[Chem. Pharm. Bull. 22(8)1864—1871(1974)]

UDC 547.861'821.057.09 : 615.28.011.5.015.11

Studies on Chemotherapeutic Agents. II.[1] Synthesis and Antibacterial Activity of 5-Substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]-pyrazine-7-carboxylic Acids

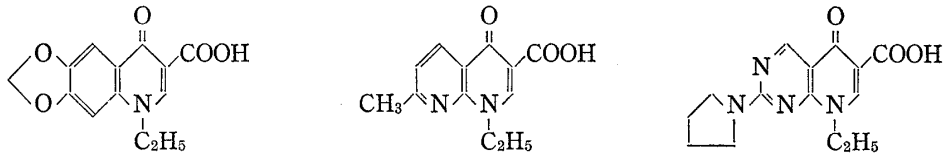
Hideo Nakao, Masami Fukushima, Hiroaki Yanagisawa, and Shin-ichi Sugawara

Central Research Laboratories, Sankyo Co., Ltd.[2]

(Received February 16, 1974)

A series of 5-substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acids were synthesized and evaluated as antibacterial agents. The most active compound *in vitro* against *Escherichia coli* and *Staphylococcus aureus* was 5-ethyl-3-(1-pyrrolidinyl)-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acid (XVIe). Structure-activity relationships are discussed.

In the previous paper,[1] the preparation of sulfonamide or phosphonic acid analogs of oxolinic acid (I) and nalidixic acid (II) was reported and it was found that these analogs showed no significant antibacterial activity.



I II III

On the other hand, piromidic acid (III)[3] in which the pyridine nucleus in II was replaced by a pyrimidine nucleus shows antibacterial activity and has been used clinically. Therefore, our attention was directed next to the compounds containing the pyrido[2,3-*b*]pyrazine skeleton in which the pyrimidine nucleus in III was replaced by a pyrazine nucleus.

Since a search of the literature revealed that the synthesis and antibacterial activity of 5-substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acids have not been reported, we decided to synthesize these compounds, and in the following the results of our investigations are presented.

Chemistry

As a related compound R. Albrecht, *et al.*[4] has reported that 8-hydroxy-3-methoxypyrido-[2,3-*b*]pyrazine-7-carboxylic acid (VI) was obtained from 2-amino-6-methoxypyrazine (IV) *via* diethyl 6-methoxypyrazin-2-ylaminomethylenemalonate (V) by the Gould–Jacobs reaction. On the other hand, D.L. Trepanier, *et al.*[5] reported that diethyl 2-pyrazinylaminomethylenemalonate (VIII) cyclized to ethyl 4-oxo-4H-pyrazino[1,2-*a*]pyrimidine-3-carboxylate (IX) by heating at 250° in Dowtherm.

It is known that cyclization of a 2-aminopyridine series under the conditions of the Gould–Jacobs reaction gives either 1,8-naphthyridine derivatives or pyrido[1,2-*a*]pyrimidine deriva-

1) Part I: H. Yanagisawa, H. Nakao and A. Ando, *Chem. Pharm. Bull.* (Tokyo), **21**, 1080 (1973).
2) Location: *Hiromachi, Shinagawa-ku, Tokyo*, 140, *Japan*.
3) S. Minami, T. Shono and J. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1426 (1971).
4) R. Albrecht and G.A. Hoyer, *Chem. Ber.*, **105**, 3118 (1972).
5) D.L. Trepanier, L.W. Rampy and K.L. Shriver, *J. Med. Chem.*, **11**, 1045 (1968).

