[Chem. Pharm. Bull. 16(12)2355—2361(1968)]

UDC 547.298.6.07 : 615.281.8

Syntheses of 2-Acylaminoacetamidine and 3-Acylaminopropionamidine Derivatives[1]

Takeo Ueda,[2a] Yoshihisa Okamoto,[2b] Tadakazu Tsuji, and Masako Muraoka[2c]

College of Pharmaceutical Sciences, Kitasato University,[2a] Pharmaceutical Institute, Keio Gijuku University,[2b] and Japan Women's University[2c]

(Received April 15, 1968)

For the purpose to examine as to their antiviral activities, 2-acylaminoacetamidine (II) and 3-acylaminopropionamidine hydrochlorides (III) were synthesized from the corresponding nitriles *via* ethyl imidates. The difference of the reactivity between ethyl 2-acylaminoacetimidates and ethyl 3-acylaminopropionimidates on the course of amidination were postulated. Iminoesterification of dinitrile, (*p*-cyano) benzamidopropionitrile (XVIII), was discussed by referring the infrared spectrum of a corresponding monocyano-monoester, ethyl *p*-(N-cyanoethylcarbamoyl)benzoate (XXI).

Among the compounds obtained hereof, 3-(*p*-methyl)benzamidopropionamidine hydrochloride was found to have an inhibitory effect on influenza virus in mice and in membrane culture.

It has been known that Noformycin,[3] Mixoviromycin,[4] and Distamycin A[5] showed *in vivo* activities on influenza virus, and possess commonly the carboxamidopropionamidine moiety in their structures. From these facts, the authors tested the antiviral activities of five known compounds, *i.e.*, hydrochlorides of acetamidine, butyramidine, octanamidine, 3-aminopropionamidine, and 3-benzamidopropionamidine. Among these compounds, 3-benzamidopropionamidine hydrochloride[6a] (I) was found to possess a borderline activity on influenza virus. This finding prompted us to synthesize carboxamidopropionamidine derivatives for the purpose to screen as to their antiviral activities. This paper describes the syntheses of 2-acylaminoacetamidine (II) and 3-acylaminopropionamidine hydrochlorides (III).

The synthetic routes of the above series of compounds are shown in Chart 1. 2-Acylaminoaceto- (IV) and 3-acylaminopropionitriles (V) were prepared from acyl chloride and aminoacetonitrile bisulfate or 3-aminopropionitrile by Schotten–Baumann's method. The conversion of nitriles, IV or V, to amidine hydrochlorides, II or III, *via* ethyl imidates, (VI) or (VII), was carried out by the method of Pinner,[7] which was consisted of the treatment of nitrile with hydrogen chloride and ethanol to afford ethyl imidate, followed by the ammonolysis of the ethyl imidate with ethanolic ammonia. Compounds obtained hereof are shown in Table I—IV.

1) Papers read at the 24th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, Apl. 1967.
2) Location: *a*) *Shirogane-Sanko-cho, Minato-ku, Tokyo*; *b*) *Shinanomachi, Shinjuku-ku, Tokyo*; *c*) *Mejirodai, Bunkyo-ku, Tokyo.*
3) R.L. Peck, H.M. Shafer, and F.J. Wolf, U.S. Patent 2804463 (1957) [*C.A.*, **52**, 8474*d* (1958)].
4) S. Nakamura and H. Umezawa, *J. Antibiotics* (Tokyo), Ser. A, **14**, 163 (1963).
5) F. Arcamone, S. Penco, P. Orezzi, V. Nicolella, and A. Pirelli, *Nature*, **203**, 1064 (1964); J. Fournel, P. Ganter, F. Koenig, Y.de Ratuld, and G.H. Werner, *Antimicrobial Agents and Chemotherapy*, **1965**, 599.
6) *a*) A.A. Goldberg and W. Kelly, *J. Chem. Soc.*, **1947**, 1372; *b*) *Idem, ibid.*, **1947**, 1369; *c*) M. Mengelberg, *Chem. Ber.*, **89**, 1185 (1956).
7) A.W. Dox, "Organic Syntheses," Coll. Vol. I, ed. by A.H. Blatt, John Wiley and Sons, Inc., New York, N.Y., 1932, p. 5.

