Notes

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Kaname Takagi, Yoko Kurokawa, and Takeo Ueda : Syntheses and Antiviral Effect of (Amidinocarbamoyl)pyridine Derivatives.

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Recently, our group found that some salts of guanidine and some of guanidine related compounds exerted inhibitory effects on several pathogenic viruses in tissue culture. These findings suggested that it was of interest to search for antiviral agents more effective than guanidine itself, among guanidine related compounds.

Isonicotinic acid hydrazide is well-known as an antituberculosal chemotherapeutic drug. Several compounds having hydrazino moiety other than isonicotinic acid hydrazide also were found to have effects nearly equal to that of the latter.[1,2]

These findings suggest that the acid hydrazide part contributes to the generation of antituberculosal activity in the structure of isonicotinic acid hydrazide, while isonicotinoyl part serves as an auxiliary group. This assumption suggests that an effective agent on microbes except tubercle bacillus may be created by the combination of isonicotinoyl group with a biologically active group other than hydrazino group. Thus, the authors conceived an idea to make antiviral agent by combining isonicotinoyl rest with guanidino group.

This report describes the syntheses and antiviral effect of (amidinocarbamoyl)-pyridine derivatives, particularly their quaternary ammonium salts.

Syntheses of (Amidinocarbamoyl)pyridine and Its Quaternary Ammonium Salts

Of the three isomers of (amidinocarbamoyl)pyridine, β- and γ-compounds were reported by Mameli, *et al.*[3] by the reaction of pyridinecarboxylic acid ethyl ester with free guanidine. α-Compound was prepared, with ease, according to the method of Mameli.

These three isomers were screened as to their inhibitory effect on the K-2211 strain of common cold virus and the Tokyo 57 strain of influenza A virus in tissue culture. As the result obtained, 4-(amidinocarbamoyl)pyridine was found to have the most marked effect, nearly equal to that of guanidne nitrate on the K-2211 strain, while 2- and 3-(amidinocarbamoyl)pyridine, less effective than guanidine nitrate. And any of the three isomers was not effective on the influenza virus.

The purpose of this study was to find antiviral agents more effective than guanidine nitrate. From this point of view, even the effectiveness of the 4-(amidinocarbamoyl)-pyridine was judged unsatisfactory for our purpose.

Accordingly, attempts were made to synthesize the derivatives of (amidinocarbamoyl)-pyridine, its quaternary ammonium salts.

The quaternary ammonium iodides and bromides of 3- and 4-(amidinocarbamoyl)-pyridine were easily obtained by the reaction of 3- and 4-(amidinocarbamoyl)pyridine with alkyl iodide and bromide, as shown in Chart 1. However, the reaction between 2-(amidinocarbamoyl)pyridine and alkyl iodide did not afford any objective quaternary salt.

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[1] T. Matsukawa, S. Ban, K. Shirakawa, M. Yoneda: Yakugaku Zasshi, **73**, 159 (1953).

[2] Y. Hamada: *Ibid.*, **79**, 908 (1959).

[3] E. Mameli, F. d Angeli: Il Farmaco (Pavia). Ed. Ssi., **11**, 124 (1956) (C. A., **50**, 13896 (1956)).

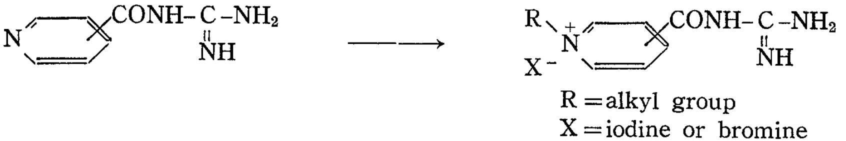

Chart 1.

The ammonium chlorides were found, with difficulty, obtainable directly by the reaction of (amidinocarbamoyl)pyridine with alkyl chloride. These salts, however, were found obtainable by the conversion of the pyridinium iodides with silver chloride.

On the other hand, 1-methyl-4-(amidinocarbamoyl)pyridinium iodide was also obtained by the reaction of guanidine with ethyl ester of 1-methyl-4-carboxypyridinium iodide,[4] which had been prepared through the reaction of ethyl isonicotinate with methyl iodide, as shown in Chart 2.