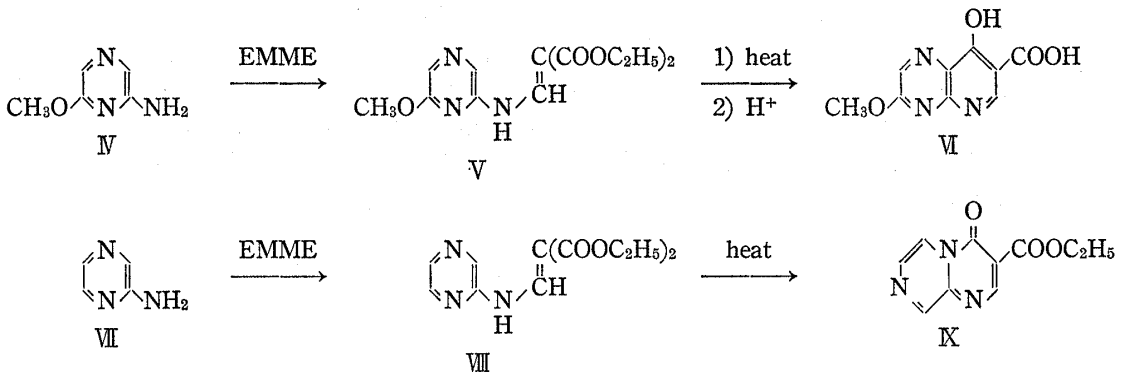


Chart 1

tives.[6] Actually the naphthyridine is formed when the 6-substituent on the pyridine nucleus is an amino, methyl or ethoxy group; whereas the pyrido[1,2-*a*]pyrimidine is produced when the substituent is a 4-methyl, 5-methyl, 5-chloro or 4-ethoxy group.

On the basis of these facts, it was anticipated that 2-aminopyrazines having a substituent at position 6 would furnish pyrido[2,3-*b*]pyrazine derivatives by the Gould–Jacobs reaction.

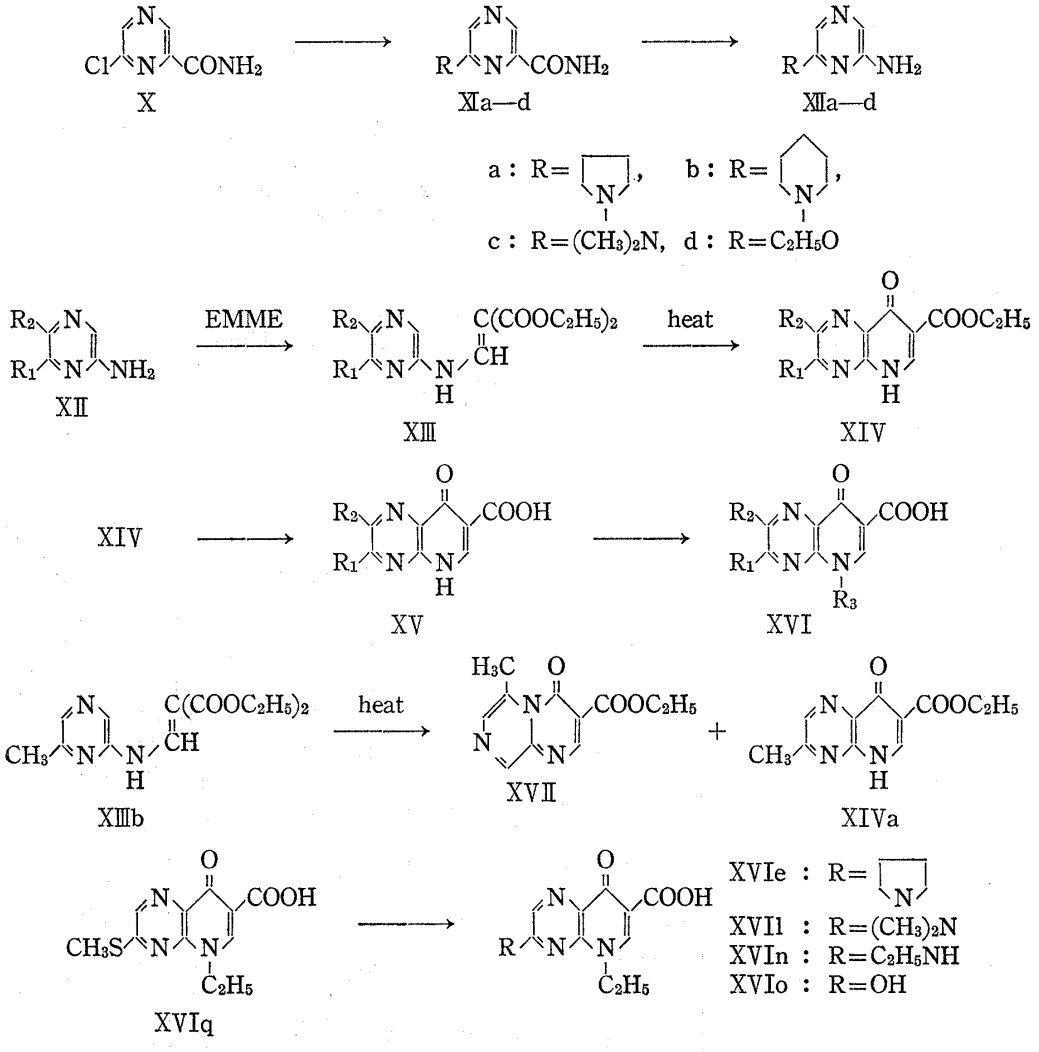


Chart 2

6) M.J. Weiss and C.R. Hauser, "Heterocyclic Compounds," Vol. 7, ed. by R.C. Elderfield, John Willey & Sons, New York, N.Y., 1961, p. 203.