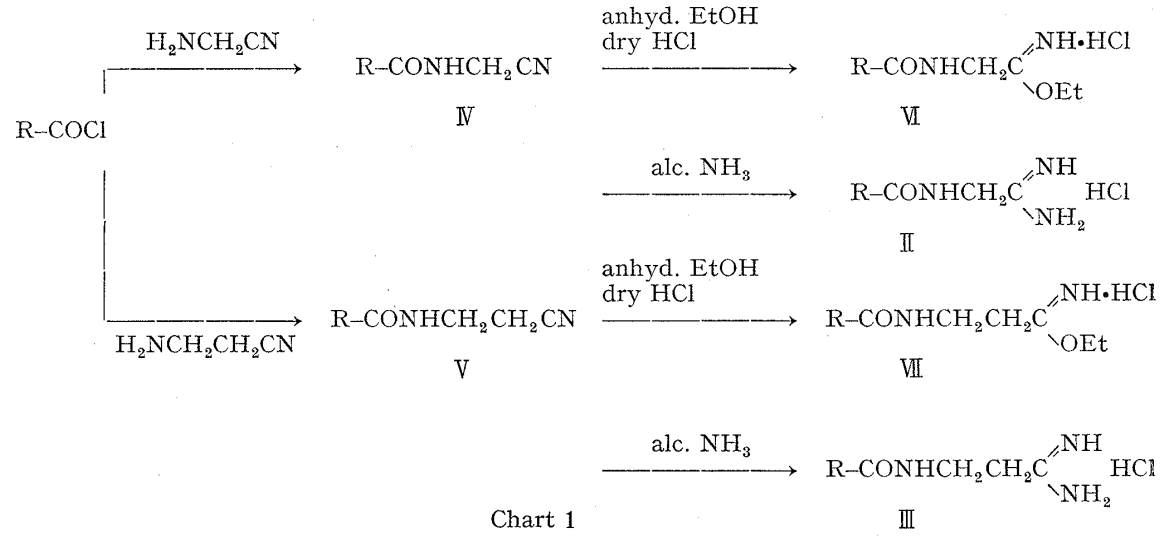
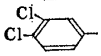
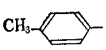
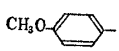
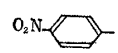
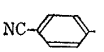
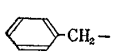
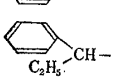
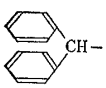
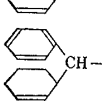
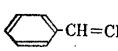
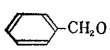
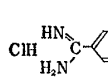


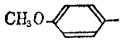
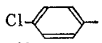
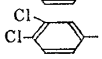
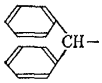
Chart 1

