[Chem. Pharm. Bull. 13(10)1183~1193(1965)] UDC 547.722.5.07

# 152. Akio Fujita, Tadatsugu Yamamoto, Shinsaku Minami, and Hideji Takamatsu: Studies on Nitrofuran Derivatives. IV.*1 Synthesis of 2- or 4-[2-(5-Nitro-2-furyl)vinyl]pyrimidine Derivatives.

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

We previously[1] 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)~(IV) 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-posion 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 (IVa), 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.

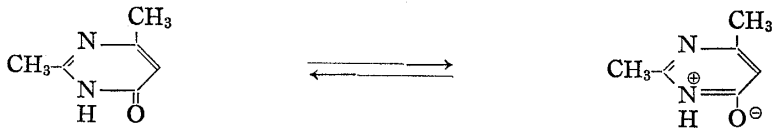


Chart 1.

*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 Ⅸ is predominantly reacted with Ⅴa as expected from the H. R. Snyder's report, the following procedures were attempted. When Ⅴa was reacted with Ⅸ 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 Ⅸ. 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.

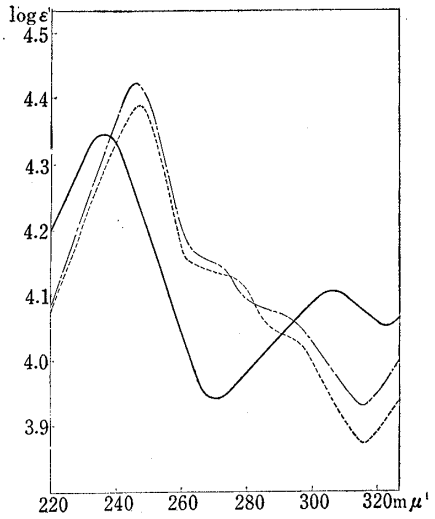



Fig. 1. Ultraviolet Spectra

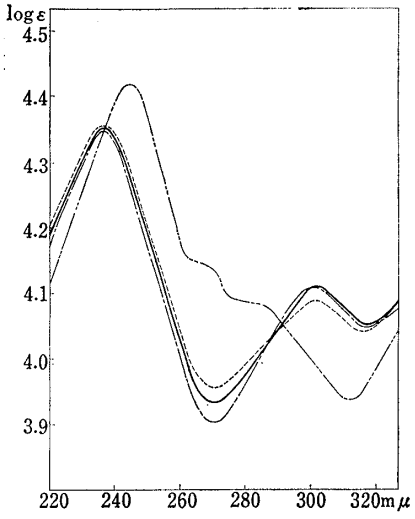
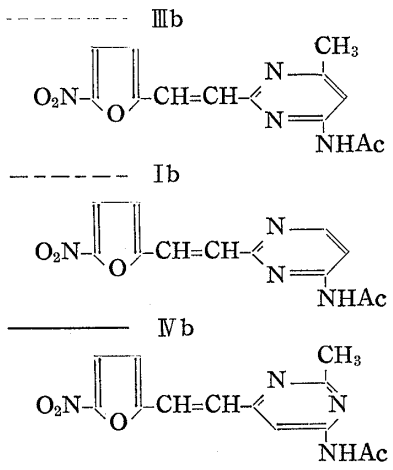
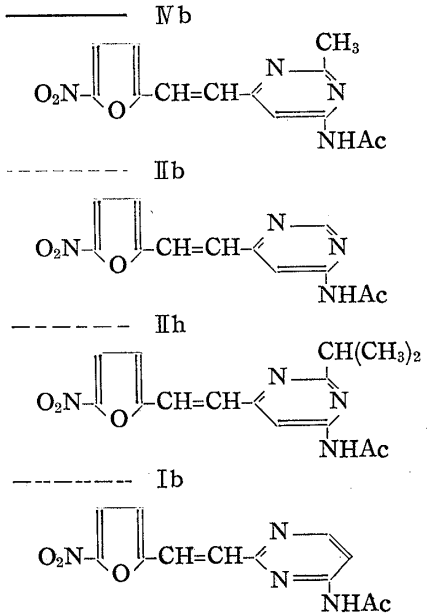



