EtOH–ethyl ether to give 3.6 g (35%) of IIIf as a colorless powder.

Methanesulfonic Acid Salt of 4-Guanidino-1-naphthoic Acid (IIIb) (Method M)—IIIf (3.5 g) was added to a solution of 1.2 g of NaOH in 10 ml of EtOH and 10 ml of water, and the mixture was stirred overnight at room temperature. The mixture was neutralized with 10% HCl and the resulting precipitates were collected. A suspension

TABLE VIII. Physical Data



Compd. No.	NO ₂	COOR	R	mp (°C)	Method	IR $\nu_{\max}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd	(Found)	
								C	H	N
VIIa	5	1	H	145—146 ^{a)}	C	1700				
VIIb	7	1	H	161—162 ^{b)}	C	1700				
VIIc	6	2	H	194—195 ^{c)}	D	1698				
VIIe	7	5	H	219—221 ^{d)}	D	1683				
VII ^{f)}	8	5	H	155—157 ^{e)}	D	1690				
VIIg	5	1	C ₆ H ₅	98—99	E	1742	C ₁₇ H ₁₅ NO ₄	68.68 (68.56)	5.09 5.10	4.71 4.71)
VIIh	7	1	C ₆ H ₅	94—94.5	E	1740	C ₁₇ H ₁₅ NO ₄	68.68 (68.48)	5.09 5.05	4.71 4.69)
VIIi	6	2	C ₆ H ₅	123—124	E	1742	C ₁₇ H ₁₅ NO ₄	68.68 (68.61)	5.09 5.06	4.71 4.65)
VIIj	6	5	C ₆ H ₅	99—99.5	G	1752	C ₁₇ H ₁₅ NO ₄	68.68 (68.63)	5.09 5.13	4.71 4.64)
VIIk	7	5	C ₆ H ₅	101—102	G	1737	C ₁₇ H ₁₅ NO ₄	68.68 (68.72)	5.09 5.10	4.71 4.64)
VIII	8	5	C ₆ H ₅	109—110	G	1734	C ₁₇ H ₁₅ NO ₄	68.68 (68.72)	5.09 5.11	4.71 4.64)
VIIIm	5	1	ECP ^{f)}	58—59	E	1750, 1712	C ₂₀ H ₁₉ NO ₆	65.03 (64.81)	5.18 5.19	3.79 3.71)
VIIIn	7	1	ECP	154—155	E	1755, 1720	C ₂₀ H ₁₉ NO ₆	65.03 (64.76)	5.18 5.16	3.79 3.71)

a) Lit.,^{4e)} 143 °C. b) Lit.,^{4e)} 163 °C. c) Lit.,^{4f)} 196.5—197.0 °C.d) Lit.,^{4g)} 212—214 °C. e) Lit.,^{4h)} 161—162 °C. f) ECP = *p*-ethoxycarbonylphenyl.

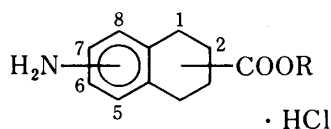
of the precipitates in 25 ml of DMF was treated with 1.4 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected and recrystallized from EtOH to give 2.3 g (71%) of IIIb as a colorless powder.

***p*-Toluenesulfonic Acid Salt of Phenyl 4-Guanidino-1-naphthoate (IIIj) (Method N)**—A mixture of 1.3 g of IIIb, 1.2 g of phenol and 1.0 g of dicyclohexylcarbodiimide in 20 ml of dry pyridine was stirred overnight at room temperature, then filtered. The filtrate was concentrated to half the initial volume and the concentrate was poured into satd. NaHCO₃. The precipitates were collected and washed with water and acetone. A suspension of the precipitates in 10 ml of MeOH was treated with 0.7 g of *p*-toluenesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected. Recrystallization from MeOH–ethyl ether gave 0.4 g (21%) of IIIj as a colorless powder.

4-(Benzyloxycarbonyl)amino-1-naphthoic Acid (XVI)—Benzyloxycarbonyl chloride (3.6 g) was added to a solution of 3.8 g of 4-amino-1-naphthoic acid in 50 ml of 0.5 N KOH under ice cooling with stirring. The mixture was stirred for 3 h at room temperature, then washed with ethyl ether and neutralized with 10% HCl. The precipitates were collected and recrystallized from DMF–water to give 3.5 g (54%) of XVI as a colorless powder, mp 245—246 °C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3260 (NH), 1682 (C=O), 1532, 1233. ¹H-NMR (*d*₆-DMSO) δ : 5.26 (2H, s, –CH₂–), 7.01—9.24 (11H, m), 10.00 (1H, s, NH), 13.01 (1H, br, COOH). Anal. Calcd for C₁₉H₁₅NO₄: C, 71.02; H, 4.70; N, 4.36. Found: C, 71.02; H, 4.69; N, 4.34.