TABLE I. 2-Acylaminoaceto- and 3-Acylaminopropionitrile
R–CONH(CH$_2$)$_n$CN

R	n	Yield [a] (%)	Appearance (Recryst. solvt.)	mp (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
	1	44	needles(EtOH)	138—139 [b]	$C_9H_8ON_2$	67.48	5.03	17.49			
	2	50	needles(iso–PrOH)	93— 95 [c]	$C_{10}H_{10}ON_2$	68.95	5.79	16.08			
	1	37	powders(dil. EtOH)	107—110	$C_9H_7ON_2Cl$	55.54	3.63	14.39			14.25
	2	78	needles(MeOH)	62— 65	$C_{10}H_9ON_2Cl$	57.56	4.35	13.43			13.24
	1	62	needles(EtOH)	146—148	$C_9H_7ON_2Cl$	55.54	3.63	14.39			14.52
	2	36	needles(EtOH)	153—155	$C_{10}H_9ON_2Cl$	57.56	4.35	13.43			13.22
	1	65	needles(EtOH)	143—145	$C_9H_6ON_2Cl_2$	47.19	2.64	12.22			11.98
	1	38	powders(dil. EtOH)	148—150	$C_{10}H_{10}ON_2$	68.95	5.79	16.08			15.58
	2	78	needles(EtOH)	108—109	$C_{11}H_{12}ON_2$	70.18	6.43	14.88			14.75
	1	48	powders(dil. EtOH)	147—148	$C_{10}H_{10}O_2N_2$	63.15	5.30	14.73			14.51
	2	83	plates(EtOH)	122—125	$C_{11}H_{12}O_2N_2$	64.69	5.92	13.72			13.56
	1	32	needles(EtOH)	139—141	$C_9H_7O_3N_3$	52.68	3.44	20.48			20.38
	2	45	powders(EtOH)	145—147	$C_{10}H_9O_3N_3$	54.79	4.14	19.17			18.98
	2	50	plates(EtOH)	127 (decomp.)	$C_{11}H_9ON_3$	66.32	4.55	21.10			20.74
	2	68	needles(EtOH)	107—108	$C_{11}H_{12}ON_2$	70.18	6.43	14.88	70.45	6.40	14.74
	1	78	powders(EtOH)	50— 52	$C_{12}H_{14}ON_2$	71.26	6.98	13.85			13.45
	2	42	needles(dil. EtOH)	67— 69	$C_{13}H_{16}ON_2$	72.19	7.46	12.95	72.08	7.38	13.02
	1	52	needles(dil .EtOH)	152—154	$C_{16}H_{14}ON_2$	76.78	5.64	11.19			11.40
	2	70	needles(EtOH)	132—134	$C_{17}H_{16}ON_2$	77.25	6.10	10.60			10.53
	2	87	needles(MeOH)	165—169	$C_{17}H_{20}ON_2$	76.08	7.51	10.44	75.80	7.51	10.48
	2	50	plates(EtOH)	120—121	$C_{12}H_{12}ON_2$	71.98	6.04	13.99	71.68	6.17	13.91
	1	61	powders(EtOH)	61— 62 [d]	$C_{10}H_{10}O_2N_2$	63.15	5.30	14.73			
	2		powders (EtOH+ether)	199—203	$C_{11}H_9ON_4Cl\cdot\frac{1}{2}H_2O$	50.48	5.39	21.41	50.98	5.94	20.96

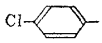
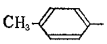
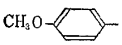
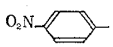
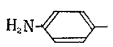
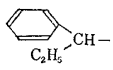
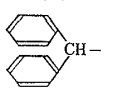
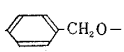
a) from corresponding carboxylic acid
b) lit. 6b) mp 142—144°
c) lit. 6b) mp 98°
d) lit. 6c) mp 62°

Table II. Ethyl 2-Acylaminoacetimidate Hydrochloride
R-CONHCH$_2$C(=NH)OEt·HCl

R	Yield[a] (%)	Appearance (Recryst. solvt.)	mp (°C)	Formula	Analysis (%) Calcd. N	Found N
	89	powders (anhyd. EtOH+ether)	131—133	$C_{12}H_{17}O_2N_2Cl$	10.91	10.58
	90	powders (anhyd. EtOH+ether)	136—138	$C_{12}H_{17}O_3N_2Cl$	10.23	10.67
	80	powders (anhyd. EtOH+ether)	134—136	$C_{11}H_{14}O_2N_2Cl_2$	10.10	10.41
	88	needles (anhyd. EtOH+ether)	138—139	$C_{11}H_{14}O_2N_2Cl_2$	10.10	10.19
	86	powders (anhyd. EtOH+ether)	140—142	$C_{11}H_{13}O_2N_2Cl_3$	8.98	8.89
	40	powders (anhyd. EtOH+ether)	133—135	$C_{18}H_{21}O_2N_2Cl$	8.41	8.71