Fig. 2. Ultraviolet Spectra



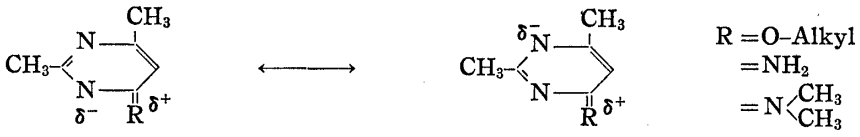



Chart 2.

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), (IIIf) 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° (IVb), was obtained in a good yield when Va and XVII were heated in acetic anhydride at 120~130°. From ultraviolet absorption spectrum of IVb 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 IVb was assumed to be 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-acetamidopyrimidine. Finally the structure of IVb was confirmed by converting the deacetylated compound of IVb,

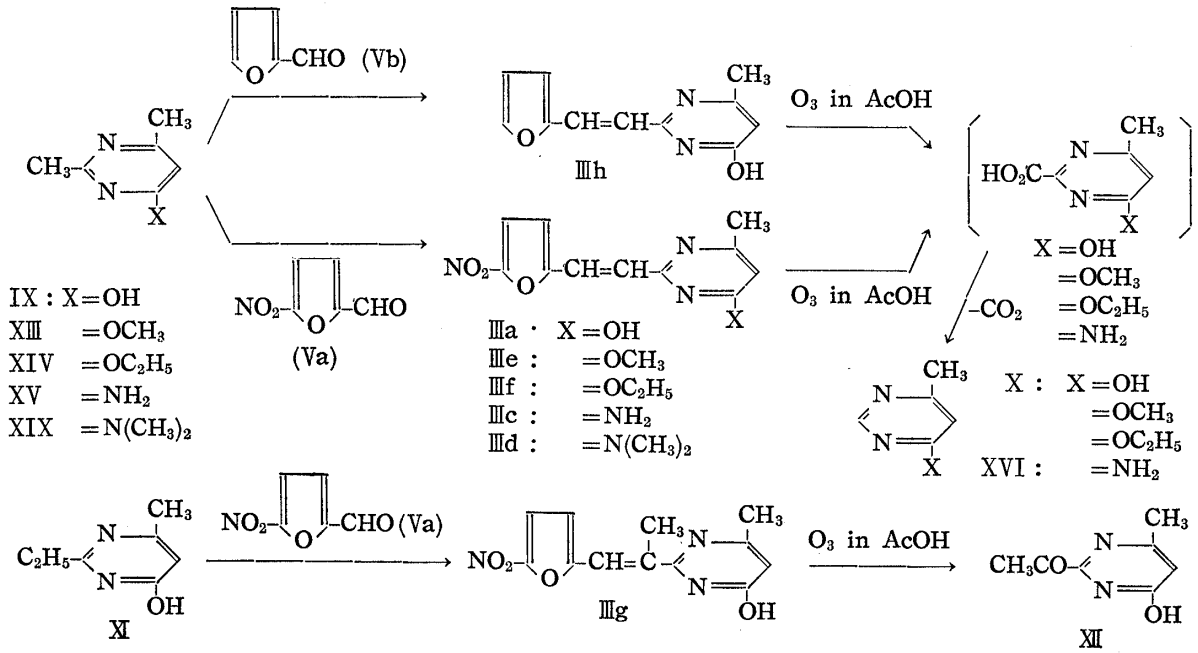



Chart 3.

