

152. Akio Fujita, Tadatsugu Yamamoto, Shinsaku Minami, and
Hideji Takamatsu : Studies on Nitrofuranyl Derivatives.

IV.*¹ Synthesis of 2- or 4-[2-(5-Nitro-2-furyl)vinyl]pyrimidine Derivatives.

(Research Laboratory, Dainippon Pharmaceutical Co., Ltd.*²)

We previously¹⁾ had reported that the [2-(5-nitro-2-furyl)vinyl]pyridine derivatives had a strong antibacterial activity. In this paper we present the synthesis of 2- or 4-[2-(5-nitro-2-furyl)vinyl]pyrimidine derivatives (I)~(V) by the condensation of 5-nitro-2-furaldehyde (Va) with 2- or 4-methylpyrimidine, 2- or 4-methyl-6-substituted pyrimidine, 2,4-dimethylpyrimidine and 2,4-dimethyl-6-substituted pyrimidines and their antibacterial activities. In this study it has been found that Va is preferably condensed with the 4-methyl group of 2,4-dimethylpyrimidine (VI) and in the reaction of Va with 2,4-dimethylpyrimidines containing a functional group in the 6-position, the active hydrogen reactivity between the 2-methyl group and 4-methyl group is affected by the group in the 6-position.

Although it is well known that the methyl group in the 2- or 4-position of pyrimidine exhibits an active hydrogen reactivity in its reaction with aromatic aldehydes, studies on the comparative reactivity between the 2- and 4-methyl group of VI in this reaction have not yet been unequivocally investigated: Ochiai, *et al.*²⁾ reported that benzaldehyde was condensed with the methyl group in the 2-position of 2,4,6-trimethylpyrimidine (VII). However, H. R. Sullivan, *et al.*,³⁾ later described that benzaldehyde was reacted with the methyl group in the 4-position of VII after reinvestigation of the Ochiai's procedure. We therefore wished to establish the relative reactivity between the 2- and 4-methyl group of VI in the condensation reaction with Va.

When Va (1 mole) and VI (1 mole) were heated in acetic anhydride at 110~120°, the yellow crystalline product (Va), m.p. 208~210°, was obtained. This structure was confirmed to be 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]pyrimidine by converting it to 2-methylpyrimidine (VIII) by ozonization and subsequent decarboxylation as shown in Chart 4. From this result it was likely that the methyl group in the 4-position of VI would be more active than the 2-methyl group. On the other hand, in respect to the reactivity of the 2- or 4-methyl group of 2,4-dimethyl-6-pyrimidinol (IX), it was reported by H. R. Snyder, *et al.*,⁴⁾ that the 2-methyl group was more active because of the resonance structure of IX as shown in Chart 1 in the Mannich reaction with piperidine and formalin.

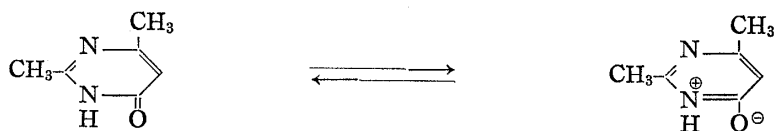


Chart 1.

*¹ Presented at the 83th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1964.
Part III. A. Fujita, *et al.* : This Bulletin, 13, 1177 (1965).

*² Ebie-kami 2, Fukushima-ku, Osaka (藤田昭夫, 山本格次, 南 新作, 高松秀二).

1) Part II. A. Fujita, *et al.* : Yakugaku Zasshi, 85, 565 (1965).

2) E. Ochiai, K. Somei : Yakugaku Zasshi, 58, 397 (1938).

3) H. R. Sullivan, *et al.* : J. Am. Chem. Soc., 77, 1559 (1955).

4) H. R. Snyder, *et al.* : *Ibid.*, 76, 118 (1954).

To establish whether the 2-methyl group of K is predominantly reacted with Va as expected from the H. R. Snyder's report, the following procedures were attempted. When Va was reacted with K in acetic anhydride, a yellowish crystalline product (IIIa), m.p. 225° (decomp.), $C_{11}H_9O_4N_3$, was obtained in a good yield. Since the ultraviolet absorption spectrum of IIIa was similar to that of 2-[2-(5-nitro-2-furyl)vinyl]-6-pyrimidinol (Ie) prepared by the reaction of Va with 2-methyl-6-pyrimidinol, it was postulated that Va was condensed with the methyl group in the 2-position of K. In addition, the structure of IIIa was characterized to be 2-[2-(5-nitro-2-furyl)vinyl]-4-methyl-6-pyrimidinol by converting it to 4-methyl-6-pyrimidinol (X) as shown in Chart 3. Similarly, in the condensation of Va with 2-ethyl-4-methyl-6-pyrimidinol (XI), 2-[1-methyl-2-(5-nitro-2-furyl)vinyl]-4-methyl-6-pyrimidinol (IIIg) was produced, whose structure was confirmed with the isolation of 2-acetyl-4-methyl-6-pyrimidinol (XII) by ozonization of IIIg followed by decarboxylation. From the above result it was clarified that the hydrogen reactivity of the methyl or methylene group in the 2-position of 6-pyrimidinols was considerably greater than the one in the 4-position in this reaction.