a) from 2-acylaminoacetonitrile

Table III. 2-Acylaminoacetamidine Hydrochloride
R-CONHCH$_2$C (=NH)NH$_2$·HCl

R	Yield[a] (%)	Appearance (Recryst. solvt.)	mp (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
	77	needles (EtOH)	184—185[c]	$C_9H_{12}ON_3Cl$	50.59	5.66	19.67	—	—	—
	56	powders (EtOH+ether)	184 (decomp.)	$C_9H_{11}ON_3Cl_2$	43.56	4.47	16.94	43.63	4.21	17.00
	69	powders (EtOH+ether)	205 (decomp.)	$C_9H_{11}ON_3Cl_2$	43.56	4.47	16.94	43.34	4.33	16.96
	57	powders (EtOH+ether)	195 (decomp.)	$C_9H_{10}ON_3Cl_3$	38.25	3.57	14.87	38.43	3.53	14.77
	74	powders (EtOH+ether)	213 (decomp.)	$C_{10}H_{14}ON_3Cl$	52.75	6.20	18.45	52.26	6.28	18.27
	72	powders (EtOH+ether)	188—190	$C_{10}H_{14}O_2N_3Cl$	49.28	5.79	17.24	49.20	5.71	17.27
	64	plates (EtOH)	248—250	$C_9H_{11}O_3N_4Cl$	41.79	4.29	21.66	41.66	3.92	21.94
	62[b]	plates (EtOH)	235—237	$C_9H_{13}ON_4Cl$	47.27	5.73	24.50	47.09	5.80	24.30
	40	needles (EtOH+ether)	156 (decomp.)	$C_{12}H_{18}ON_3Cl$	56.35	7.09	16.43	56.35	6.85	16.65
	29	needles (EtOH+ether)	208 (decomp.)	$C_{16}H_{18}ON_3Cl$	63.26	5.97	13.83	62.60	6.02	14.10
	64	powders (EtOH+EtOAc)	75— 77[d]	$C_{10}H_{14}O_2N_3Cl$	49.28	5.79	17.24	—	—	—

a) from 2-acylaminoacetonitrile
b) from (*p*-nitro)benzamidoacetamidine hydrochloride
c) lit. 6a) mp 186—188°
d) lit. 6c) mp 78°

Table IV. 3-Acylaminopropionamidine Hydrochloride
R-CONHCH$_2$CH$_2$C(=NH)NH$_2$·HCl

R	Yield[a] (%)	Appearance (Recryst. solvt.)	mp (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
C$_6$H$_5$-	60	plates (EtOH)	180—183[c]	$C_{10}H_{14}ON_3Cl$	52.75	6.20	18.46			
2-ClC$_6$H$_4$-	30	needles (EtOH)	205—208	$C_{10}H_{13}ON_3Cl_2$	45.81	5.00	16.03	46.05	4.95	16.19
4-ClC$_6$H$_4$-	42	needles (EtOH)	206 (decomp.)	$C_{10}H_{13}ON_3Cl_2$	45.81	5.00	16.03	46.01	5.13	16.03
4-CH$_3$C$_6$H$_4$-	35	needles (EtOH)	220—223	$C_{11}H_{16}ON_3Cl$	54.65	6.67	17.39	54.94	6.93	17.34
4-CH$_3$OC$_6$H$_4$-	23	needles (EtOH)	211—214	$C_{11}H_{16}O_2N_3Cl$	51.26	6.26	16.31	51.80	5.92	15.96
4-O$_2$NC$_6$H$_4$-	21	prisms (EtOH)	157 (decomp.)	$C_{10}H_{13}O_3N_4Cl$	44.04	4.81	20.54	44.20	4.82	20.33
4-H$_2$NC$_6$H$_4$-	60[b]	needles (EtOH+MeOH)	205 (decomp.)	$C_{10}H_{15}ON_4Cl$	49.48	6.23	23.09	49.58	6.25	24.11
C$_6$H$_5$CH$_2$-	24	plates (EtOH+ether)	144—145	$C_{11}H_{16}ON_3Cl$	54.65	6.67	17.39	53.85	6.57	17.64
(C$_6$H$_5$)(C$_2$H$_5$)CH-	24	needles (EtOH+ether)	143—144	$C_{13}H_{20}ON_3Cl$	57.88	7.47	15.58	57.82	7.14	15.58
(C$_6$H$_5$)$_2$CH-	68	needles (EtOH+MeOH)	244—246	$C_{17}H_{20}ON_3Cl$	64.24	6.34	13.22	64.71	6.35	13.33
(C$_6$H$_5$)$_2$CH-	52	needles (EtOH+ether)	55 (decomp.)	$C_{17}H_{24}ON_3Cl\cdot\frac{1}{2}H_2O$	61.71	7.31	12.70	62.05	7.35	12.66
C$_6$H$_5$CH=CH-	68	needles (EtOH)	92— 93	$C_{12}H_{16}ON_3Cl\cdot H_2O$	53.04	6.68	15.46	53.01	6.58	15.47
2-ClHN=C(NH$_2$)-C$_6$H$_4$-	20	powders (EtOH+ether)	255—257	$C_{11}H_{17}ON_5Cl_2\cdot H_2O$	40.75	5.91	21.60	40.68	5.96	21.11

a) from 3-acylaminopropionitrile *b*) from (*p*-nitro)benzamidopropionamidine hydrochloride *c*) lit. 6*a*) mp 178—180°

The compounds thus obtained were characterized by the inspection of the data of elemental analysis and in frared spectra (IR spectra).

