(MeOH–soluble part) was allowed to stand at room temperature and colorless crystals (**25**) which separated out were collected, 11 mg, mp 175° (decomp.). **25**: *Anal.* Calcd. for $C_{15}H_{12}O_4NBr$: C, 51.45; H, 3.45; N, 4.00. Found: C, 51.23; H, 3.53; N, 3.89. **26**: *Anal.* Calcd. for $C_{15}H_{12}O_4NBr$: C, 51.45; H, 3.45; N, 4.00. Found: C, 52.16; H, 3.40; N, 3.60.

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Pyrido[2,3-*d*]pyrimidine Antibacterial Agents. I. 8-Alkyl-5,8-dihydro-5-oxopyrido[2,3-*d*]-pyrimidine-6-carboxylic Acids and Related Compounds

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Since nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-napthyridine-3-carboxylic acid)[2] was developed as an useful antibacterial agent for gram-negative microorganisms, attention has been focussed on the compounds consisting of pyrido[2,3-*d*]pyrimidine skeleton which has one more nitrogen atom than 1,8-naphthyridine ring system in nalidixic acid. Thus Lesher[3] has reported that 8-ethyl-2-methyl- and 2,4,8-trimethyl-5,8-dihydro-5-oxopyrido-[2,3-*d*]pyrimidine-6-carboxylic acids exhibit *in vivo* activity against *Klebsiella pneumoniae* and *Salmonella typhimurium* in mice on oral administration. In the similar interest Nishigaki, *et al.*[4] also have described on the synthesis of 2-methyl-4-substituted-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids.

We have studied at the almost same time as Lehser on 8-alkyl-2-substituted- and -2,4-di-substituted-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (VI)[5] and the related compounds. This paper deals with the synthesis and structure–antibacterial activity relationship of these compounds.

Condensation of 4-aminopyrimidines (I) with diethyl ethoxymethylenemalonate gave readily diethyl N-(4-pyrimidinyl)aminomethylenemalonates (II). Then II was subjected to thermal cyclization in refluxing diphenyl ether to afford ethyl 5,8-dihydro-5-oxopyrido[2,3-*d*]-pyrimidine-6-carboxylates (III). Hydrolysis and ethylation of III gave easily 5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (IV) and ethyl 8-ethyl-5,8-dihydro-5-oxopyrido-[2,3-*d*]pyrimidine-6-carboxylates (V), respectively.

The structure of V was supported by their infrared spectra in the dilute chloroform solution which showed the absorption bands at 1729 and 1690 cm^{-1} for Va, and at 1720 and 1680 cm^{-1} for Vc, indicative of the presence of β-ketoester function.

1) Location: *Enoki-cho 33-94, Suita, Osaka.*
2) G.Y. Lesher, E.J. Eroelish, M.D. Gruett, J.H. Bailey, and R.P. Brundage, *J. Med. Pharm. Chem.*, **5**, 1063 (1962).
3) G.Y. Lesher, U. S. Patent 3320257 (1967) [*C. A.*, **68**, 49643 (1968)].
4) S. Nishigaki, K. Ogiwara, K. Senga, S. Fukazawa, K. Aida, Y. Machida, and F. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **18**, 1385 (1970).
5) S. Minami, T. Shono, M. Shimizu, and Y. Takase, Japan. Patent, Pub., 25911/67 (1967)[*C. A.*, **69**, 52185 (1968)].

The subsequent alkylation of IV afforded VI, of which VIh and VIi were prepared alternatively by hydrolysis of Vc and Vd, respectively.

In the case of alkaline treatment of Va, Vb, VIa and VIb, it was found that the substitution reactions on the 2-methylthio and 2-methoxy groups with hydroxy anion occured to give all 8-ethyl-5,8-dihydro-2-hydroxy-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (VIIc) while neither methylthio nor methoxy group of IIIa, IIIb, IVa and IVb was affected under the same condition.

Va and VIa underwent desulfurizarion with Raney Ni to yield VIIb and VIIa, respectively. On treating 2- and 4-hydroxy compounds (VIIc and VIg) with phosphorus oxychloride the corresponding 2- and 4-chloro derivatives (VIId and VIIe) were obtained.