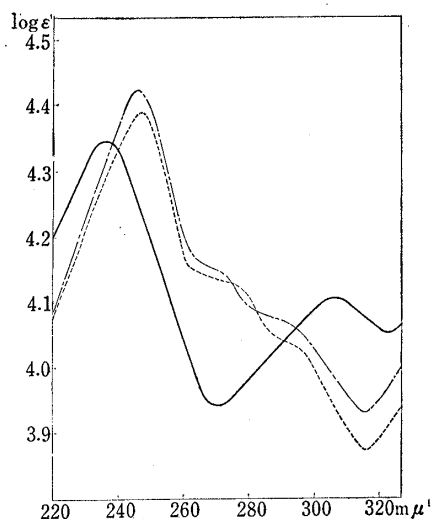


Fig. 1. Ultraviolet Spectra

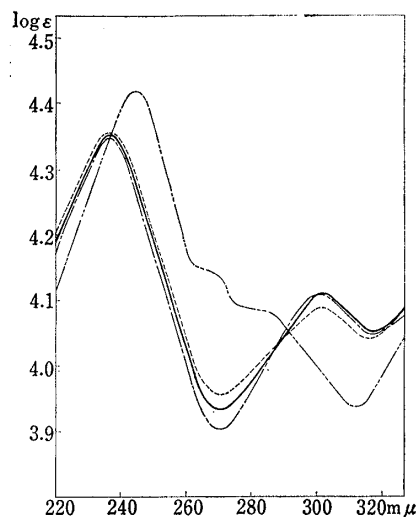
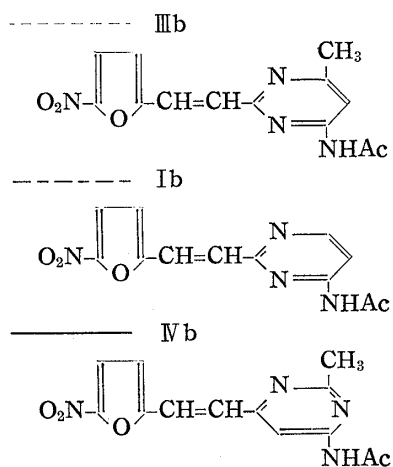
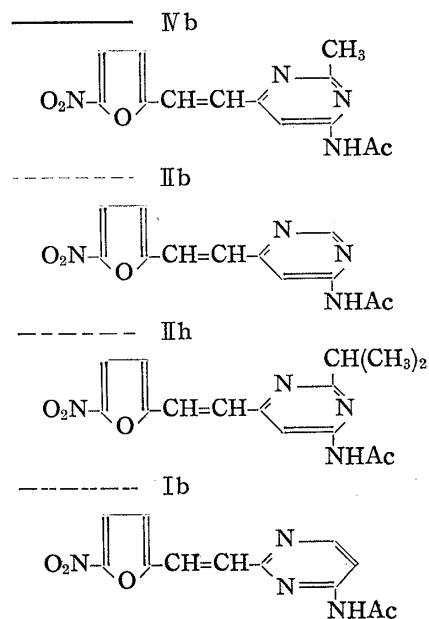


Fig. 2. Ultraviolet Spectra



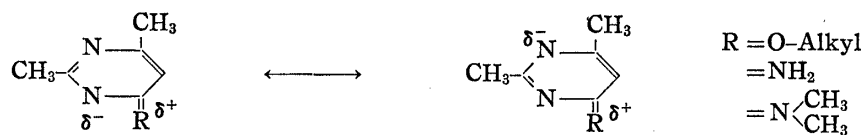


Chart 2.

It was also speculated that in the reaction of Va with 2,4-dimethylpyrimidines having a functional group such as the alkoxy, amino or dimethylamino, the reactivity of the 2-methyl group of these pyrimidines would be more active than the 4-methyl group owing to their resonance hybrid making to decrease the +M-effect of the ring-nitrogen in the 1- or 3-position of pyrimidine as shown in Chart 2. To give a proof of this assumption, the following examinations were carried out. When Va was reacted with 6-methoxy or ethoxy-2,4-dimethylpyrimidine (XIII), (XIV), 2-[2-(5-nitro-2-furyl)vinyl]-4-methyl-6-methoxy pyrimidine (IIIe), (IIIc) were obtained respectively, whose structures were determined as shown in Chart 3. In the reaction of 6-amino-2,4-dimethylpyrimidine (XV) with Va in acetic acid containing conc. sulfuric acid, a yellowish crystalline substance (IIIc), m.p. 218~219° (decomp.) was produced. The structure of IIIc was characterized to be 2-[2-(5-nitro-2-furyl)vinyl]-4-methyl-6-aminopyrimidine as shown in the following procedures. The ultraviolet absorption spectrum of the acetate (IIIb) of IIIc was similar to that of 2-[2-(5-nitro-furyl)vinyl]-6-acetamidopyrimidine (Ib) as shown in Fig. 1 and finally IIIc was converted to 4-methyl-6-aminopyrimidine (XVI) by ozonization and subsequent decarboxylation as shown in Chart 3. If a hydrogen of the amino-group in the 6-position of XV is substituted by an electron attracting group such as the acetyl group making to reduce the -M-effect of the amino group, in contrast with the above result it was presumed that Va might be condensed with the methyl group in the 4-position of 6-acetamido-2,4-dimethylpyrimidine (XVII). A yellowish crystalline product, $C_{13}H_{12}O_4N_4$, m.p. 208~209° (Nb), was obtained in a good yield when Va and XVII were heated in acetic anhydride at 120~130°. From ultraviolet absorption spectrum of Nb being alike to those of 4-[2-(5-nitro-2-furyl)vinyl]-6-acetamidopyrimidine (IIb) and the 2-isopropyl isomer (IIh) (Fig. 2), the structure of Nb was assumed to be 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-acetamidopyrimidine. Finally the structure of Nb was confirmed by converting the deacetylated compound of Nb,