Bose, *et al.*[8] prepared (*o*-carbamoyl)benzamidoacetamidine hydrochloride (IX) from ethyl phthalimidoacetimidate (VIII) and ethanolic ammonia, and also reported that VIII was hydrolyzed to ethyl phthalimidoacetate (X) by the treatment of water.

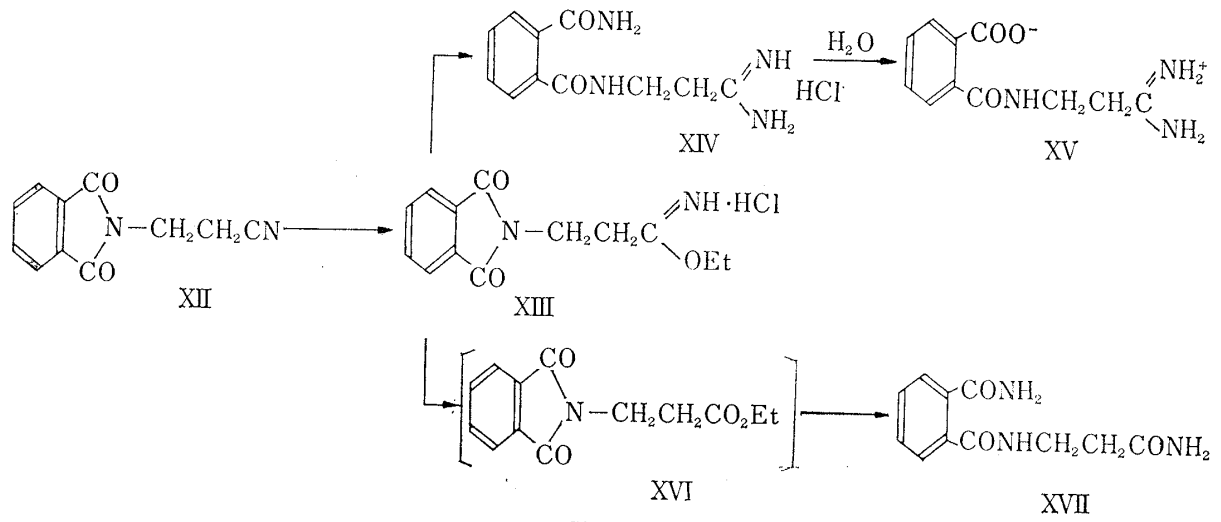


Chart 2

By the application of this method, ammonolysis of ethyl phthalimidopropionimidate (XIII) was attempted to obtain (*o*-carbamoyl)benzamidopropionamidine (XIV). Then, it was found, as shown in Chart 2, (*o*-carbamoyl)benzamidopropionamide (XVII) was yielded as a by-product (9.6%) along with XIV (32.0%) in this reaction. The formation of XVII might be due to the sequence of the following reaction: ethyl imidate XIII was decomposed to the corresponding ester (XVI) by the action of a trace amount of water which was contained in ethanol, and then this ester (XVI) was converted to XVII with ammonia. As it was confirmed that any amount of acetamide derivative was not detected in the ammonolysis of ethyl phthalimidoacetimidate (VIII) under the same condition as the case of XIII, it may be said that VIII might be more stable against the hydrolysis than XIII. This assumption might be further extended to the relationship of stability between VI and VII, since the hygroscopic property of the latter was, in general, observed to be more than that of the former, and the higher yield of II than that of III from corresponding nitriles, IV and V, was obtained.

Next, syntheses of several compounds of 3-acylaminopropionamidine having additional amidino group or ureido group were investigated as follows. In order to obtain (*p*-amidino) benzamidopropionamidine dihydrochloride (XXIII), (*p*-cyano)benzamidopropionitrile (XVIII) was treated with two equivalent amounts of hydrogen chloride and ethanol. In this reaction, however, there was obtained a product (XIX), in which only one nitrile group attached to the benzene ring in XVIII was converted to ethyl imidate. Product (XIX) was derived to a corresponding monocyanomonoester (XXI) by the hydrolysis. As the infrared (IR) spectrum of XXI showed the aromatic ester bands at 1721, 1273, and 1105 cm^{-1}, XIX was concluded to be ethyl *p*-(N-cyanoethylcarbamoyl)benzimidate hydrochloride. The conversion of XVIII to ethyl diimidate (XX) was furnished in addition of excess amounts of hydrogen chloride and ethanol to XVIII or XIX. The treatment of ethyl imidates, (XIX) and (XX), with ammonia afforded the corresponding amidine derivatives, (XXII) and (XXIII), as expected. These synthetic routes are shown in Chart 3.