Among the 2-aminopyrazines employed as starting materials, 6-(1-pyrrolidinyl), 6-(1-piperidinyl), 6-dimethylamino and 6-ethoxy derivatives (XIIa—d) were prepared by the Hofmann rearrangement of the corresponding amides (XIa—d) which were obtained from 6-chloropyrazinecarboxamide (X). All of the 2-aminopyrazines investigated reacted readily with diethyl ethoxymethylenemalonate (EMME) at 110° to give diethyl 2-pyrazinylaminomethylenemalonates (XIIIa—i) (Table I).

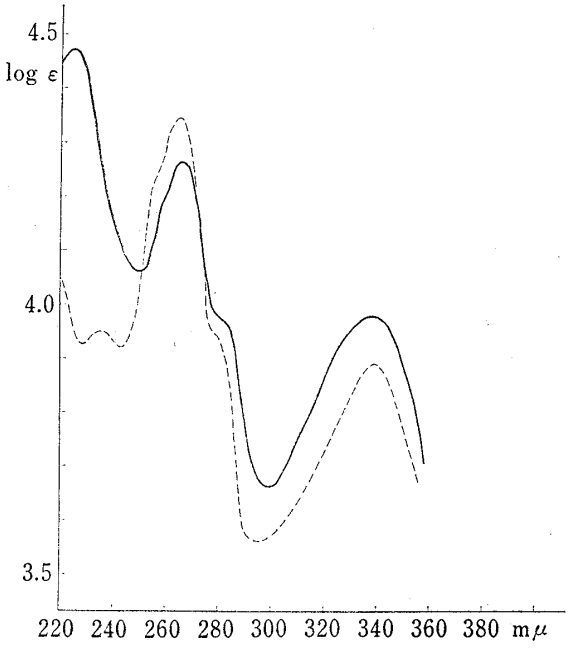


Fig. 1. UV Spectra of VI (——) and XIVa (------) in EtOH

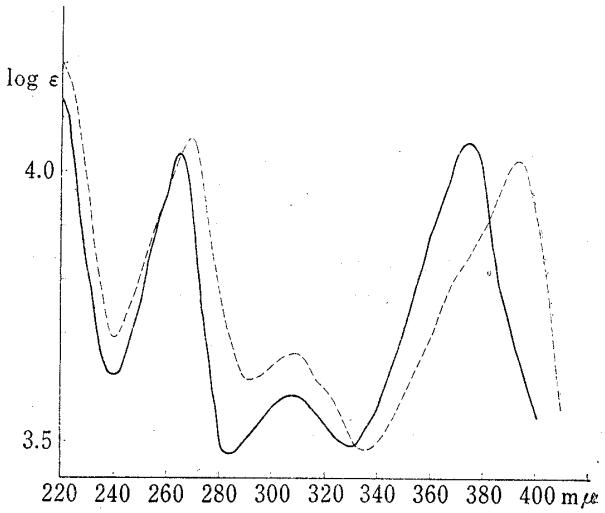
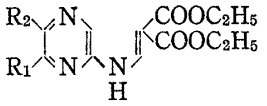


Fig. 2. UV Spectra of IX (——) and XVII (------) in EtOH

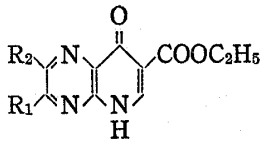
TABLE I. Diethyl 2-Pyrazinylaminomethylenemalonates