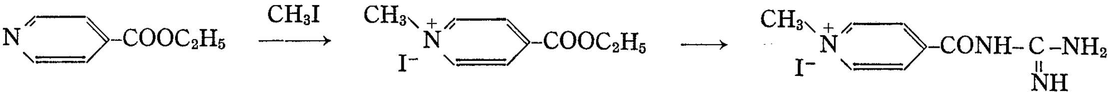

Chart 2.

The reaction between ethyl ester of 1-methyl-3-carboxypyridinium iodide[5] and guanidine did not afford objective 1-methyl-3-(amidinocarbamoyl)pyridinium iodide, but gave exclusively guanidinium salt of 1-methyl-3-carboxypyridinium iodide, as shown in Chart 3.

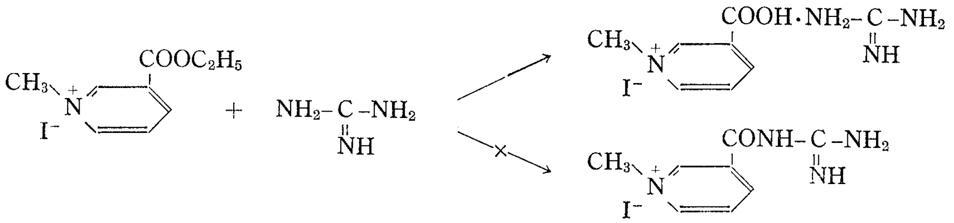

Chart 3.

The compounds synthesized are listed in Table I and II.

As described in the screening part, some of the ammonium halides synthesized exerted effects on the common cold virus more than guanidine nitrate, but all of the ammonium halides, not any effect on the influenza virus. In practice, it is desired to obtain an agent possessing antiviral effect on both common cold and influenza virus. Along this line, attempts were made to introduce higher alkanoyl groups into the structure of (amidinocarbamoyl)pyridine. This exploitation, however, failed to obtain any of 1-acyl-4-(amidinocarbamoyl)pyridinium halide.

Hereupon, the authors conceived an idea to introduce N,N-dialkylaminoethyl rest into (amidinocarbamoyl)pyridine. For this purpose, the objective compounds were, in success, synthesized by the reaction of chlorides of dimethylaminoethyl and diethylaminoethyl with 4-(amidinocarbamoyl)pyridine in anhydrous butanol. The compounds synthesized are listed in Table III.

Screening Tests with Synthesized Compounds on Common Cold Virus

The K-2211 strain of common cold virus, which was given by Prof. W. J. Mogabgab, Tulane University to the authors, was passaged in the culture with Hep. No. 2 cells, and employed for a viral material. The Hep. No. 2 cells were used for host cells.

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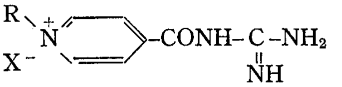
[4] T. Tsukamoto, T. Komori: This Bulletin, **3**, 245 (1955). W. Ciusa, A. Buccelli: Gazz. chim. ital., 88, 393 (1958) (C. A., **53**, 20059 (1959)).

[5] M. R. Lamborg, R. M. Burton, N. O. Kaplan: J. Am. Chem. Soc., **79**, 6173 (1957).

TABLE I. 

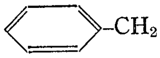
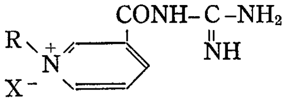
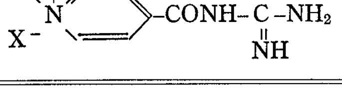
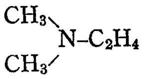
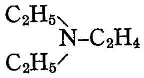
R	X	m.p. (°C)	Yield	Recryst. solvent	Mol. formula	N (%) Calcd.	N (%) Found
CH_3	I	246	80	ethanol-water	$C_8H_{11}ON_4I$	18.30	18.33
〃	Br	241	25	ethanol-ether	$C_8H_{11}ON_4Br$	21.62	21.33
〃	Cl	227～229	43	ethanol	$C_8H_{11}ON_4Cl$	26.10	26.04
C_2H_5	I	221～223	40	ethanol-water	$C_9H_{13}ON_4I$	17.50	17.69
〃	Br	206～207	22	ethanol-ether	$C_9H_{13}ON_4Br$	20.51	20.75
〃	Cl	215～216	26	〃	$C_9H_{13}ON_4Cl$	24.50	24.39
C_3H_7	I	185～186	11	ethanol-water	$C_{10}H_{15}ON_4I$	16.77	16.87
〃	Br	222～223	30	ethanol-ether	$C_{10}H_{15}ON_4Br$	19.51	19.46
C_4H_9	I	173～175	13	ethanol	$C_{11}H_{17}ON_4I$	16.09	16.30
C_6H_{13}	〃	180～182	10	〃	$C_{13}H_{21}ON_4I$	14.89	14.63
〃	Br	85～88	60	ethanol-ether	$C_{13}H_{21}ON_4Br$	17.02	16.88
C_8H_{17}	〃	95～98	30	〃	$C_{15}H_{25}ON_4Br$	15.69	15.58
$C_{10}H_{21}$	I	123～126	14	ethanol	$C_{17}H_{29}ON_4I$	12.96	12.78
〃	Br	111～113	71	ethanol-ether	$C_{17}H_{29}ON_4Br$	14.54	14.50
$C_{12}H_{25}$	〃	108～112	45	〃	$C_{19}H_{33}ON_4Br$	13.55	13.76
$C_{14}H_{29}$	〃	123～126	33	〃	$C_{21}H_{37}ON_4Br$	12.69	12.73
	〃	202	50	ethanol	$C_{14}H_{15}ON_4Br$	16.72	16.55