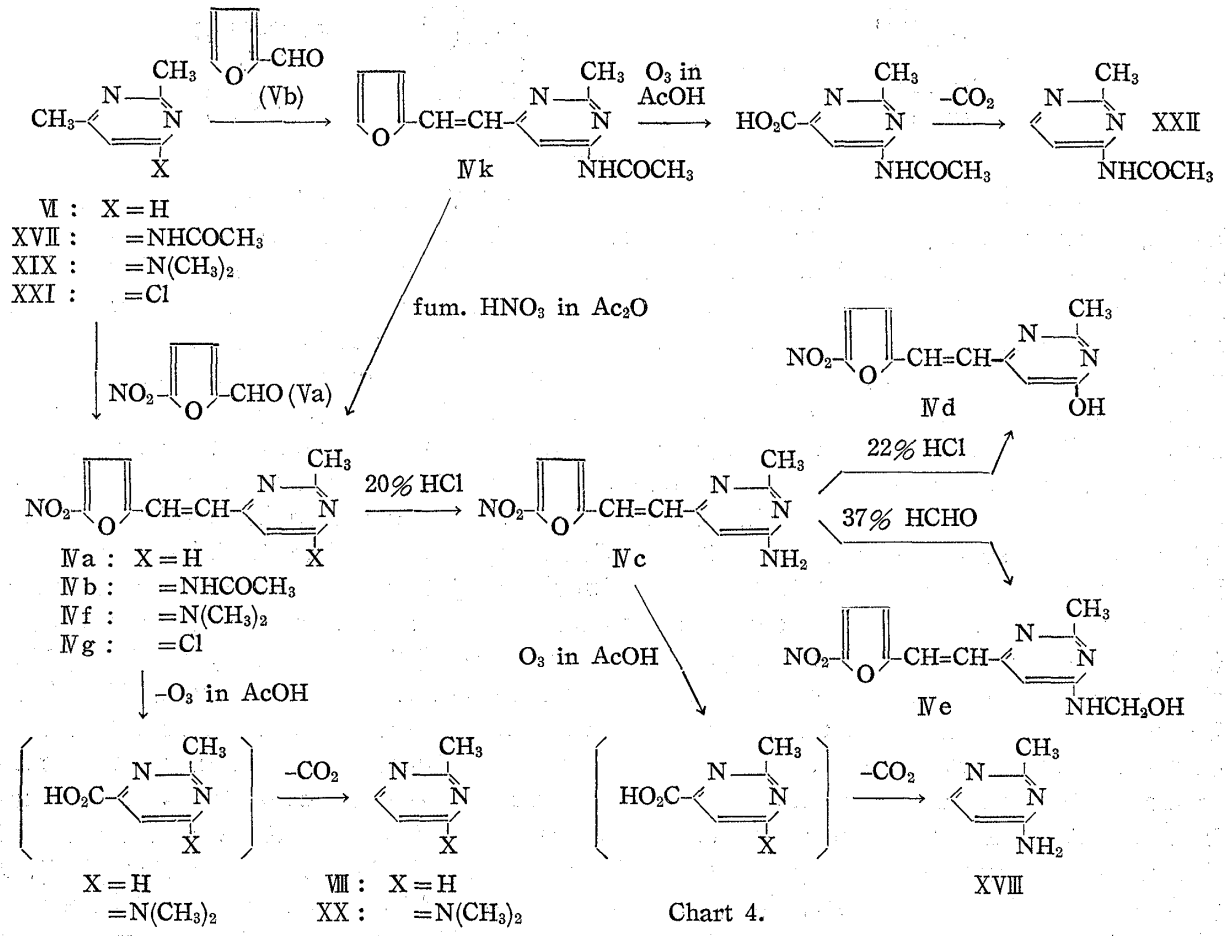



Chart 4.

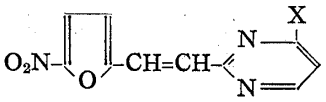
2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-aminopyrimidine(Ⅳc), to 2-methyl-6-aminopyrimidine (XVIII) as shown in Chart 4.