Compound XIII	R_1	R_2	mp (°C)	Recrystn. solvent	Yield (%)	Formula	Analysis (%) Calcd. (Found) C	H	N
a	Cl	H	95	cyclohexane	40	$C_{12}H_{14}O_4N_3Cl$	48.09 (47.93)	4.71 (4.65)	14.02 (13.93)
b	CH_3	H	96	cyclohexane	62	$C_{13}H_{17}O_4N_3$	55.90 (56.18)	6.14 (6.14)	15.05 (15.22)
c	CH_3	CH_3	118	cyclohexane 6 / benzene 1	92	$C_{14}H_{19}O_4N_3$	57.32 (57.42)	6.53 (6.57)	14.33 (14.16)
d		H	123	cyclohexane	70	$C_{16}H_{22}O_4N_4$	57.47 (57.05)	6.63 (6.59)	16.76 (16.39)
e		H	103	cyclohexane	50	$C_{17}H_{24}O_4N_4$	58.60 (58.66)	6.94 (6.99)	16.09 (16.36)
f	$N(CH_3)_2$	H	133	cyclohexane 10 / EtOH 1	57	$C_{14}H_{20}O_4N_4$	54.53 (54.37)	6.54 (6.40)	18.17 (18.44)
g	OC_2H_5	H	126	benzene 1 / *n*-hexane 1	57	$C_{14}H_{19}O_5N_3$	54.36 (54.02)	6.19 (6.22)	13.59 (13.61)
h	SCH_3	H	117	cyclohexane	76	$C_{13}H_{17}O_4N_3S$	50.16 (50.42)	5.50 (5.50)	13.50 (13.83)
i	SC_2H_5	H	83	cyclohexane	46	$C_{14}H_{19}O_4N_3S$	51.68 (51.76)	5.89 (5.88)	12.92 (12.85)

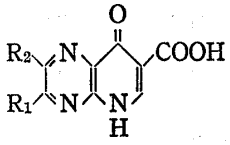
With the exception of the 6-chloro derivative (XIIIa), which failed to cyclize yielding only tars, cyclization of XIII proceeded in refluxing Dowtherm A to give pyrido[2,3-*b*]-pyrazine derivatives (XIVa—h) (Table II).

TABLE II. Ethyl 5,8-Dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylates



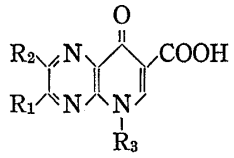
Compound XIV	R_1	R_2	mp (°C)	Recrystn. solvent	Reaction time (min)	Yield (%)	Formula	Analysis (%) Calcd. (Found) C	H	N
a	CH_3	H	280 (dec.)	EtOH	10	10	$C_{11}H_{11}O_3N_3$	56.65 (56.98)	4.75 (4.68)	18.02 (18.07)
b	CH_3	CH_3	270 (dec.)	EtOH	15	15	$C_{12}H_{13}O_3N_3$	58.29 (58.52)	5.30 (5.26)	17.00 (16.65)
c		H	>300	DMF	7	85	$C_{14}H_{16}O_3N_4$	58.32 (58.11)	5.59 (5.61)	19.44 (19.41)
d		H	280 (dec.)	70%EtOH	5	38	$C_{15}H_{18}O_3N_4$	59.59 (59.20)	6.00 (5.86)	18.53 (19.08)
e	$N(CH_3)_2$	H	>280	DMF	6	51	$C_{12}H_{14}O_3N_4$	54.95 (54.71)	5.38 (5.33)	21.37 (21.34)
f	OC_2H_5	H	282 (dec.)	60%EtOH	6	76	$C_{12}H_{13}O_4N_3$	54.75 (54.89)	4.98 (5.08)	15.96 (15.53)
g	SCH_3	H	270 (dec.)	EtOH	7	53	$C_{11}H_{11}O_3N_3S$	49.81 (49.60)	4.18 (4.30)	15.84 (15.84)
h	SC_2H_5	H	280 (dec.)	DMF	6	64	$C_{12}H_{13}O_3N_3S$	51.61 (51.69)	4.69 (4.69)	15.05 (14.82)

TABLE III. 5,8-Dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic Acids



Compound XV	R_1	R_2	mp (°C)	Recrystn. solvent	Yield (%)	Formula	Analysis (%) Calcd. (Found) C	H	N
a	CH_3	H	>300	DMF	41	$C_9H_7O_3N_3$	52.68 (52.50)	3.44 (3.56)	20.48 (20.30)
b	CH_3	CH_3	>300	DMF	62	$C_{10}H_9O_3N_3$	54.79 (54.89)	4.14 (4.12)	19.17 (19.03)
c		H	>300	DMF	88	$C_{12}H_{12}O_3N_4$	55.38 (55.33)	4.65 (4.69)	21.53 (21.31)
d		H	>280	DMF	57	$C_{13}H_{14}O_3N_4$	56.93 (56.92)	5.15 (5.06)	20.43 (20.55)
e	$N(CH_3)_2$	H	>280	DMSO	87	$C_{10}H_{10}O_3N_4$	51.28 (51.00)	4.30 (4.41)	23.92 (23.73)
f	OC_2H_5	H	>280	85% AcOH	53	$C_{10}H_9O_4N_3$	51.06 (51.40)	3.86 (3.75)	17.87 (17.80)
g	SCH_3	H	>280	DMF	68	$C_9H_7O_3N_3S$	45.57 (45.61)	2.98 (3.13)	17.72 (17.74)
h	SC_2H_5	H	>280	DMF	62	$C_{10}H_9O_3N_3S$	47.81 (48.00)	3.61 (3.61)	16.73 (16.47)