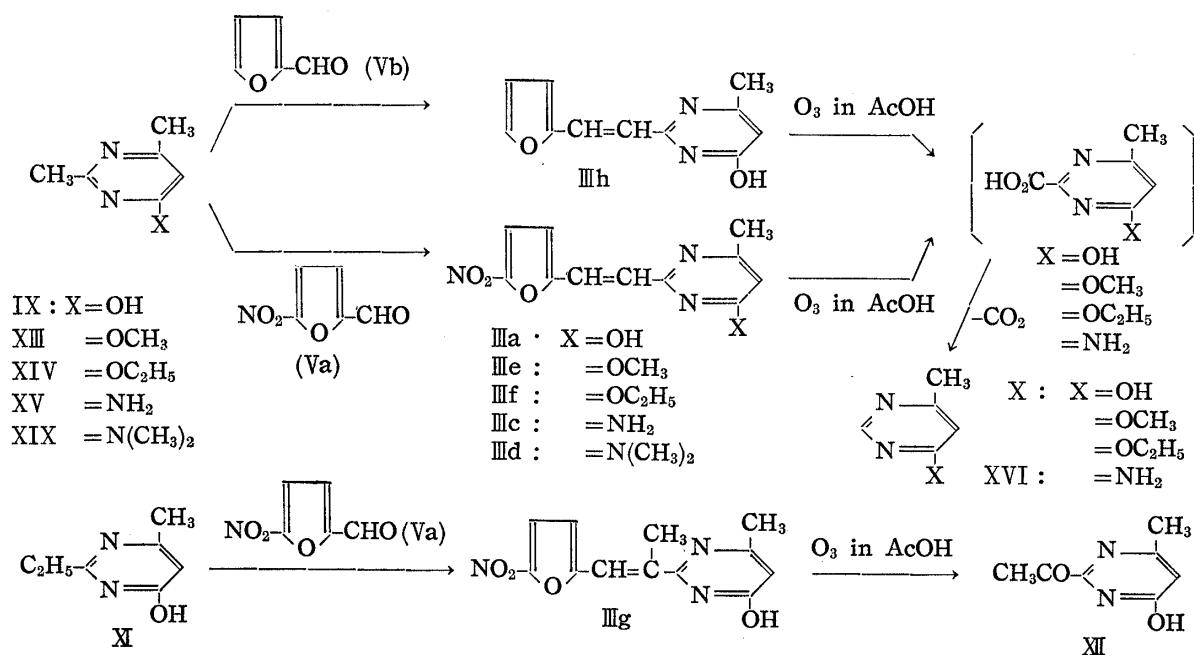
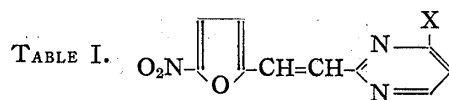
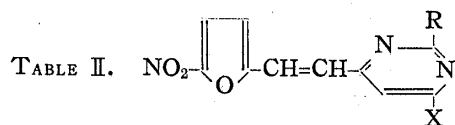


Chart 3.



No.	X	m.p. (°C)	Recryst. solvent	Appear- ance	Formula	Elemental analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
Ia	H	216~217	CH ₃ CN	yellow needles	C ₁₀ H ₇ O ₃ N ₃	55.30	3.25	19.35	55.31	3.54	19.18
Ib	NHAc	221~222	"	"	C ₁₂ H ₁₀ O ₄ N ₄	52.55	3.68	20.43	52.88	3.81	20.70
Ic ^{a)}	NH ₂	252~253	"	yellow powder	C ₁₀ H ₈ O ₃ N ₄	51.72	3.47	24.13	51.44	3.74	24.17
Id	N(CH ₃) ₂	206~207.5	"	yellow needles	C ₁₂ H ₁₂ O ₃ N ₄	55.38	4.65	21.53	55.15	4.86	21.43
Ie	OH	265	Acetone	"	C ₁₀ H ₇ O ₄ N ₃	51.51	3.03	18.02	51.43	3.08	18.05
If	OCH ₃	138~140	CH ₃ CN	"	C ₁₁ H ₉ O ₄ N ₃	53.44	3.67	17.00	53.38	3.64	16.85

a) Ic was obtained by hydrolysis of Ib with 20% ethanolic hydrochloric acid for 2 hr.