Hydrolysis of Ⅳc with ethanolic hydrochloric acid gave 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-pyrimidinol (Ⅳd) and the treatment of Ⅳc with 37% formalin in ethanol afforded 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-hydroxymethylaminopyrimidine (Ⅳe).

In the reaction of Va with 6-dimethylamino-2,4-dimethylpyrimidine (XIX) in acetic anhydride, unexpectedly 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-dimethylaminopyrimidine (Ⅳf) was predominantly obtained with expected 2-[2-(5-nitro-2-furyl)vinyl]-4-methyl-6-dimethylaminopyrimidine (Ⅲd) in the ratio of the products being 3:1. The structure of Ⅳf was unequivocally characterized by converting it to 2-methyl-6-dimethylaminopyrimidine (XX) as shown in Chart 4. The possibility that Ⅲd was the geometrical isomer of Ⅳf was neglected by the presence of a *trans* vinyl proton in the nuclear magnetic resonance*[3] spectra of Ⅲd and Ⅳf, that is, the signals of the vinyl proton of Ⅲd were found at 7.40 p.p.m., 7.75 p.p.m. (J=16.0 c.p.s.) and those of Ⅳf at 7.43 p.p.m., 7.80 p.p.m. (J=16.0 c.p.s.). (The values are expressed in 10 p.p.m. (TMS=0 p.p.m.)).

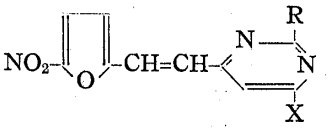
Since the −M-effect of the halogen group is smaller than those of the alkoxy and amino groups, in the reaction of Va with 6-chloro-2,4-dimethylpyrimidine (XXI) it was assumed that the active hydrogen reactivity of the 4-methyl group was more active, as found in XVII. In this reaction 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-chloropyrimidine (Ⅳg) was obtained, whose structure was confirmed by hydrolysis to Ⅳd and

*[3] The NMR spectrum were measured in $CDCl_3$ at 60 Mc by Varian A-60 NMR spectrometer. Tetramethylsilane was used at internal standard.

Table I. 

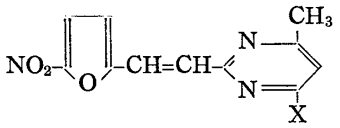
| No. | X | m.p. (°C) | Recryst. solvent | Appearance | Formula | Elemental analysis (%) Calcd. C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ia | H | 216~217 | $CH_3CN$ | yellow needles | $C_{10}H_7O_3N_3$ | 55.30 | 3.25 | 19.35 | 55.31 | 3.54 | 19.18 |
| Ib | NHAc | 221~222 | 〃 | 〃 | $C_{12}H_{10}O_4N_4$ | 52.55 | 3.68 | 20.43 | 52.88 | 3.81 | 20.70 |
| Ic[a] | $NH_2$ | 252~253 | 〃 | yellow powder | $C_{10}H_8O_3N_4$ | 51.72 | 3.47 | 24.13 | 51.44 | 3.74 | 24.17 |
| Id | $N(CH_3)_2$ | 206~207.5 | 〃 | yellow needles | $C_{12}H_{12}O_3N_4$ | 55.38 | 4.65 | 21.53 | 55.15 | 4.86 | 21.43 |
| Ie | OH | 265 | Acetone | 〃 | $C_{10}H_7O_4N_3$ | 51.51 | 3.03 | 18.02 | 51.43 | 3.08 | 18.05 |
| If | $OCH_3$ | 138~140 | $CH_3CN$ | 〃 | $C_{11}H_9O_4N_3$ | 53.44 | 3.67 | 17.00 | 53.38 | 3.64 | 16.85 |

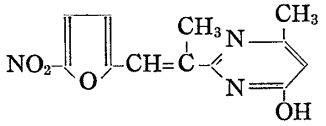
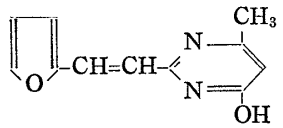
a) Ic was obtained by hydrolysis of Ib with 20% ethanolic hydrochloric acid for 2 hr.