TABLE IV. 5-Substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic Acids



Compound XVI	R_1	R_2	R_3	mp (°C)	Recrystn. solvent	Yield (%)	Formula	Analysis (%) Calcd. (Found) C	H	N
a	CH_3	CH_3	C_2H_5	265 (dec.)	EtOH	35	$C_{12}H_{13}O_3N_3$	58.29 (58.62)	5.30 (5.40)	17.00 (16.99)
b	CH_3	H	CH_3	260 (dec.)	DMF	38	$C_{10}H_9O_3N_3$	54.79 (54.77)	4.14 (4.01)	19.17 (19.36)
c	CH_3	H	C_2H_5	260 (dec.)	EtOH	37	$C_{11}H_{11}O_3N_3$	56.65 (56.86)	4.75 (4.92)	18.02 (17.66)
d		H	CH_3	>280	DMF	60	$C_{13}H_{14}O_3N_4$	56.93 (57.17)	5.15 (5.20)	20.43 (20.48)
e		H	C_2H_5	>280	DMF	75	$C_{14}H_{16}O_3N_4$	58.32 (58.34)	5.59 (5.51)	19.44 (19.27)
f		H	*n*-C_3H_7	>280	DMF	55	$C_{15}H_{18}O_3N_4$	59.59 (59.59)	6.00 (5.93)	18.53 (18.57)
g		H	$CH_2CH{=}CH_2$	>280	DMF	62	$C_{15}H_{16}O_3N_4$	59.99 (59.84)	5.37 (5.43)	18.66 (18.30)
h		H	$CH_2C{\equiv}CH$	280	EtOH	37	$C_{15}H_{14}O_3N_4$	60.39 (60.42)	4.73 (4.66)	18.78 (18.41)
i		H	$CH_2COOC_2H_5$	278 (dec.)	60% EtOH	50	$C_{16}H_{18}O_5N_4$	55.48 (54.66)	5.24 (5.30)	16.18 (15.41)
j		H	C_2H_5	>280	EtOH	37	$C_{15}H_{18}O_3N_4$	59.59 (59.29)	6.00 (6.05)	18.53 (18.55)
k	$N(CH_3)_2$	H	CH_3	>280	DMF	59	$C_{11}H_{12}O_3N_4$	53.22 (53.20)	4.87 (5.27)	22.57 (22.17)
l	$N(CH_3)_2$	H	C_2H_5	>280	DMF	56	$C_{12}H_{14}O_3N_4$	54.94 (54.62)	5.38 (5.26)	21.37 (21.50)
m	$N(CH_3)_2$	H	*n*-C_3H_7	>280	DMF	54	$C_{13}H_{16}O_3N_4$	56.51 (56.71)	5.84 (5.89)	20.28 (20.42)
n	NHC_2H_5	H	C_2H_5	>280	DMF	38	$C_{12}H_{14}O_3N_4$	54.95 (54.90)	5.38 (5.21)	21.37 (21.31)
o	OH	H	C_2H_5	>280	DMF	35	$C_{10}H_9O_4N_3$	51.06 (51.06)	3.86 (3.98)	17.87 (18.18)
p	OC_2H_5	H	C_2H_5	260	60% EtOH	37	$C_{12}H_{13}O_4N_3$	54.75 (54.78)	4.98 (5.22)	15.96 (15.61)
q	SCH_3	H	C_2H_5	270 (dec.)	EtOH	52	$C_{11}H_{11}O_3N_3S$	49.81 (49.89)	4.18 (4.16)	15.84 (15.72)
r	SC_2H_5	H	C_2H_5	232	EtOH	59	$C_{12}H_{13}O_3N_3S$	51.61 (51.26)	4.69 (4.76)	15.05 (15.23)

Interestingly, in spite of the presence of a substituent at the 6-position, cyclization of XIIIb afforded pyrazino[1,2-*a*]pyrimidine (XVII) besides pyrido[2,3-*b*]pyrazine (XIVa). In the case of 2-amino-6-methylpyridine, the sole product from cyclization reported is 1,8-naphthyridine.[7] The structures of these cyclized products (XIV and XVII) were confirmed by the similarity of their ultraviolet (UV) spectra with those of the known compounds VI and IX, respectively (Fig. 1 and 2).