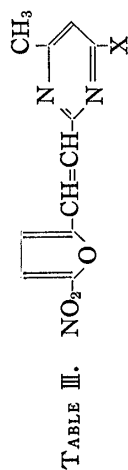


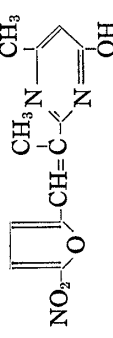
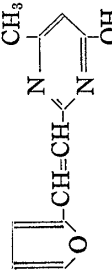
No.	R	X	m.p. (°C)	Recryst. solvent	Appear- ance	Formula	Elemental analysis (%)					
							Calcd.			Found		
							C	H	N	C	H	N
IIa	H	H	223~ 224	Dioxane	yellow needles	C ₁₀ H ₇ O ₃ N ₃	55.30	3.25	19.39	55.04	3.54	19.02
IIb	"	NHAc	235~ 236.5	CH ₃ CN	"	C ₁₂ H ₁₀ O ₄ N ₄	52.55	3.68	20.43	52.30	3.73	20.82
IIc ^{a)}	"	NH ₂	265	"	yellow plates	C ₁₀ H ₈ O ₃ N ₄	51.72	3.47	24.13	51.57	3.46	23.80
IId	"	OH	300	"	yellow needles	C ₁₀ H ₇ O ₄ N ₃	51.51	3.03	18.02	51.74	3.00	18.02
IIe	"	N(CH ₃) ₂	267~ 268	Acetone	"	C ₁₂ H ₁₂ O ₃ N ₄	55.38	4.65	21.53	55.43	4.68	21.15
IIf	"	OCH ₃	217~ 218	CH ₃ CN	yellow plates	C ₁₁ H ₉ O ₄ N ₃	53.44	3.67	17.00	53.67	3.76	16.71
IIg	"	OC ₂ H ₅	158~ 159	"	yellow needles	C ₁₂ H ₁₁ O ₄ N ₃	55.17	4.24	16.09	55.11	4.44	15.71
IIh	CH(CH ₃) ₂	NHAc	221~ 222	EtOH	"	C ₁₅ H ₁₆ O ₄ N ₄	56.96	5.10	17.71	57.20	5.10	17.32
IIi ^{b)}	"	NH ₂	229~ 230	iso-PrOH	"	C ₁₃ H ₁₄ O ₃ N ₄	56.93	5.15	20.43	56.93	5.84	20.51
IIj ^{c)}	"	OH	285	EtOH	"	C ₁₃ H ₁₃ O ₄ N ₃	56.72	4.76	15.27	56.66	4.88	15.05

a) IIc was obtained by hydrolysis of IIb with 20% ethanolic hydrochloric acid for 2 hr.

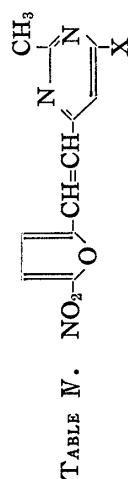
b) IIi was obtained by hydrolysis of IIh with 20% ethanolic hydrochloric acid for 2 hr.

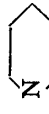
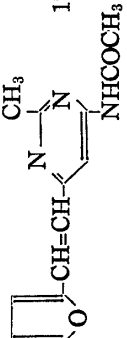
c) IIj was obtained by hydrolysis of IIi with 22% ethanolic hydrochloric acid for 78 hr.



No.	X	m.p. (°C)	Recryst. solvent	Appearance	Formula	Elemental analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
IIIa	OH	255	AcOH	yellow plates	$C_{11}H_9O_4N_3$	53.44	3.67	17.00	53.17	3.67	16.67
IIIb ^{a)}	NHAc	227~229	Acetone	"	$C_{13}H_{13}O_4N_4$	54.16	4.20	19.44	53.89	4.04	19.16
IIIc	NH ₂	218~219	CH ₃ CN	"	$C_{11}H_{10}O_3N_4$	53.66	4.09	22.76	53.64	4.85	22.07
IIId	N(CH ₃) ₂	224.5~225.5	EtOH	yellow needles	$C_{13}H_{14}O_3N_4$	56.93	5.15	20.43	56.81	5.21	20.41
IIIe	OCH ₃	202~204	CH ₃ CN	"	$C_{12}H_{11}O_4N_3$	55.17	4.24	16.09	55.02	3.99	15.89
IIIf	OC ₂ H ₅	207~209	"	yellow plates	$C_{13}H_{13}O_4N_3$	56.72	4.76	15.27	56.51	4.98	14.99
IIIg		265~268	HCON(CH ₃) ₂	"	$C_{12}H_{11}O_4N_3$	55.17	4.24	16.09	55.16	4.36	16.26
IIIh		203~205	EtOH	"	$C_{11}H_{10}O_2N_2$	65.33	4.98	13.86	65.32	5.07	13.63

a) IIIb was obtained by acetylation of IIIc.



No.	X	m.p. (°C)	Recryst. solvent	Appearance	Formula	Elemental analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
Na	H	208~210	MeOH	yellow plates	$C_{11}H_9O_3N_3$	57.14	3.92	18.18	56.57	4.15	17.88
Nb	NHAc	208~209	CH_3CN	yellow needles	$C_{13}H_{12}O_4N_4$	54.16	4.20	19.44	53.70	4.37	19.22
Nc	NH ₂	251~251.5	"	"	$C_{11}H_{10}O_3N_4$	53.66	4.09	22.76	53.49	4.16	22.69
Nd	OH	303	Acetone	yellow plates	$C_{11}H_9O_4N_3$	53.44	3.67	17.00	53.36	3.78	16.96
Ne	NHCH ₂ OH	244~246	"	"	$C_{12}H_{12}O_4N_4$	52.17	4.38	20.28	52.10	4.34	20.34
Nf	N(CH ₃) ₂	231~232.5	EtOH	yellow needles	$C_{13}H_{14}O_3N_4$	56.93	5.15	20.43	56.85	5.07	20.11
Ng	Cl	193~195	"	yellow plates	$C_{11}H_8O_3N_3Cl$	49.73	3.04	15.82	50.35	3.22	16.19
Nh	NHNH ₂	237~239	"	"	$C_{11}H_{11}O_3N_5$	50.57	4.24	26.81	50.72	4.31	25.80
Ni		226~228	"	yellow needles	$C_{13}H_{16}O_4N_4$	56.96	5.10	17.71	56.73	5.20	17.47
Nj		171~172	EtOH+H ₂ O	"	$C_{16}H_{18}O_3N_4$	61.13	5.77	17.83	61.08	5.99	17.57
Nk		174.5~175.5	CH_3CN	yellow powder	$C_{13}H_{13}O_3N_5$	64.18	5.39	17.28	64.27	5.40	17.29