Table II. 

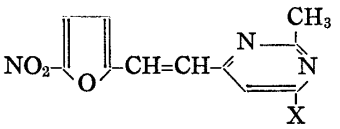
| No. | R | X | m.p. (°C) | Recryst. solvent | Appearance | Formula | Elemental analysis (%) Calcd. C | H | N | Found C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IIa | H | H | 223~224 | Dioxane | yellow needles | $C_{10}H_7O_3N_3$ | 55.30 | 3.25 | 19.39 | 55.04 | 3.54 | 19.02 |
| IIb | 〃 | NHAc | 235~236.5 | $CH_3CN$ | 〃 | $C_{12}H_{10}O_4N_4$ | 52.55 | 3.68 | 20.43 | 52.30 | 3.73 | 20.82 |
| IIc[a] | 〃 | $NH_2$ | 265 | 〃 | yellow plates | $C_{10}N_8O_3N_4$ | 51.72 | 3.47 | 24.13 | 51.57 | 3.46 | 23.80 |
| IId | 〃 | OH | 300 | 〃 | yellow needles | $C_{10}H_7O_4N_3$ | 51.51 | 3.03 | 18.02 | 51.74 | 3.00 | 18.02 |
| IIe | 〃 | $N(CH_3)_2$ | 267~268 | Acetone | 〃 | $C_{12}H_{12}O_3N_4$ | 55.38 | 4.65 | 21.53 | 55.43 | 4.68 | 21.15 |
| IIf | 〃 | $OCH_3$ | 217~218 | $CH_3CN$ | yellow plates | $C_{11}H_9O_4N_3$ | 53.44 | 3.67 | 17.00 | 53.67 | 3.76 | 16.71 |
| IIg | 〃 | $OC_2H_5$ | 158~159 | 〃 | yellow needles | $C_{12}H_{11}O_4N_3$ | 55.17 | 4.24 | 16.09 | 55.11 | 4.44 | 15.71 |
| IIh | $CH(CH_3)_2$ | NHAc | 221~222 | EtOH | 〃 | $C_{15}H_{16}O_4N_4$ | 56.96 | 5.10 | 17.71 | 57.20 | 5.10 | 17.32 |
| IIi[b] | 〃 | $NH_2$ | 229~230 | iso-PrOH | 〃 | $C_{13}H_{14}O_3N_4$ | 56.93 | 5.15 | 20.43 | 56.93 | 5.84 | 20.51 |
| IIj[c] | 〃 | OH | 285 | EtOH | 〃 | $C_{13}H_{13}O_4N_3$ | 56.72 | 4.76 | 15.27 | 56.66 | 4.88 | 15.05 |

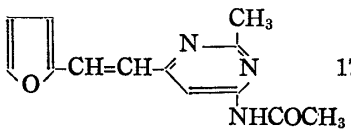
a) IIc was obtained by hydrolysis of IIb with 20% ethanolic hydrochloric acid for 2 hr.
b) IIi was obtained by hydrolysis of IIh with 20% ethanolic hydrochloric acid for 2 hr.
c) IIj was obtained by hydrolysis of IIi with 22% ethanolic hydrochloric acid for 78 hr.

TABLE III. 

| No. | X | m.p. (°C) | Recryst. solvent | Appearance | Formula | Elemental analysis (%) Calcd. C | Calcd. H | Calcd. N | Found C | Found H | Found 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 | 〃 | $C_{13}H_{12}O_4N_4$ | 54.16 | 4.20 | 19.44 | 53.89 | 4.04 | 19.16 |
| IIIc | $NH_2$ | 218～219 | $CH_3CN$ | 〃 | $C_{11}H_{10}O_3N_4$ | 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 | $CH_3CN$ | 〃 | $C_{12}H_{11}O_4N_3$ | 55.17 | 4.24 | 16.09 | 55.02 | 3.99 | 15.89 |
| IIIf | $OC_2H_5$ | 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.