The *in vitro* antibacterial activities of these compounds were tested against *Escherichia coli* and *Staphylococcus aureus* by the serial tube dilution method.[6]

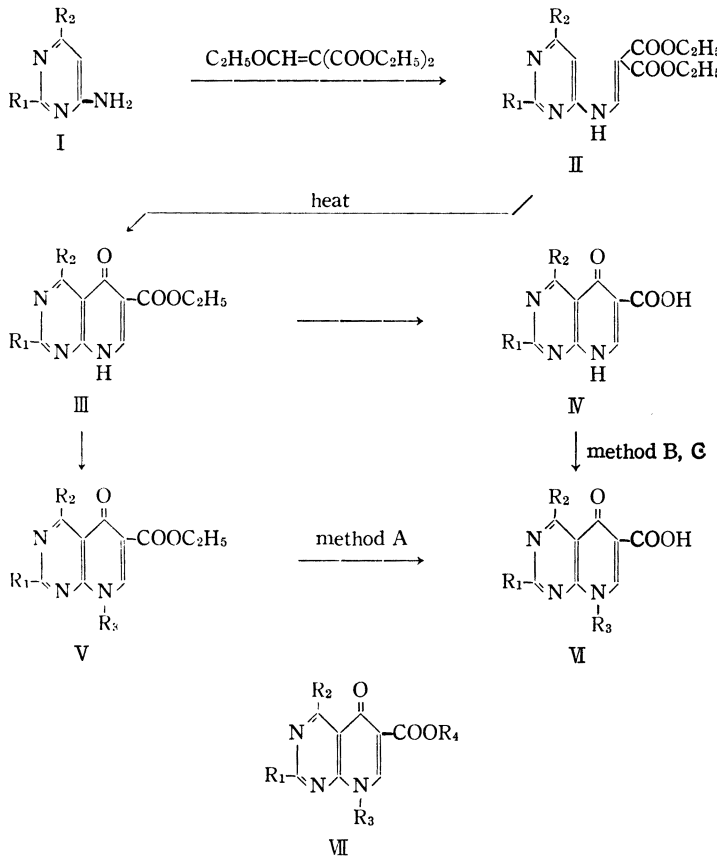


Chart 1

The minimum inhibitory concentrations were listed in Table I. The activity against *Escherichia coli* were generally superior to that against *Stapylococcus aureus*. The structure–activity relationship for *Escherichia coli* was as follows.

Alkylation of IV at the position 8 to VI was resulted in the enhancement of the activity and, however, the esters (V) was brought about the loss of it. The introduction of the electron–

6) K. Fujimoto, *Chemotherapy*, **15**, 228 (1967).

releasing groups such as methylthio (VIa) and methoxy (VIb) groups at the position 2 of VIIa greatly enhaced the activity, while that of the electron-attracting groups such as chloro (VIId) and hydroxy (VIIc)[7] groups caused the decrease of it. On the other hand, the further replacement of hydrogen at the position 4 of VIa with hydroxy (VIg) or chloro (VIIe), and of VIi with methyl (VIh) dropped the activity. Among the compounds in this series, VIa was the most active.

As a result, it is conceivable from these findings that both of 5-oxo-6-carboxy and 8-alkyl groups may be essential for the activity and the presence of an electron-releasing substituent at the position 2 important to the enhancement of the acitivity.

Experimental[8]

4-Aminopyrimidine (I)——The following 4-aminopyrimidines used as the starting material were prepared according to the literatures; 2-methoxy-,[9a] 2-methylthio-,[9b] 2-methylthio-6-hydroxy-,[9c] 2-methyl-,[9d] and 2,6-dimethyl-4-aminopyrimidines.[9e]

TABLE I. *In Vitro* Antibacterial Activity (Minim. Inhib. Conc., μg/ml)

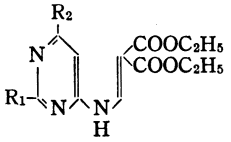
Compd.	*Staphylococcus aureus*	*Escherichia coli*
IIIa	>100	>100
b	>100	>100
c	>100	>100
d	>100	>100
e	>100	30
IVa	>100	>100
b	>100	>100
c	>100	>100
d	>100	>100
e	>100	>100
Va	>100	>100
b	>100	>100
c	>100	>100
d	>100	>100
VIa	30	3
b	>100	10
c	>100	30
d	>100	10
e	>100	30
f	>100	30
g	>100	10
h	>100	100
i	>100	30
VIIa	>100	30
b	>100	>100
c	>100	>100
d	>100	>100
e	>100	>100

7) The infrared spectra of 2-hydroxypyrido[2,3-*d*]pyrimidines suggest that the oxo-structures are predominant in solid phase: W.J. Irwin and D.G. Wibberley, "Advances in Heterocyclic Chemistry," Vol. 10, ed. by A.R. Katritzky and A.J. Boulton, Academic Press, New York, 1969, p. 185.