TABLE II. 

R	X	m.p. (°C)	Yield	Recryst. solvent	Mol. formula	N (%) Calcd.	N (%) Found
CH_3	I	199～200	53	ethanol-water	$C_8H_{11}ON_4I$	18.30	18.32
〃	Br	200～201	48	ethanol	$C_8H_{11}ON_4Br$	21.62	21.71
〃	Cl	199～201	23	〃	$C_8H_{11}ON_4Cl$	26.10	25.95
C_2H_5	I	155～158	21	〃	$C_9H_{13}ON_4I$	17.50	17.69
〃	Br	189～190	31	〃	$C_9H_{13}ON_4Br$	20.51	20.49
〃	Cl	180～181	25	〃	$C_9H_{13}ON_4Cl$	24.50	24.71

TABLE III. 

R	X	m.p. (°C)	Yield	Mol. formula	N (%) Calcd.	N (%) Found
 $(CH_3)_2N\text{-}C_2H_4$	Cl	239～240	27	$C_{11}H_{18}ON_5Cl_2$	22.73	22.82
 $(C_2H_5)_2N\text{-}C_2H_4$	〃	235～236	43	$C_{13}H_{23}ON_5Cl_2$	20.83	20.94

Prior to the experiments, the stocked K-2211 strain was inoculated into an assay tube in which the monosheet of the Hep. No. 2 cells had been established, and then the tube was incubated at 33° for two days by using a roller drum. The completely degenerated cells and culture fluid were frozen and thawed together three times and then the mixture was centrifuged at 3,000 r.p.m. for ten minutes. The supernatant was used as the material for the inoculation. The $TCID_{50}$ of this material was $10^{-6.5}$/ml.

As the growth medium to prepare the monosheet of the Hep. No. 2 cells, the YLA medium supplemented with 15% bovine serum and as the maintenance medium for viral cultivation, the LH basal medium which was consisted of 0.25 percent of lactalbumine hydrolysate and Hank's balanced salt solution (among medium components, the concentration of sodium bicarbonate was modified to 0.03 percent), supplemented with 5 percent bovine serum, were used.

After the determination of the nontoxic concentration of the compounds on the Hep. No. 2 cells, 0.1 ml. of ten times the nontoxic concentration of each of the compounds and 0.8 ml. of the maintenance medium was added to each of assay tubes, in which the monosheet of the Hep. No. 2 cells had been established, and soon later, 0.1 ml. of the undiluted viral material was inoculated into the tubes. To the tubes of the control group, 0.1 ml. of phosphate buffered saline was added instead of the solution of each of the compounds. These tubes of both the treated and control groups were incubated at 33° by using a roller drum until all of the tubes of the control group showed the maximum cytophatic effect.

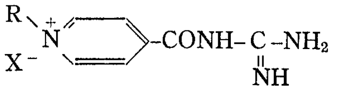
At that time when the tubes of the control group showed the maximum cytophatic effect, the degree of the cytophatic effect of each of the tubes of the tested group was recorded as follows:

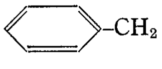
Degree	0 :	None of C. P. E.
	0.5 :	Appearance of C. P. E. in 10~15% of microscopic field.
	1 :	Appearance of C. P. E. in about 25% of microscopic field.
	2 :	Appearance of C. P. E. in about 50% of microscopic field.
	3 :	Appearance of C. P. E. in about 75% of microscopic field.
	4 :	Appearance of C. P. E. in 100% of microscopic field.