Phenyl 4-Benzyloxycarbonylamino-1-naphthoate (XVII)—A mixture of 2.0 g of XVI in 20 ml of SOCl₂ was refluxed for 0.5 h, then concentrated *in vacuo*. The residue was added to a solution of 1 g of phenol in 20 ml of pyridine. The mixture was stirred for 3 h at room temperature, then poured into water. The precipitates were collected and recrystallized from EtOAc–hexane to give 2.0 g (81%) of XVII as a colorless powder, mp 141—142 °C. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3300 (NH), 1725 (ester C=O), 1690 (amide C=O), 1530, 1225. ¹H-NMR (*d*₆-DMSO) δ : 5.27 (2H, s, –CH₂–), 7.04—9.18 (16H, m), 10.14 (1H, s, NH). Anal. Calcd for C₂₅H₁₉NO₄: C, 75.55; H, 4.82; N, 3.52. Found C,

TABLE IX. Physical Data

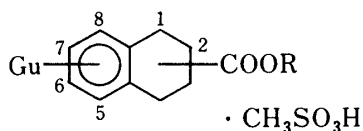


Compd. No.	NH ₂	COOR	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd (Found)		
								C	H	N
IVa	5	1	C ₆ H ₅	210—213 (d)	I	1744	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (67.10)	5.97 5.96	4.61 4.60
IVb	7	1	C ₆ H ₅	149—150	I	1741	^{b)}			
IVc	6	2	C ₆ H ₅	218—222 (d)	I	1735	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.90)	5.97 5.96	4.61 4.64
IVd	7	5	C ₆ H ₅	179—181	I	1734	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.89)	5.97 5.97	4.61 4.69
IVe	8	5	C ₆ H ₅	>270	I	1734	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.91)	5.97 6.05	4.61 4.51
IVf	5	1	ECP ^{a)}	206—209 (d)	I	1742, 1715	C ₂₀ H ₂₁ NO ₄ ·HCl	63.91 (63.84)	5.90 5.87	3.73 3.72
IVg	7	1	ECP	173—175	I	1765, 1712	^{c)}			

a) ECP = *p*-ethoxycarbonylphenyl.

b, c) The structures were confirmed as the guanidino derivatives (obtained in the next step).

TABLE X. Physical Data



Compd. No.	Gu ^{a)}	COOR	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd (Found)		
								C	H	N
Va	5	1	C ₆ H ₅	156—158	J	1742	^{c)}			
Vb	7	1	C ₆ H ₅	189—190	J	1763	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.16)	5.72 5.74	10.36 10.33
Vc	6	2	C ₆ H ₅	184—185	J	1751	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.30)	5.72 5.74	10.36 10.39
Vd	7	5	C ₆ H ₅	197—198	J	1731	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.25)	5.72 5.71	10.36 10.35
Ve	8	5	C ₆ H ₅	161—162	J	1722	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.22)	5.72 5.70	10.36 10.35
Vf	5	1	ECP ^{b)}	177—178	J	1763, 1705	C ₂₁ H ₂₃ N ₃ O ₄ ·CH ₄ O ₃ S	55.34 (55.29)	5.70 5.70	8.80 8.87
Vg	7	1	ECP	159—160	J	1760, 1718	C ₂₁ H ₂₃ N ₃ O ₄ ·CH ₄ O ₃ S	55.34 (55.02)	5.70 5.66	8.80 8.77

a) Gu = $-\text{NH}-\text{C}(\text{NH}_2)=\text{NH}$ b) ECP = *p*-ethoxycarbonylphenyl.c) The structure of Va was confirmed by the following spectral data: IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3320, 3150 (—NH), 1742 (C=O), ¹H-NMR (*d*₆-DMSO) δ : 1.47—3.00 (6H, br m), 2.42 (3H, s, CH₃), 4.18 (1H, br, H-1), 6.66—8.01 (12H, m), 9.30 (1H, s, NH); ¹³C-NMR (*d*₆-DMSO): 19.4, 23.8, 25.5 (—CH₂—CH₂—CH₂), 44.0 (—CH—).

75.48; H, 4.81; N, 3.55.

Methanesulfonic Acid Salt of Phenyl 4-Amino-1-naphthoate (IIf) (Method O)—Hydrogen gas was passed into a mixture of 1 g of XVII and 0.5 g of 10% Pd-C in 30 ml of DMF containing 0.3 g of methanesulfonic acid with stirring for 5 h at room temperature. The mixture was filtered, then ethyl ether was added to the filtrate to give an amorphous powder. Recrystallization from MeOH-ethyl ether gave 0.7 g (77%) of IIf as a colorless powder.

Enzyme Inhibition—Bovine trypsin was purchased from Sigma Chemical Co., St. Louis, USA, and dissolved in 0.1 M borate buffer containing 0.01 M CaCl₂, pH 8.5. Human plasmin was purchased from Green Cross Co., Osaka, Japan, and porcine kallikrein from Bayer, and they were each dissolved in 0.1 M borate buffer, pH 8.5. Bovine thrombin was purchased from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan, and dissolved in 0.02 M phosphate buffer, pH 7.4. Human Cl esterase was prepared by the method of Sumi *et al.*^{5a)} The rates of hydrolysis of TAME by trypsin, plasmin, kallikrein, and thrombin were determined as described by Muramatsu *et al.*,^{5b)} and that of ATEE by Cl esterase was assayed as described by Tamura *et al.*,^{5c)} at a substrate concentration of 10 mM.

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