converting to Vf on treatment with dimethylamine. 2-Methyl-4-[2-(5-nitro-2-furyl)-vinyl]-6-hydrazinopyrimidine (Nh), 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-morpholinopyrimidine (Ni), 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-piperidinopyrimidine (Nj) were prepared from Vg by treating with hydrazine hydrate, morpholine and piperidine respectively. Moreover, with the interest to know whether 2-furaldehyde (Vb) is similarly condensed with 2,4-dimethylpyrimidines having a functional group in the 6-position as observed in the reaction of Va, the condensations of Vb with X and with XVII were investigated. 2-[2-(2-Furyl)vinyl]-4-methyl-6-pyrimidinol (IIIh) and 2-methyl-4-[2-(2-furyl)vinyl]-6-acetamidopyrimidine (Vk) were obtained, whose structures were determined by converting them to X and 2-methyl-6-acetamidopyrimidine (XXII) respectively as shown in Charts 3 and 4 and by identification of the nitrated product of Vk with Vb. From these results described above, in particular, the formation of Vf and Va, the comparative reactivity between 2- and 4-methyl group of 2,4-dimethylpyrimidine containing the functional groups in the 6-position could not be elucidated only by the electronic interpretation. We will give a full account for this evidence in the near future.

All compounds obtained were listed in Tables I, II, III, and IV. Though the detail for the antibacterial activities *in vitro* and *in vivo* and the structure activity relationship of 2- or 4-[2-(5-nitro-2-furyl)vinyl]pyrimidine derivatives synthesized will be published elsewhere, in almost of the compounds there are significant antibacterial activities *in vitro* for a gram-positive and gram-negative bacteria, which are not varied with the substituent of 2-(5-nitro-2-furyl)vinyl group in the 2- or 4-position of pyrimidine, and the activities are increased by the functional group at the 6-position in pyrimidine in the order of the acetamido, amino and hydroxy, however, the presence of the alkoxy group in place of the hydroxy group in pyrimidine diminishes the activities.

Experimental^{*4}

4-Methyl-6-ethoxypyrimidine—To the solution of 4-methyl-6-chloropyrimidine⁵⁾ (4.3 g.) in absolute EtOH (5 ml.) was added a solution of Na (1.4 g.) in absolute EtOH (20 ml.) with ice cooling, and the mixture was heated in a water bath for 15 min. The residue obtained by concentration was diluted with water (100 ml.) and extracted with ether (150 ml.). Ethereal extract was dried with Na₂SO₄ and evaporated. The oily residue was distilled to give a colorless oil, 4-methyl-6-ethoxypyrimidine (3.8 g.), b._p 85°. Picrate (from EtOH), m.p. 105~108°. *Anal.* Calcd. for C₇H₁₀ON₂·C₆H₃O₇N₃: C, 42.51; H, 3.57; N, 19.07. Found: C, 42.47; H, 3.67; N, 19.01.

2-Isopropyl-4-methyl-6-acetamidopyrimidine—The mixture of 2-isopropyl-4-methyl-6-pyrimidinol⁴⁾ (10 g.) and POCl₃ (60.5 g.) was heated under reflux for 40 min. The residue obtained by evaporation of an excess of POCl₃ under reduced pressure was poured into ice-water, basified with 10% Na₂CO₃ and extracted with benzene (150 ml.). The extract was dried with Na₂SO₄ and evaporated. The residue was distilled to afford a colorless oil (8.4 g.), b._p 91~92°. 2-Isopropyl-4-methyl-6-chloropyrimidine, which used in the subsequent experiment without purification.

The mixture of 2-isopropyl-4-methyl-6-chloropyrimidine (18.0 g.) and 13% absolute ethanolic NH₃ (50 ml.) was heated at 120° in an autoclave for 6 hr. The reaction mixture was concentrated under reduced pressure. The product separated was filtered, washed with water and recrystallized from iso-PrOH to give colorless needles, 2-isopropyl-4-methyl-6-aminopyrimidine (9.0 g.) m.p. 212~212.5°. *Anal.* Calcd. for C₈H₁₃N₃: C, 63.54; H, 8.66; N, 27.79. Found: C, 63.13; H, 8.61; N, 28.22. The acetate m.p. 122~124° (hexane). *Anal.* Calcd. for C₁₀H₁₅ON₃: C, 62.15; H, 7.82; N, 21.75. Found: C, 62.36; H, 7.93; N, 21.82.

6-Ethoxy-2,4-dimethylpyrimidine (XIV)—It was prepared from 6-chloro-2,4-dimethylpyrimidine (XXI)⁶⁾ by treating with NaOC₂H₅, b._p 92~94°. Picrate, m.p. 124~125°. *Anal.* Calcd. for C₈H₁₂ON₂·C₆H₃O₇N₃: C, 44.10; H, 3.97; N, 13.37. Found: C, 43.98; H, 3.91; N, 13.19.

*4 All melting points were uncorrected.

5) J. R. Marshall: J. Chem. Soc., 1951, 1004.

6) E. Ochiai, Y. Ito: Yakugaku Zasshi, 57, 579 (1935).