Four assay tubes were used for one tested compound, and the average value of C.P.E. score of the four tubes was shown as the C.P.E. score of the given compound.

The experimental results are shown in Table Ⅳ.

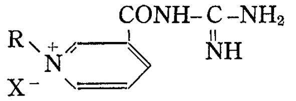
TABLE Ⅳ.



R	X	Results[a]	R	X	Results[a]
CH_3	I	0/3	C_6H_{13}	I	4/4
〃	Br	3.75/4	〃	Br	4/4
〃	Cl	3.5/4	C_8H_{17}	〃	4/4
C_2H_5	I	0/3	$C_{10}H_{21}$	I	4/4
〃	Br	3.5/4	〃	Br	4/4
〃	Cl	3.25/4	$C_{12}H_{25}$	〃	4/4
C_3H_7	I	4/4	$C_{14}H_{29}$	〃	4/4
〃	Br	3/4		〃	4/4
C_4H_9	I	4/4			

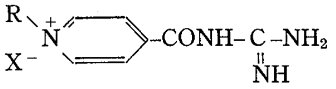
a) Average C.P.E. score of a treated group/Average C.P.E. score of the control group.

TABLE V.



R	X	Results
CH_3	I	0.5/3
〃	Br	3.5/4
〃	Cl	3/4
C_2H_5	I	4/4
〃	Br	4/4
〃	Cl	4/4

TABLE VI.



R	X	Results
CH_3⟍ $N-C_2H_4$ CH_3⟋	Cl	3/4
C_2H_5⟍ $N-C_2H_4$ C_2H_5⟋	〃	4/4

As described above, compounds of (amidinocarbamoyl)pyridine and its quaternary ammonium salt were synthesized for the purpose of finding antiviral agents. Any of the compounds of the former series was not so effective on the virus, but some of the compounds of the latter series exerted an effect on the virus. This fact suggested that the quarterization of the pyridine ring supported the antiviral activity.

As can be understood in the tables, some compounds were found effective on the virus among the series of 1-alkyl-(N-amidinocarbamoyl)pyridinium halide. Of these compounds, it was observed that 1-methyl- and 1-ethyl-4-(amidinocarbamoyl)pyridinium iodide were the most markedly effective on the virus. Besides, the carbon number of alkyl, the position of N-amidinocarbamoyl group and the kind of halogen were found important for the generation of antiviral effect.

At present, it may be said that the selection of methyl or ethyl group as alkyl and iodine atom as halogen were favorable for the construction of antiviral compounds in this series.

On the comparison of the effect of 1-methyl-4-(amidinocarbamoyl)pyridinium iodide with that of 1-methyl-3-(amidinocarbamoyl)pyridinium iodide, it may be suggested that the introduction of amidinocarbamoyl group into 4 position of the pyridine ring might enhance the antiviral effect.

The effectiveness of these two effective compounds was significantly stronger than that of guanidine nitrate and its other salts. This fact suggested that the effect of these compounds was not always due to that of guanidine decomposed from them in the tissue culture liquids.

The detailed investigation along this problem will be discussed in the future.

Experimental

(Amidinocarbamoyl)pyridine——To an ethanolic solution of guanidine, prepared from 3.8 g. of guanidinium chloride and 0.8 g. of Na in 25 ml. of anhyd. EtOH, was added 5 g. of ethyl pyridinecarboxylate. The mixture was allowed to stand some days and the crystals deposited were collected, and washed with cold EtOH and recrystallized from EtOH.

2-(Amidinocarbamoyl)pyridine, m.p. 185~186°. Yield, 64%. *Anal.* Calcd. for $C_7H_8ON_4$: C, 51.21; H, 4.91; N, 34.13. Found: C, 50.81; H, 4.81; N, 34.35.

3-(Amidinocarbamoyl)pyridine, m.p. 195~197°. Yield, 62%. *Anal.* Calcd. for $C_7H_8ON_4$: N, 34.13. Found: N, 34.32.