Hydrolysis of XIV gave the corresponding acids (XV), which were converted to 5-alkyl-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acids (XVI) by alkylation. The physical and analytical data are shown in Table III and IV, respectively.

7) G.R. Lappin, *J. Am. Chem. Soc.*, **70**, 3348 (1948).

3-(Substituted)amino-5-ethyl-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acids (XVIe, l and n) were prepared alternatively by treating the 3-methylthio derivative (XVIq) with an appropriate amine. On the other hand, alkaline hydrolysis of XVIq gave the corresponding 3-hydroxy analogue (XVIo).

Biological Activity

The *in vitro* antibacterial activity of pyrido[2,3-*b*]pyrazine derivatives obtained in this study was tested by the serial agar dilution method. The minimum inhibitory concentrations are listed in Table V. Most of the 5-substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acids exhibited activity, but all of the 5-unsubstituted acids and the ethyl esters showed no activity. The most effective substituent at position 5 was an ethyl group. Replacement of the ethyl by other substituents caused a decrease in the activity to varying degrees. Most effective as a substituent at position 3 was the 1-pyrrolidinyl group. Dimethylamino, 1-piperidinyl, alkylthio and alkoxy groups also were effective. Replacement of the ethoxy by hydroxy group resulted in a decrease of the activity. Interestingly this is similar to previously reported results observed in pyrido[2,3-*d*]pyrimidine series.[3] In this series of compounds, 5-ethyl-3-(1-pyrrolidinyl)-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic acid (XVIe) was the most active. As shown in Table V, activity of XVIe was similar to that of II.

Table V. Antibacterial Activity (M.I.C., mcg/ml)

Compound	Organism						
	Staph. aureus 209P	*E. coli* NIHJ	*Sh. flexneri* 2a	*Klebsiella* 806	*Proteus vulgaris*	*Salm. enteritidis*	*Pseud. aeruginosa*
I	1.5	0.8	0.1	1.5	0.05	0.2	25
II	25	3.1	0.8	6.2	0.4	3.1	100
III	6.2	25	6.2	25	0.4	25	>100
XVc	>100	>100	>100	>100	>100	>100	>100
XVIa	>100	100	25	100	100	100	>100
XVIb	>100	>100	>100	>100	>100	>100	>100
XVIc	>100	25	25	50	50	50	>100
XVId	50	50	25	50	25	50	>100
XVIe	6.2	1.5	0.8	1.5	0.8	3.1	25
XVIf	12.5	12.5	3.1	12.5	3.1	12.5	>100
XVIg	50	25	6.2	25	3.1	25	>200
XVIh	50	50	6.2	50	6.2	25	>200
XVIi	>100	>100	>100	>100	>100	>100	>100
XVIj	25	25	12.5	50	3.1	50	>200
XVIk	50	50	25	>100	50	>100	>100
XVIl	50	3.1	0.8	3.1	1.5	3.1	100
XVIm	50	6.2	1.5	12.5	3.1	6.2	200
XVIn	100	50	12.5	100	25	50	>200
XVIo	>100	>100	>100	>100	>100	>100	>100
XVIp	100	6.2	3.1	12.5	6.2	6.2	>100
XVIq	50	3.1	0.8	3.1	1.5	3.1	200
XVIr	25	6.2	1.5	6.2	3.1	6.2	>200

Experimental

6-(1-Pyrrolidinyl)pyrazinecarboxamide (XIa)——A mixture of 6-chloropyrazinecarboxamide[8] (X) (2.5 g), pyrrolidine (3 ml) and EtOH (25 ml) was refluxed for 3.5 hr with stirring. After cooling, the separated crystals were collected and recrystallized from 60% EtOH (150 ml) to yield 2.2 g of pale yellow crys-

8) M. Asai, *Yakugaku Zasshi*, **81**, 1475 (1961).

tals, mp 277°. *Anal.* Calcd. for $C_9H_{12}ON_4$: C, 56.23; H, 6.29; N, 29.15. Found: C, 56.53; H, 6.21; N, 29.09.