4-Methyl-6-acetamidopyrimidine—It was obtained by acetylation of 4-methyl-6-aminopyrimidine (XVI)⁶⁾ by the usual method, m.p. 124~125°. *Anal.* Calcd. for C₇H₉ON₃: C, 55.61; H, 6.00; N, 27.80. Found: C, 55.47; H, 5.87; N, 27.83.

2 or 4-[2-(5-Nitro-2-furyl)vinyl]pyrimidines (Table I, Ia~If, Table II, IIa~IIj)—To the solution of 2-methylpyrimidines^{7,8)} or 4-methylpyrimidines^{5,8,9)} (0.01 mol.) in acetic anhydride (3.0 g.) was added 5-nitro-2-furaldehyde (Va) (0.01 mol.) and heated in an oil bath at 120~130° for 7 hr. The solution was concentrated under reduced pressure, diluted with water and basified with powdered NaHCO₃. The crystalline product separated was filtered, washed with water, dried and purified by recrystallization (yield, Ia~If, 10~60%; yield, IIa~IIj, 20~60%).

4-Methyl-2-[2-(5-nitro-2-furyl)vinyl]-6-substituted Pyrimidines (Table III, IIIa, IIIe, IIIf, IIIg)—To the suspension of 2,4-dimethyl-6-pyrimidinol (K)¹⁰⁾ (0.01 mol.), 6-alkoxy-2,4-dimethylpyrimidine (XIII) (XIV)¹¹⁾ (0.01 mol.) or 2-ethyl-4-methyl-6-pyrimidinol (X)¹⁰⁾ (0.01 mol.) in acetic anhydride (3.0 g.) was added Va (0.01 mol.). The mixture was heated in an oil bath at 130° for 4 hr. After concentration under reduced pressure, the residue was basified with 2% aqueous NaHCO₃. The crystals were filtered, washed with water, dried and purified by recrystallization to give the desired products (yield, IIIa 70%; IIIe, IIIf, IIIg 30%).

2-[2-(5-Nitro-2-furyl)vinyl]-4-methyl-6-amino or acetamido-pyrimidine (Table III, IIIc, IIIf)—To the solution of 6-amino-2,4-dimethylpyrimidine (XV)⁶⁾ (0.02 mol.) in gl. AcOH containing conc. H₂SO₄ (2.0 g.) was added Va (0.02 mol.). The mixture was heated in a water bath at 80° for 5 hr. After being concentrated, the residue was diluted with water, neutralized with 2% aqueous NaHCO₃. The crystals were filtered and recrystallized from CH₃CN to give IIIc (0.45 g.), as yellow prisms, m.p. 218~219°. The acetate (IIIf) (0.2 g.) m.p. 227~229° (decomp.) (from acetone).

2-Methyl-4-[2-(5-nitro-2-furyl)vinyl]pyrimidine (Table IV, IVa)—The mixture of 2,4-dimethylpyrimidine (VI)¹²⁾ (2.39 g.) and Va (3.12 g.) in acetic anhydride (6.77 g.) was heated at 120° in an oil bath for 1.5 hr. and kept overnight at room temperature. The mixture was concentrated under reduced pressure and basified with 2% aqueous NaHCO₃. The crystalline mass separated was filtered, washed with ether, dried and recrystallized from MeOH to give the contaminated products (1.8 g.), m.p. 206~207° (decomp.), which shows three spots on their thin-layer chromatography in EtOH on silica gel. This was purified by chromatography in CHCl₃ on silica gel (25 g.). The CHCl₃ elute (150 ml.) afforded IVa (1.6 g.), which recrystallized from MeOH as yellow prisms, m.p. 208~210° (decomp.) and showed only one spot on its thin-layer chromatography in EtOH on silica gel.

2-Methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-substituted Pyrimidines (Table IV, IVb~IVe, IVg)—Va (0.01 mol.) was added to the solution of 6-acetamido-2,4-dimethylpyrimidine (XVII)¹³⁾ or 6-chloro-2,4-dimethylpyrimidine (XXI)⁶⁾ (0.01 mol.) in acetic anhydride and the solution was heated at 120~130° for 3 hr. After cooling, crystalline product separated was filtered, washed with water, dried and purified by recrystallization to give IVb (2.5 g.) and IVg (1.1 g.). The suspension of IVb (2.0 g.) in 20% ethanolic hydrochloric acid was heated under reflux for 2 hr. The residue, after evaporation of the solvent, was neutralized with 10% aqueous Na₂CO₃ and the crystals separated were filtered, washed with water and recrystallized from CH₃CN to give IVc (1.6 g.). Hydrolysis of IVc for 50 hr. with 20% ethanolic hydrochloric acid gave IVd (yield, 71%). The mixture of IVc (2.0 g.), 37% formalin (20 ml.) and EtOH (10 ml.) was warmed at 80~85° for 3 hr. with stirring. The crystalline mass separated by dilution with water was filtered, washed with water and recrystallized from acetone to give IVe (0.4 g.) as yellow prisms, m.p. 244~246° (decomp.).

Hydrolysis and Amination of IVg (Table IVh~IVj)—The suspension of IVg (0.265 g.) in 10% ethanolic hydrochloric acid (20 ml.) was heated for 2.5 hr. The residue obtained by removal of the solvent was neutralized with 10% aqueous Na₂CO₃ and the crystalline mass separated was filtered, washed with water and recrystallized from acetone to give the product which was identical with IVd described before by the comparison of their IR spectra and a mixed melting point determination. IVg (0.1 g.) was heated with 30% methanolic dimethylamine (0.4 ml.) at 50° for 1.5 hr. After cooling, the crystalline mass separated was filtered, washed with water and crystallized from CH₃CN to afford IVf (0.04 g.), which was identical with the sample prepared by the condensation of XIX with Va by admixture and the comparison of their IR spectra as described below.