TABLE Ⅳ. 

| No. | X | m.p. (°C) | Recryst. solvent | Appearance | Formula | Elemental analysis (%) Calcd. C | Calcd. H | Calcd. N | Found C | Found H | Found N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ⅳa | H | 208～210 | MeOH | yellow plates | $C_{11}H_9O_3N_3$ | 57.14 | 3.92 | 18.18 | 56.57 | 4.15 | 17.88 |
| Ⅳb | 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 |
| Ⅳc | $NH_2$ | 251～251.5 | 〃 | 〃 | $C_{11}H_{10}O_3N_4$ | 53.66 | 4.09 | 22.76 | 53.49 | 4.16 | 22.69 |
| Ⅳd | OH | 303 | Acetone | yellow plates | $C_{11}H_9O_4N_3$ | 53.44 | 3.67 | 17.00 | 53.36 | 3.78 | 16.96 |
| Ⅳe | $NHCH_2OH$ | 244～246 | 〃 | 〃 | $C_{12}H_{12}O_4N_4$ | 52.17 | 4.38 | 20.28 | 52.10 | 4.34 | 20.34 |
| Ⅳf | $N(CH_3)_2$ | 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 |
| Ⅳg | Cl | 193～195 | 〃 | yellow plates | $C_{11}H_8O_3N_3Cl$ | 49.73 | 3.04 | 15.82 | 50.35 | 3.22 | 16.19 |
| Ⅳh | $NHNH_2$ | 237～239 | 〃 | 〃 | $C_{11}H_{11}O_3N_5$ | 50.57 | 4.24 | 26.81 | 50.72 | 4.31 | 25.80 |
| Ⅳi |  | 226～228 | 〃 | yellow needles | $C_{15}H_{16}O_4N_4$ | 56.96 | 5.10 | 17.71 | 56.73 | 5.20 | 17.47 |
| Ⅳj |  | 171～172 | EtOH+$H_2O$ | 〃 | $C_{16}H_{18}O_3N_4$ | 61.13 | 5.77 | 17.83 | 61.08 | 5.99 | 17.57 |
| Ⅳk |  | 174.5～175.5 | $CH_3CN$ | yellow powder | $C_{13}H_{13}O_2N_3$ | 64.18 | 5.39 | 17.28 | 64.27 | 5.40 | 17.29 |