Two propionamidine derivatives having ureido group (XXV and XXVII) in their structures were synthesized from phenylcarbamidopropionitrile (XXIV) and diphenylcarbamidopropionitrile (XXVI) respectively by the usual method.[7] Their synthetic courses from diphenylcarbamoyl chloride and phenylisocyanate were shown in Chart 4.

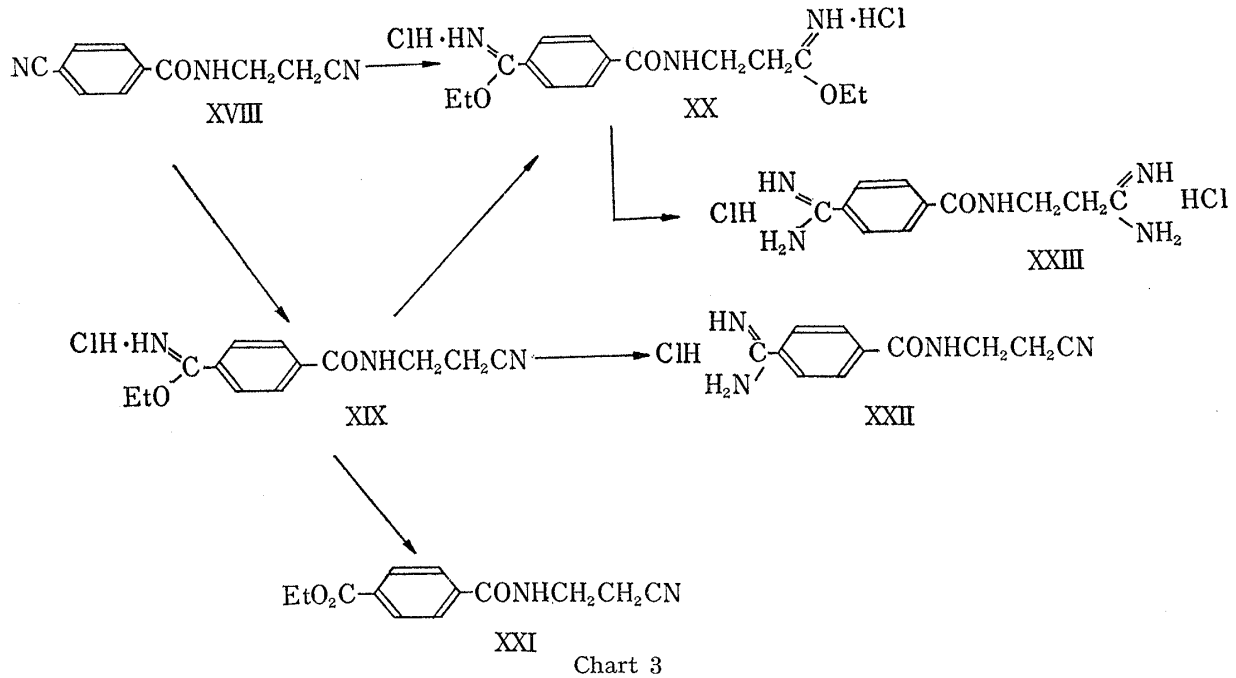


Chart 3

8) A.K. Bose, F. Greer, J.S. Gots, and C.C. Price, *J. Org. Chem.*, **24**, 1309 (1959).