6-(1-Piperidinyl)pyrazinecarboxamide (XIb)——By the same procedure to that for XIa, reaction of X (5 g) and piperidine (7 ml) gave 5.3 g of XIb, mp 216°. *Anal.* Calcd. for $C_{10}H_{14}ON_4$: C, 58.23; H, 6.84; N, 27.17. Found: C, 58.00; H, 6.88; N, 27.02.

6-Dimethylaminopyrazinecarboxamide (XIc)——Reaction of X (37.4 g) and 40% aqueous dimethylamine (200 ml) in EtOH (280 ml) gave XIc by the same procedure to that for XIa. Yield, 34 g, mp 248°. *Anal.* Calcd. for $C_7H_{10}ON_4$: C, 50.59; H, 6.07; N, 33.72. Found: C, 50.35; H, 6.05; N, 33.60.

6-Ethoxypyrazinecarboxamide (XId)——To a solution of Na (2.8 g) in EtOH (200 ml) was added X (15 g) and the mixture was refluxed for 1.5 hr. After cooling the separated crystals were collected, washed with water and recrystallized from EtOH to give 11 g of XId as colorless needles, mp 171°. *Anal.* Calcd. for $C_7H_9O_2N_3$: C, 50.29; H, 5.43; N, 25.14. Found: C, 50.29; H, 5.42; N, 25.25.

2-Amino-6-(1-pyrrolidinyl)pyrazine (XIIa)——To a solution of KOH (18 g) in H_2O (165 ml) was added dropwise Br_2 (10.5 g) at 0° with stirring. To the cold solution was added XIa (18 g) followed by EtOH (30 ml) after 5 min. The mixture was stirred at 0° for 3.5 hr. After 130 ml of EtOH was added, the mixture was gradually heated to 75° and stirred at this temperature for 3.5 hr. The mixture was concentrated to volume of about 100 ml under reduced pressure and then the separated crystals were collected and recrystallized from benzene to give colorless prisms, mp 157°, yield 2 g. *Anal.* Calcd. for $C_8H_{12}N_4$: C, 58.51; H, 7.37; N, 34.12. Found: C, 58.49; H, 7.34; N, 33.89.

The mother liquor from collection of crude crystals of XIIa was made alkaline and extracted with CH_2Cl_2. Concentration of the extract gave 3 g of XIIa, which was used in following step without purification.

2-Amino-6-(1-piperidinyl)pyrazine (XIIb)——By the similar procedure to that for XIIa, from XIb (4.9 g) was obtained XIIb (2.3 g), mp 129°. *Anal.* Calcd. for $C_9H_{14}N_4$: C, 60.65; H, 7.92; N, 31.44. Found: C, 60.73; H, 7.78; N, 31.24.

2-Amino-6-dimethylaminopyrazine (XIIc)——To a solution of KOH (36.2 g) in H_2O (325 ml) was added dropwise Br_2 (6.75 ml) at 0—3° with stirring. To the cold solution was added XIc (20 g) and the mixture was stirred at 0° for 3 hr. After addition of EtOH (200 ml), the mixture was heated to 75° and stirred for 3 hr. Then the reaction mixture was concentrated to volume of about 100 ml under reduced pressure. The separated crystals were collected, washed with cold water and dried yielding 7.5 g of XIIc.

The above mother liquor was extracted with $CHCl_3$ and evaporation of $CHCl_3$ gave second crop (4 g) of XIIc. For analysis a part of the product was recrystallized from cyclohexane–ethanol (10: 1), mp 156°. *Anal.* Calcd. for $C_6H_{10}N_4$: C, 52.15; H, 7.30; N, 40.55. Found: C, 52.18; H, 7.15; N, 40.41.

2-Amino-6-ethoxypyrazine (XIId)——This compound was prepared from XId by the similar method to that for 2-amino-6-methoxypyrazine (IV).[9] Yield, 55%, mp 76° (from benzene–hexane). *Anal.* Calcd. for $C_6H_9ON_3$: C, 51.78; H, 6.52; N, 30.20. Found: C, 51.43; H, 6.33; N, 30.19. The following 2-aminopyrazines were prepared according to the literature. 6-Methyl,[10] 5,6-dimethyl,[10] 6-chloro,[9] 6-methylthio[11] and 6-ethylthio[11] derivatives.