converting to Ⅳf on treatment with dimethylamine. 2-Methyl-4-[2-(5-nitro-2-furyl)-vinyl]-6-hydrazinopyrimidine (Ⅳh), 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-morpholino-pyrimidine (Ⅳi), 2-methyl-4-[2-(5-nitro-2-furyl)vinyl]-6-piperidinopyrimidine (Ⅳj) were prepared from Ⅳg by treating with hydrazine hydrate, morpholline 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 Ⅸ 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 (Ⅳk) 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 Ⅳk with Ⅳb. From these results described above, in particular, the formation of Ⅳf and Ⅳa, 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 becteria, 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-chloropyrimidine[5] (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 $Na_2SO_4$ and evaporated. The oily residue was distillated to give a colorless oil, 4-methyl-6-ethoxypyrimidine (3.8 g.), b.$p_3$ 85°. Picrate (from EtOH), m.p. 105~108°. *Anal.* Calcd. for $C_7H_{10}ON_2 \cdot C_6H_3O_7N_3$ : 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-pyrimidinol[4] (10 g.) and $POCl_3$ (60.5 g.) was heated under reflux for 40 min. The residue obtained by evaporation of an excess of $POCl_3$ under reduced pressure was poured into ice-water, basified with 10% $Na_2CO_3$ and extracted with benzene (150 ml.). The extract was dried with $Na_2SO_4$ and evaporated. The residue was distillated to afford a colorless oil (8.4 g.), b.$p_{10}$ 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 $NH_3$ (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 $C_8H_{13}N_3$ : 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 $C_{10}H_{15}ON_3$ : 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 $NaOC_2H_5$, b.$p_{35}$ 92~94°. Picrate, m.p. 124~125°. *Anal.* Calcd. for $C_8H_{12}ON_2 \cdot C_6H_3O_7N_3$ : 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)[5] by the usual method, m.p. 124~125°. *Anal.* Calcd. for $C_7H_9ON_3$: 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 $NaHCO_3$. 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 (IX)[10] (0.01 mol.), 6-alkoxy-2,4-dimethylpyrimidine (XIII) (XIV)[11] (0.01 mol.) or 2-ethyl-4-methyl-6-pyrimidinol (XI)[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 $NaHCO_3$. 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, IIIb)**——To the solution of 6-amino-2,4-dimethylpyrimidine (XV)[6] (0.02 mol.) in gl. AcOH containing conc. $H_2SO_4$ (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, neutrallized with 2% aqueous $NaHCO_3$. The crystals were filtered and recrystallized from $CH_3CN$ to give IIIc (0.45 g.), as yellow prisms, m.p. 218~219°. The acetate (IIIb) (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 $NaHCO_3$. 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 $CHCl_3$ on silica gel (25 g.). The $CHCl_3$ 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 neutrallized with 10% aqueous $Na_2CO_3$ and the crystals separated were filtered, washed with water and recrystallized from $CH_3CN$ 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 neutrallized with 10% aqueous $Na_2CO_3$ 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 $CH_3CN$ 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. Pfleiderer, 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, Ⅳg (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 Ⅳh (yield 35%), Ⅳi (yield 70%) and Ⅳj (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, IIId)**——To the solution of 6-dimethylamino2,4-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. $NaHCO_3$ to give the crystalline product. Recrystallization from $CH_3CN$ gave the contaminated products m.p. 210~217° (0.46 g.), which show two spots on their thin-layer chromatography in acetone-$CHCl_3$ (1:1) on silica gel and thus was chromatographed in $CHCl_3$ on alumina (15 g.). The first $CHCl_3$ elute (25 ml.) afforded Ⅳf, as yellow needles (EtOH), m.p. 231~232.5° (0.3 g.), undepressed on admixture with the sample obtained by dimethylamination of Ⅳg. The second $CHCl_3$ elute (40 ml.) affored Ⅲd, 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 (Ⅸ) (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., $d \fallingdotseq 1.502$) and acetic anhydride (15 ml.) at −10~−15° during 4 hr. with stirring. The reaction mixture was poured into ice-water (200 ml.) and neutrallized 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 Ⅳb described above.

**Ozonization of [2-(5-Nitro-2-furyl or 2-furyl)vinyl]pyrimidines**——Ozone (7.7 mol.) (3.01% in $O_2$/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% $H_2O_2$ 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 $C_8H_9O_3N_3$: 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 $C_7H_9ON_3$: 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 $C_6H_7O_2N_3$: 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 $C_7H_8O_2N_2$: 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 $C_{13}H_{13}O_3N_5$: C, 54.35; H, 4.56; N, 24.38. Found: C, 54.16; H, 4.61; N, 24.52.

From Ⅳf 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 dimethylamination of 2-methyl-6-chloropyrimidine.[7] Picrate, *Anal.* Calcd. for $C_7H_{11}N_3 \cdot C_6H_3O_7N_3$: 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 Ⅳa 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 menbers of the elemental analytical center of this Laboratory for analytical data.

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**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 founded to be more active, while in the ones substituted by the acetamido, dimethylamino and chloro group, the 4-methyl group was more reactive.

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## 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*.

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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-$^{14}$C (DL-Phe-2-$^{14}$C), L-tyrosine-$^{14}$C (u) (L-Tyr-$^{14}$C(u)), and DL-trypto-

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8) *Idem* : Cancer Research, **19**, 388 (1959).
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