8) Melting points were taken in an open capillary tube and uncorrected. Yields in the Tables are of purified product and are not maximal.
Infrared spectra were recorded on a Hitachi Model EPI-S2 infrared spectrophotometer.

9) *a*) G. Spiteller and H. Bretschneider, *Monatsh. Chem.*, **92**, 183 (1961); *b*) H.L. Wheeler and H.S. Bristol, *Am. Chem. J.*, **33**, 437 (1905); *c*) B.R. Baker, J.P. Joseph, and R.E. Schaub, *J. Org. Chem.*, **19**, 631 (1954); *d*) S. Gabriel, *Chem. Ber.*, **37**, 3641 (1904); *e*) T.L. Cairns, A.W. Larchar, and B.C. Mckusick, *J. Am. Chem. Soc.*, **74**, 5633 (1952).

TABLE II. Diethyl N-(4-Pyrimidinyl)aminomethylenemalonates



Compd.	R_1	R_2	mp (°C)	Recrystn. solvent	Yield (%)	Formula		Analysis (%) C	H	N	S
IIa	SCH_3	H	106—107	EtOH	53	$C_{13}H_{17}O_4N_3S$	Calcd.	50.15	5.50	13.50	10.30
							Found	49.88	5.80	13.10	10.20
IIb	OCH_3	H	102—103	EtOH	63	$C_{13}H_{17}O_5N_3$	Calcd.	52.87	5.80	14.23	—
							Found	52.47	5.70	14.20	—
IIc	CH_3	CH_3	123—125[a]	EtOH	44	$C_{14}H_{19}O_4N_3$	Calcd.	57.32	6.53	14.33	—
							Found	57.04	6.80	14.47	—
IId	CH_3	H	102—103[b]	EtOH	75	$C_{13}H_{17}O_4N_3$	Calcd.	55.90	6.14	15.05	—
							Found	56.30	6.13	14.91	—
IIe	SCH_3	OH	228—230	EtOH	39	$C_{13}H_{17}O_5N_3S$	Calcd.	47.70	5.23	12.84	9.80
							Found	47.96	5.47	12.97	10.01

a) lit.[9] mp 108—110° *b*) lit.[9] mp 103.8—104.5°

Diethyl N-(2- and 2,6-Di-substituted-4-pyrimidinyl)aminomethylenemalonates (II)——An equimolar mixture of an appropriate 4-aminopyrimidine and diethyl ethoxymethylenemalonate was heated at 120—140° with stirring for 3 hr during which the liberated EtOH was removed by distillation under ordinary pressure. After the mixture was allowed to cool, the solid separated was collected and recrystallized from an appropriate solvent (Table II).

Ethyl 2-Substituted- and 2,4-Di-substituted-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylates (III)——II was added to about a 10-fold volume of boiling diphenylether. The mixture was heated to reflux for 10—30 min, cooled, and diluted with a 5-fold volume of *n*-hexane. The precipitates were collected, washed with EtOH and recrystallized (Table III).

2-Substituted- and 2,4-Di-substituted-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids (IV)——A suspension of III (0.01 mole) in 7% aqueous NaOH (30 ml) was heated at 100° until it became clear. After cooling the alkaline solution was acidified with AcOH, and the precipitates were collected, washed with water and recrystallized (Table III).

Ethyl 8-Ethyl-5,8-dihydro-2-substituted- and -2,4-di-substituted-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylates (V)——A mixture of III (0.10 mole), EtI (0.15 mole), 20% aqueous K_2CO_3 (30 ml) and DMF (200 ml) was heated at 95—100° with stirring for 1 hr. After cooling, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. To the residue was added an aqueous NaCl saturated solution and the mixture was extracted with $CHCl_3$. The extract was washed with water. After evaporation of the solvent, the resulting solid was collected and recrystallized (Table III).

8-Alkyl-5,8-dihydro-2-substituted- and -2,4-di-substituted-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids (VI) (Table III)——Method A: Hydrolysis of Vc and Vd: In the same treatment as with hydrolysis of III, Vc and Vd (0.01 mole) were hydrolysed with 5% aqueous NaOH (20 ml), giving VIh and VIi, respectively.