7) S. Gabriel: *Ber.*, **37**, 3638 (1904).

8) W. Pfeiderer, H. Mosthaf: *Ibid.*, **90**, 728 (1957).

9) K. Miyagi, H. Kataoka: *Yakugaku Zasshi*, **60**, 367 (1940).

10) A. Pinner: *Ber.*, **22**, 1616 (1889).

11) R. Andrisano: *Gazz. chim. ital.*, **81**, 398 (1951). (*C. A.*, **46**, 5053 (1952)).

12) T. Matsukawa, B. Ota: *Yakugaku Zasshi*, **70**, 137 (1950).

13) A. Pinner: *Ber.*, **22**, 1600 (1889).

Similarly, Ng (0.01 mol.) was treated with each of ethanolic hydrazine hydrate, morpholine or piperidine (0.02 mol.) under reflux for 1 hr. After cooling, the separated crystalline mass was purified similarly described as above to give Nh (yield 35%), Ni (yield 70%) and Nj (yield 65.4%).

2-Methyl-4-[2-(5-nitro-2-furyl)vinyl-6-dimethylamino]pyrimidine (Table IV, IVf) and 2-[2-(5-Nitro-2-furyl)vinyl]-4-methyl-6-dimethylaminopyrimidine (Table III, IIIId)—To the solution of 6-dimethylamino-2,4-dimethylpyrimidine (XIX)¹⁴⁾ (0.01 mol.) in acetic anhydride (0.03 mol.) was added Va (0.01 mol.) and the solution was heated in an oil bath at 130° for 2 hr. After cooling, the mixture was concentrated under reduced pressure, basified with 2% aq. NaHCO₃ to give the crystalline product. Recrystallization from CH₃CN gave the contaminated products m.p. 210~217° (0.46 g.), which show two spots on their thin-layer chromatography in acetone-CHCl₃ (1:1) on silica gel and thus was chromatographed in CHCl₃ on alumina (15 g.). The first CHCl₃ elute (25 ml.) afforded Vf, as yellow needles (EtOH), m.p. 231~232.5° (0.3 g.), undepressed on admixture with the sample obtained by dimethylation of Ng. The second CHCl₃ elute (40 ml.) afforded IIIId, which was crystallized from EtOH as yellow needles m.p. 224.5~225.5° (0.1 g.).

2 or 4-[2-(2-Furyl)vinyl]pyrimidine (Table IV, IIIh, IVk)—a) The mixture of 2-furaldehyde (Vb) (3.20 g.), 2,4-dimethyl-6-pyrimidinol (X) (4.0 g.) and acetic anhydride (56.9 g.) was heated in an oil bath at 140° for 12 hr. The crystalline residue obtained by concentration of the reaction mixture under reduced pressure was recrystallized from EtOH to give IIIh (0.6 g.).

b) The mixture of Vb (9.65 g.), XVII (16.52 g.) and acetic anhydride (110 g.) was heated at 150~155° for 54 hr. On cooling, the crystalline mass separated was filtered, washed with hot water and recrystallized to give IVk (7.4 g.). The solution of IVk (1.0 g.) in 50 ml. of acetic anhydride was added in portion to the mixture of fum. nitric acid (3.0 g., $d_4^{20}=1.502$) and acetic anhydride (15 ml.) at -10~-15° during 4 hr. with stirring. The reaction mixture was poured into ice-water (200 ml.) and neutralized with 40% NaOH giving a crystalline mass, which was recrystallized from acetone to give IVb (0.86 g.) as yellowish needles, m.p. 209~210° which was identical with Nb described above.

Ozonization of [2-(5-Nitro-2-furyl or 2-furyl)vinyl]pyrimidines—Ozone (7.7 mol.) (3.01% in O₂/min.) was let into the solution of the vinylpyrimidines obtained above (1 mol.) in acetic acid at 0~2°. To the ozonized solution was added a 3% H₂O₂ aqueous solution (2 mol.) or preferably water, and the solution was heated in a water bath for 10 min. The solution was concentrated to dryness under reduced pressure and the residual pyrimidine carboxylic acids, from which in a few cases the pure carboxylic acids were obtained as shown below, was submitted to subsequent decarboxylation under reduced pressure (2~3 mm. Hg) without purification. The crystalline mass thus obtained was purified by recrystallization or distillation to give pyrimidines, which or whose picrates were identical with authentic sample by the comparison of the IR spectra and mixed melting point.

From IVk 2-methyl-6-acetamido-4-pyrimidinecarboxylic acid, m.p. 247~249° (from MeOH). *Anal.* Calcd. for C₈H₉O₃N₃: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.03; H, 4.82; N, 21.08. 2-Methyl-6-acetamidopyrimidine (XXII), m.p. 141~142° (from hexane). *Anal.* Calcd. for C₇H₉O₂N₃: C, 55.61; H, 6.00; N, 27.80. Found: C, 55.82; H, 6.10; N, 28.18, which was identical with the authentic sample obtained by acetylation of XVIII.⁷⁾

From IIIc 4-methyl-6-amino-2-pyrimidinecarboxylic acid, m.p. 245~246° (decomp.) (from EtOH). *Anal.* Calcd. for C₆H₇O₂N₃: C, 47.05; H, 4.61; N, 27.44. Found: C, 47.08; H, 4.96; N, 27.40. 4-Methyl-6-aminopyrimidine (XVI), m.p. 192~193° (the authentic sample,⁵⁾ m.p. 194°).