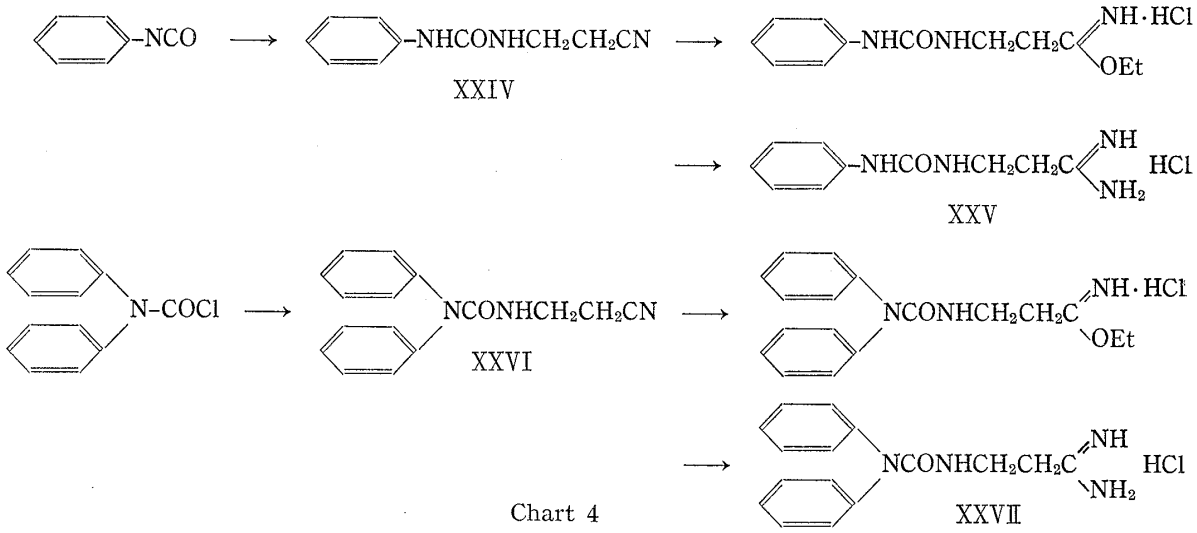


Chart 4

Compounds obtained hereof were screened as to their antiviral properties on influenza virus in mice and in membrane culture. Among them, 3-(*p*-methyl)benzamidopropionamidine hydrochloride was found to have an inhibitory effect. The effect of this compound will be evaluated by comparison with that of Adamantanamine Hydrochloride and Noformycin in the next step of our research. Those data will be reported in detail in a medical journal in the near future.

Experimental

General Procedure for Synthesis of 2-Acylaminoaceto- (IV) and 3-Acylaminopropionitriles (V) ——At the temperature between 5 to 15°, 0.043 mole of acyl chloride and 20 ml of 10% NaOH were gradually added alternately into 0.044 mole of aminoacetonitrile or 3-aminopropionitrile. After then, the precipitates were collected and washed with water.

General Procedure for Synthesis of Ethyl 2-Acylaminoaceto-(VI) and 3-Acylaminopropionimidate Hydrochrolides (VII)——To a solution (or suspension) of 0.030 mole of IV or V in 50 to 60 ml of $CHCl_3$, 0.031 mole of anhyd. EtOH was added, and then dry HCl gas was passed into the solution below 5° until 0.030 mole of HCl was absorbed. The flask was tightly stoppered and allowed to stand for one day. In such a case as crystals of VI or VII were not appeared, anhyd. ether was added to the solution to obtain the precipitates. Crystals obtained were filtered by suction, and washed with anhyd. ether.

General Procedure for Synthesis of 2-Acylaminoaceto-(II) and 3-Acylaminopropionamidine Hydrochlorides (III)——Dry NH_3 gas was passed into 40 ml of anhyd. EtOH until it contained 9% of NH_3 by weight. Then the ethanolic NH_3 solution was added with stirring to 0.023 mole of VI or VII until it dissolved. The flask was stoppered and allow to stand at a cold place. After 3 days, the solution was filtered by suction and anhyd. ether was added to the filtrate to yield the precipitates of II or III.

Phthalimidopropionitrile[9] (XII)——To a mixture of 0.13 mole phthalimide and 70 ml of acrylonitrile was added 5 ml of 40% aq. solution of trimethyl benzylammonium hydroxide. Then, the mixture was refluxed on a water bath for 2 hr. After that, the precipitates were collected and recrystallized from EtOH to give colorless needles, mp 148—150° (lit.[9] 154—155°). *Anal.* Calcd. for $C_{11}H_8O_2N_2$: N, 14.00. Found: N, 13.61. Yield, 55.1%.