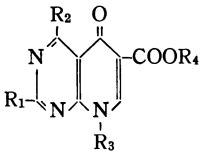
Method B: Alkylation of IV with Dialkyl Sulfate: To a solution of IV (0.03 mole) in 4% aqueous KOH was added dropwise Et_2SO_4 or Me_2SO_4 (0.02 mole) during 30 min with vigorous stirring. The mixture was maintained at the same temperature for 2 hr with stirring and allowed to stand overnight at room temperature. After acidification with AcOH, the precipitates were collected, washed with water and recrystallized to give VIa, VIb, VIg—i, or VIc.

Method C: Alkylation of IVa with Alkyl Bromide: In the same manner as in the case of alkylation of III with EtI, IVa was treated with *n*-PrBr, iso-PrBr or *n*-BuBr, respectively, to yield VId—f.

8-Ethyl-5,8-dihydro-2-hydroxy-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (VIIc)——A mixture of Va or Vb (0.01 mole) in 5% aqueous NaOH (4 ml) was heated at 90—95° for 15 min. The reaction mixture was cooled and acidified with AcOH. The resulting crystals were collected, washed with water and recrystallized to afford VIIc, which was identical in all respects with the sample prepared in same treatment of VIa with 5% aqueous NaOH.

8-Ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (VIIa) and Its Ethyl Ester (VIIb)——A mixture of VIa or Va (0.003 mole) and Raney Ni (W-4) (1.0 g) in absolute EtOH or dioxane (30 ml) was refluxed with stirring for 5 hr. The cold reaction mixture was filtered and the filtrate was concentrated under reduced pressure and the residual solid was recrystallized (Table III).

TABLE III. Ethyl 5,8-Dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylates and Their Acids