4-(Amidinocarbamoyl)pyridine, m.p. 235~236°. Yield, 60%. *Anal.* Calcd. for $C_7H_8ON_4$: N, 34.13. Found: N, 34.18.

1-Methyl- and 1-Ethyl-4-(amidinocarbamoyl)pyridinium Halide——i) A mixture of 0.8 g. of 4-(amidinocarbamoyl)pyridine amide and 1 g. of CH_3I (or C_2H_5I) in 30 ml. of anhyd. EtOH was heated on a water bath for several hours. After removal of the solvent, the precipitates deposited were collected and recrystallized from EtOH-H_2O to yellow needles. These pyridinium iodide were converted to the bromides with AgBr and to chlorides with AgCl in MeOH by the usual method.

ii) To an ethanolic solution of guanidine, prepared from 1.2 g. of guanidinium chloride and 0.23 g. of Na in 30 ml. of anhyd. EtOH, was added 3 g. of ethyl ester of 1-methyl-4-carboxypyridinium iodide. The mixture was allowed to stand overnight and the precipitates deposited were collected and recrystallized from EtOH-H_2O. The IR spectrum of this product was identical with that of 1-methyl-4-amidinocarbamoyl) pyridinium iodide obtained in i).

1-Methyl- and 1-Ethyl-3-(amidinocarbamoyl)pyridinium Halide——By the method i) described for the synthesis of 1-methyl-4-(amidinocarbamoyl)pyridinium halide.

1-Alkyl-4-(amidinocarbamoyl)pyridinium Bromide——A mixture of 1.6 g. of 4-(amidinocarbamoyl)-pyridine and 0.015 mole of alkyl bromide (alkyl=C_3H_7, C_6H_{13}~$C_{14}H_{29}$) in 50 ml. of anyhd. BuOH was heated under reflux for 2 hr. After removal of the solvent *in vacuo*, the residue was allowed to stand overnight. The precipitates deposited were collected, washed with ether and recrystallized from EtOH-ether to colorless needles.

1-Benzyl derivative was obtained by the same method using benzyl bromide.

1-Dimethylaminoethyl- and 1-Diethylaminoethyl-4-(amidinocarbamoyl)pyridinium Chloride——A mixture of 0.8 g. of 4-(amidinocarbamoyl)pyridine and 0.8 g. of hydrochloride of dimethylaminoethyl chloride (or 1 g. of hydrochloride of diethylaminoethyl chloride) in 50 ml. of anhyd. BuOH was heated under reflux for 3 hr. After cooling, the precipitates deposited were collected and recrystallized from EtOH-H_2O to colorless needles.

Guanidinium Salt of 1-Methyl-3-carboxypyridinium Iodide——A mixture of ethyl ester of 1-methyl-3-carboxypyridinium iodide and guanidine in anhyd. EtOH was treated by the method ii) described for the synthesis of 1-methyl-(4-amidinocarbamoyl)pyridinium iodide. Recrystallization from EtOH gave yellowish needles. m.p. 185~187°. *Anal.* Calcd. for $C_8H_{13}O_2N_4I$: C, 29.64; H, 4.04; N, 17.28. Found : C, 29.85; H, 3.89; N, 17.41. No depression in melting point of this product was observed upon admixture with authentic sample prepared from 1-methyl-3-carboxypyridinium iodide[6] and guanidine, and the IR spectrum of the former was identical with that of the latter.

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Seiji Miyano[*1] : Deoxygenation of Pyridine 1-Oxide by Means of Benzyl Alcohol.

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The reduction of nitro[1] and carbonyl compounds[2~4] by means of hot benzyl alcoholic solutions of potassium hydroxide has been reported by several groups. More recently the reducing properties of the reagent was well demonstrated by Sprinzak who extended the reduction to nitrogen-carbon[5] and some polarized carbon-carbon double bonds.[6]

The present report concerns the extension of this reduction to pyridine and quinoline 1-oxides. For the deoxygenation of N-O bond in pyridine 1-oxides the use of zinc and dilute acid,[7] iron and acetic acid,[8] phosphorus trihalide,[9] triphenyl phosphite,[10] tri-

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9) M. Hamana : Yakugaku Zasshi, **75**, 123, 127, 130, 135, 263 (1955); C.A., **50**, 1817, 1818, 4542 (1956).

10) *Idem* : *Ibid.*, **75**, 121 (1955); C.A., **50**, 1818 (1956).