From IIIg 2-acetyl-4-methyl-6-pyrimidinol (XII), m.p. 165° (from acetone). *Anal.* Calcd. for C₇H₉O₂N₂: C, 55.25; H, 5.30; N, 18.41. Found: C, 54.53; H, 5.35; N, 18.03. *p*-Nitrophenylhydrazone, m.p. 303° (from EtOH). *Anal.* Calcd. for C₁₃H₁₃O₃N₅: C, 54.35; H, 4.56; N, 24.38. Found: C, 54.16; H, 4.61; N, 24.52.

From Nf 2-methyl-6-dimethylaminopyrimidine (XX), b.p.₂₃ 103~105°. Picrate, m.p. 215~217° (from EtOH), which was identical with the authentic sample prepared by dimethylation of 2-methyl-6-chloropyrimidine.⁷⁾ Picrate, *Anal.* Calcd. for C₇H₁₁N₃·C₆H₅O₇N₃: C, 42.62; H, 3.85; N, 22.95. Found: C, 42.59; H, 3.70; N, 22.72.

From IIIe 4-methyl-6-methoxypyrimidine b.p.₃₀ 73~74°. Picrate, m.p. 114~115° (the authentic sample,⁵⁾ b.p.₂₁ 69°, picrate, m.p. 117°).

From IIIh 4-methyl-6-pyrimidinol (X), m.p. 149~150° (the authentic sample,⁵⁾ m.p. 150°).

From IVc 2-methyl-6-aminopyrimidine (XVIII), m.p. 204~205° (the authentic sample,⁷⁾ m.p. 205°).

From Va 2-methylpyrimidine (VIII), b.p. 130~135°. Picrate, m.p. 112~114° (the authentic sample,⁷⁾ picrate, m.p. 112~114°).

We are grateful to Dr. S. Ose, Director of this Research Laboratory, for encouragement throughout the experiment and also indebted to the members of the elemental analytical center of this Laboratory for analytical data.

14) W. Klotzer: *Monatsh.*, **87**, 536 (1956).

Summary

To test the antibacterial activity, 2- or 4-[2-(5-nitro-2-furyl)vinyl]pyrimidines were synthesized by the condensation of 5-nitro-2-furaldehyde (Va) with the active methyl group of pyrimidines.

It has been found that Va predominantly condensed with the 4-methyl group of 2,4-dimethylpyrimidine and in the reaction of Va with 2,4-dimethylpyrimidine containing a functional group in the 6-position, the active hydrogen reactivity between the 2-methyl group and 4-methyl group is affected by the presence of group in the 6-position: in 2,4-dimethylpyrimidines substituted by the hydroxy, amino and alkoxy group in the 6-position, the 2-methyl group was found to be more active, while in the ones substituted by the acetamido, dimethylamino and chloro group, the 4-methyl group was more reactive.

(Received March 27, 1965)

[Chem. Pharm. Bull.]
13(10)1193~1199(1965)

UDC 612.398.014.46

153. Osamu Tamemasa, Shoji Okada, and Yasuo Wakita : Inhibition and Stimulation of the Biosynthesis of Protein and Nucleic Acid. I.

Effect of Some Phenylalanine Analogs on the Aromatic
Amino Acid Incorporation into Proteins of Ehrlich
Mouse Ascites Tumor Cells *in vitro*.

(Shizuoka College of Pharmacy*¹)

It has been reported in recent years that many kinds of chemical compounds show inhibiting or stimulating effect on the biosynthesis of protein or nucleic acid. As a part of those studies, investigations have been undertaken on the inhibition of protein synthesis by amino acid analogs in bacteria, and it has frequently been observed that many structural analogs of amino acid depress bacterial protein synthesis.¹⁻⁴⁾ As for neoplastic cells, however, there have been only few reports on the structural analogs which inhibit the amino acid incorporation.^{2,3,5-9)}

In the present study, the effect of some phenylalanine analogs was investigated on the incorporation of aromatic amino acid into proteins of Ehrlich mouse ascites tumor cells *in vitro* with an emphasis on the difference in the response to the analogs between tumor cells and normal mouse liver cells.

Experimental

Materials—DL-Phenylalanine-2-¹⁴C (DL-Phe-2-¹⁴C), L-tyrosine-¹⁴C (u) (L-Tyr-¹⁴C(u)), and DL-trypto-

*¹ Oshika, Shizuoka (為政 脩, 岡田昌二, 脇田康夫).

- 1) A. Meister : "Biochemistry of the Amino Acids," 114 (1957), Academic Press Inc., New York.
- 2) W. Shive, C.G. Skinner : Ann. Rev. Biochem., **27**, 643 (1958).
- 3) Y. Miura : Tampakushitsu Kakusan Kôso, **6** (11), 123 (1961).
- 4) M.H. Richmond : Bacteriol. Rev., **26**, 398 (1962).
- 5) M. Rabinovitz, M.E. Olson, D.M. Greenberg : J. Biol. Chem., **210**, 837 (1954).
- 6) *Idem* : Cancer Research, **17**, 885 (1957).
- 7) *Idem* : J. Biol. Chem., **227**, 217 (1957).
- 8) *Idem* : Cancer Research, **19**, 388 (1959).
- 9) J.E. Wilson, J.R. Irvin, J.E. Suggs, K. Liu : *Ibid.*, **19**, 272 (1959).