Preparation of Diethyl 2-Pyrazinylaminomethylenemalonates (XIIIa—i)——An equimolar mixture of an appropriate 2-aminopyrazine and diethyl ethoxymethylenemalonate was heated at 120° for 3 hr. After the mixture was allowed to cool, the solid separated was recrystallized from an appropriate solvent (Table I).

Preparation of Ethyl 5,8-Dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylates (XIVa—h)——An appropriate XIII was added to about a 10-fold volume of boiling Dowtherm A. The mixture was heated for 5—20 min under reflux, then cooled and diluted with a 5-fold volume of *n*-hexane. The precipitates were collected, washed with EtOH and recrystallized (Table II).

5,8-Dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic Acids (XVa—h)——Method a) A mixture of XIV, about 10-fold volume of 5% NaOH and 4-fold volume of EtOH was refluxed until it became clear. After cooling the alkaline solution was acidified with 10% HCl, and the precipitates were collected, washed with EtOH and recrystallized.

Method b) A mixture of XIVf (1.5 g), AcOH (20 ml) and 1N-HCl (10 ml) was refluxed for 20 min. After cooling the separated crystals were collected washed with water and recrystallized from 85% AcOH yielding 700 mg of XVf as colorless leafletes, mp>280° (Table III).

5-Substituted-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylic Acids (XVIa—r)——Method a) Alky lati on of XV: To a solution of K_2CO_3 (0.5 g) in H_2O (10 ml) and N,N-dimethyl formamide (DMF) (20 ml) was dissolved XV (0.5 g) at 60° and then added alkyl bromide or iodide (1.5—2 g). The mixture was stirred at 60° for 1 hr. The separated crystals were collected, washed with water and recrystallized (Table IV).

9) G. Palamidessi and L. Bernardi, *Gazz. Chim. Ital.*, **91**, 1431 (1961).
10) R.T. Arnold and N. Bortnick, *J. Am. Chem. Soc.*, **67**, 802 (1945).
11) N. Okuda, Y. Fukuda, I. Kuniyoshi and H. Shinoda, Japan Patent 12712 (1965) [*C.A.*, **63**, 11589c (1965)].

Method b) Hydrolysis of XVIq: A mixture of XVIq (400 mg) and 10% aqueous NaOH (10 ml) was heated for 6 hr under reflux. After cooling, the reaction mixture was acidified with 10% HCl, and the precipitates were collected, washed with water and recrystallized from DMF to yield 200 mg of XVIo.

Method c) Substitution Reaction of XVIq with Amines: A mixture of 265 mg (1 mmole) of XVIq and an appropriate amine (1.5—2 mmoles) in EtOH (20 ml) was heated at 95—105° for 7 hr in a sealed tube. The solvent was evaporated and the crystalline residue was recrystallized from DMF or EtOH to yield XVIe, XVIl or XVIn (Table IV).

Cyclization of Diethyl 6-Methylpyrazin-2-yl-aminomethylenemalonate (XIIIb)——XIIIb (1 g) was added to boiling Dowtherm A (10 ml), and the mixture was refluxed for 10 min. After cooling, to the reaction mixture was added petroleum ether (50 ml), and the precipitates were collected, washed with petroleum ether and recrystallized from EtOH to give 80 mg of ethyl 3-methyl-5,8-dihydro-8-oxopyrido[2,3-*b*]pyrazine-7-carboxylate (XIVa) (Table II). UV λ_{max}^{EtOH} mμ(log ε): 235 (3.95), 265 (4.38), 278 (sh) (3.96), 339 (3.89).

On the other hand, the mother liquor from filtration of precipitates of XIVa was concentrated to almost dryness under reduced pressure, and the residue was chromatographed on a dry column of silica gel (50 g) using $CHCl_3$–AcOEt (4: 1) mixture as solvent. Extraction of the part whose *Rf* value was about 0.25 with AcOEt and recrystallization from ethanol–petroleum ether (2: 1) mixture gave 170 mg (20%) of ethyl 6-methyl-4-oxo-4H-pyrazino[1,2-*a*]pyrimidine-3-carboxylate (XVII), mp 97°. *Anal.* Calcd. for $C_{11}H_{11}O_3N_3$: C, 56.90; H, 4.73; N, 17.73. Found: C, 56.65; H, 4.75; N, 18.02. UV λ_{max}^{EtOH} mμ(log ε): 211 (4.08), 268 (4.06), 308 (3.65), 394 (4.02).

Acknowledgement The authors express their deep gratitudes to Dr. G. Sunagawa, director of this laboratories for his kind encouragement.