Compd.	R_1	R_2	R_3	R_4	mp (°C)	Recrystn. solvent	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	S (Cl)	Found C	H	N	S (Cl)
IIIa	SCH_3	H	H	C_2H_5	269—271 (decomp.)	EtOH	72	$C_{11}H_{11}O_3N_3S$	49.80	4.18	15.84	12.09	49.68	4.31	15.57	11.83
b	OCH_3	H	H	C_2H_5	235—237	EtOH	75	$C_{11}H_{11}O_4N_3$	53.01	4.45	16.86	—	52.61	4.48	16.79	—
c	CH_3	CH_3	H	C_2H_5	266—267[a]	EtOH	25	$C_{12}H_{13}O_3N_3$	58.29	5.30	17.00	—	58.33	5.54	16.81	—
d	CH_3	H	H	C_2H_5	282—284[b] (decomp.)	EtOH	11	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	—	56.56	4.94	18.07	—
e	SCH_3	OH	H	C_2H_5	261—263	EtOH–$CHCl_3$	80	$C_{11}H_{11}O_4N_3S$	46.97	3.94	14.94	11.40	47.06	4.03	14.95	11.45
IVa	SCH_3	H	H	H	279—281 (decomp.)	MeOH	78	$C_9H_7O_3N_3S$	45.57	2.98	17.72	13.49	45.35	2.96	17.34	13.43
b	OCH_3	H	H	H	>300	—[c]	68	$C_9H_7O_4N_3$	48.87	3.19	19.00	—	43.48	3.38	17.15[c]	—
c	CH_3	CH_3	H	H	266—267[d]	EtOH	75	$C_{10}H_9O_3N_3$	54.79	4.14	19.17	—	54.39	4.32	18.87	—
d	CH_3	H	H	H	260—262[e]	EtOH–AcOH	70	$C_9H_7O_3N_3$	52.68	3.44	20.48	—	52.52	3.28	20.32	—
e	SCH_3	OH	H	H	>300	—[f]	85	$C_9H_7O_4N_3S$	42.69	2.79	16.59	12.66	42.76	3.09	16.29	12.36
Va	SCH_3	H	C_2H_5	C_2H_5	145—146	EtOH	85	$C_{13}H_{15}O_3N_3S$	53.22	5.15	14.33	10.93	53.23	5.21	14.09	10.73
b	OCH_3	H	C_2H_5	C_2H_5	147—149	hexane–MeOH	47	$C_{13}H_{15}O_4N_3$	56.31	5.45	15.16	—	56.55	5.66	15.13	—
c	CH_3	CH_3	C_2H_5	C_2H_5	157—158	EtOH	54	$C_{14}H_{17}O_3N_3$	61.08	6.22	15.26	—	60.89	6.12	15.30	—
d	CH_3	H	C_2H_5	C_2H_5	138—141	C_6H_6	10	$C_{13}H_{15}O_3N_3$	59.76	5.79	16.08	—	59.36	5.73	15.81	—
VIa	SCH_3	H	C_2H_5	H	253—256	MeOH	92[g]	$C_{11}H_{11}O_3N_3S$	49.80	4.18	15.84	12.09	49.97	4.41	15.44	12.49
b	OCH_3	H	C_2H_5	H	254—256	$CHCl_3$–acetone	28[g]	$C_{11}H_{11}O_4N_3$	53.01	4.45	16.86	—	53.05	4.56	16.60	—
c	SCH_3	H	CH_3	H	295—298	EtOH	82[g]	$C_{10}H_9O_3N_3S$	47.80	3.61	16.73	12.76	47.93	3.73	16.45	12.56
d	SCH_3	H	$(CH_2)_3CH_3$	H	297—299	EtOH	67[h]	$C_{12}H_{15}O_3N_3S$	51.60	4.69	15.05	11.48	51.35	4.91	14.72	11.29
e	SCH_3	H	$CH(CH_3)_2$	H	231—233	EtOH	77[h]	$C_{12}H_{15}O_3N_3S$	51.60	4.69	15.05	11.48	51.75	4.66	14.68	11.27
f	SCH_3	H	$(CH_2)_3CH_3$	H	174—176	EtOH	57[h]	$C_{13}H_{15}O_3N_3S$	53.22	5.15	14.33	10.93	53.59	5.35	13.99	10.64
g	SCH_3	OH	C_2H_5	H	300—302	DMF	33[g]	$C_{11}H_{11}O_4N_3S$	46.98	3.94	14.94	11.37	46.72	4.08	14.68	11.18
h	CH_3	CH_3	C_2H_5	H	222—224[i]	EtOH	56,[g] 80[j]	$C_{12}H_{13}O_3N_3$	58.29	5.30	17.00	—	57.97	5.41	17.11	—
i	CH_3	H	C_2H_5	H	231—234[k]	EtOH	48,[g] 72[j]	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	—	56.43	4.69	17.81	—
VIIa	H	H	C_2H_5	H	219—222	EtOH	21	$C_{10}H_9O_3N_3$	54.79	4.14	19.17	—	54.59	4.15	19.12	—
b	H	H	C_2H_5	C_2H_5	132—133	EtOH–hexane	10	$C_{12}H_{13}O_3N_3$	58.29	5.30	17.00	—	58.38	5.26	17.00	—
c	OH	H	C_2H_5	H	287—292	EtOH	69	$C_{10}H_9O_4N_3$	51.06	3.86	17.87	—	50.66	3.97	17.47	—
d	Cl	H	C_2H_5	H	226—227	C_6H_6	16	$C_{10}H_8O_3N_3Cl$	47.35	3.18	16.56	(13.98)	47.33	3.39	16.73	(13.94)
e	SCH_3	Cl	C_2H_5	H	224—226	DMF–EtOH	17	$C_{11}H_{10}O_3N_3SCl$	44.07	3.36	14.02	10.70 (11.83)	44.30	3.52	13.78	10.54 (11.63)

a) lit.[b] mp 160—170° *b*) lit.[b] mp 282—284° *c*) This compound was difficult to purify for elemental analysis and so derived to VIb without further purification.
d) lit.[b] mp 223.4—230.6° (decomp.) *e*) lit.[b] mp 275° (decomp.) *f*) The purification was performed on alkali-acid treatment *g*) The yield is by Method B.
h) The yield is by Method C. *i*) lit.[b] mp 224.2—225.8° *j*) The yield is by Method A. *k*) lit.[b] mp 232.6—235.4°

2-Chloro- and 4-Chloro-8-ethyl-5,8-dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (VIId and VIIe)——A mixture of VIg or VIIc (0.002 mole) and $POCl_3$ (9 ml) was refluxed for 3 hr and an excess of $POCl_3$ was evaporated under reduced pressure. The residue was poured onto crushed ice. The extracts were washed with water and dried (Na_2SO_4). The crystals, after evaporation of $CHCl_3$, were recrystallized to afford the corresponding VIIe or VIId. The results were listed in Table III.

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