Ethyl Phthalimidopropionimidate Hydrochloride (XIII)——Excess amount of dry HCl was passed through into a suspension of 0.035 mole of XII in 50 ml of EtOH under cooling until the crystals were completely dissolved. After standing on the solution for 2 days, the resulted precipitates were collected, and recrystallized from anhyd. EtOH to give colorless powders, mp 108—110°. *Anal.* Calcd. for $C_{13}H_{15}O_3N_2Cl$: C, 55.22; H, 5.30; N, 9.91. Found: C, 55.00; H, 5.63; N, 10.07. Yield, 61.2%.

(*o*-Carboxy)benzamidopropionamidine Hydrochloride (XV) and (*o*-Carbamoyl)benzamidopropionamide (XVII)——In a similar way with the procedure for the preparation of III, an ethanolic NH_3 solution was added to a solution of 0.01 mole of XIII in EtOH. After standing for 3 days, anhyd. ether was added and the resulted precipitates, XVII, was separated from the filtrate by suction, recrystallized from EtOH to give colorless crystals, mp 210—212°. *Anal.* Calcd. for $C_{11}H_{13}O_3N_3$: C, 56.16; H, 5.57. Found: C, 56.16; H, 5.55.

9) A. Galat, *J. Am. Chem. Soc.*, **67**, 1414 (1959).

The filtrate was evaporated and the hygroscopic substance (XIV) was remained. It was dissolved with H_2O and filtered. After a few minutes, the crystals (XV) were separated from the filtrate, washed with EtOH and dried for 3 hr at 100° *in vacuo*. mp 188—189°. *Anal.* Calcd. for $C_{11}H_{13}O_3N_3 \cdot \frac{1}{2}H_2O$: C, 54.09; H, 5.78; N, 17.21. Found: C, 54.77; H, 5.52; N, 17.82. Yield, 32.0%.

Ethyl *p*-(N-Cyanoethylcarbamoyl)benzoate (XXI)——The crystals of XIX were dissolved with H_2O, and on standing oily substances were appeared, dried at room temperature *in vacuo*. Recrystallized from EtOH to give hygroscopic crystals. IR ν_{max}^{KBr} cm^{-1}: 2260 (C≡N), 1721 (C=O), 1273 (C–O–C), 1105 (C–O–C).

Phenylcarbamidopropionitrile (XXIV)——3-Aminopropionitrile (0.06 mole) was gradually added to 0.06 mole of phenylisocyanate under cooling. The resulted precipitates were collected and recrystallized from EtOH–MeOH to give fine needles, mp 144—145°. *Anal.* Calcd. for $C_{10}H_{11}ON_3$: C, 63.47; H, 5.86; N, 22.21. Found: C, 63.48; H, 5.89; N, 22.22. Yield, 78.7%.

Phenylcarbamidopropionamidine Hydrochloride (XXV)——This compound was synthesized from XXIV by the usual method.[7] Recrystallized from EtOH–ether to give colorless powders, mp 154—155°. *Anal.* Calcd. for $C_{10}H_{15}ON_4Cl$: C, 49.48; H, 6.23; N, 23.09. Found: C, 49.30; H, 6.39; N, 23.10. Yield from XXIV, 39.2%.

Diphenylcarbamidopropionitrile (XXVI)——Diphenylcarbamoyl chloride (0.039 mole) was gradually added to a solution of 0.04 mole of 3-aminopropionitrile in 40 ml of anhyd. pyridine under cooling, and the whole was heated on a water bath for 1 hr. After that, excess pyridine was removed under diminished pressure, and then the residue was poured into an ice–water. Crystals thus obtained were recrystallized from EtOH to give colorless needles, mp 152.5°. *Anal.* Calcd. for $C_{16}H_{15}ON_3$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.51; H, 5.74; N, 15.74. Yield, 71.61%.

Diphenylcarbamidopropionamidine Hydrochloride (XXVII)——This compound was obtained from XXVI by the application of Pinner's method.[7] Recrystallization from EtOH–MeOH gave colorless needles, mp 223—224°. *Anal.* Calcd. for $C_{16}H_{19}ON_4Cl$: C, 60.28; H, 6.01; N, 17.58. Found: C, 60.53; H, 5.80; N, 17.52. Yield from XXVI 51.0%.

Acknowledgement The authors thank Mr. T. Kato, Kitasato University, for the technical assistance, and Miss H. Yoda, this Institute, for the elemental analysis.