Chem. Pharm. Bull. 13(5) 529~534 (1965)

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70. Koji Miura, Masao Ikeda, Tomiji Oohashi, Yoshiko Igarashi, and Ikuko Okada : Chemical and Chemotherapeutical Studies on the Furan Derivatives. XXXVIII.*1 Synthesis and Antitumor Effect of Methylol Derivatives of Nitrofurylvinyl-Aminoheterocyclics.

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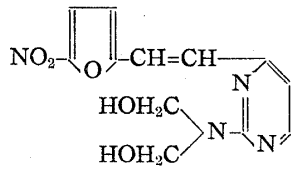
As in the succeeding previous report,[1] six methylol derivatives of nitrofurylvinylogues were prepared and examined as to their antitumor effect on Ehrlich ascites carcinoma in mice. These compounds were prepared from nitrofurylvinyl-aminoheterocyclics with formalin or paraformaldehyde. Except Ⅳ in Table I, the other five compounds were proved to have an antitumor effect at the suitable dose and have small toxicity to mice.

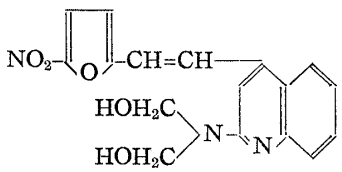
Table I. Methylol Derivatives of Nitrofurylvinyl-Aminoheterocyclics

Compd. No.	Name	Initials	Preparing method
Ⅰ	2-Bis(hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl]-pyrimidine	D-ran-methylol	A
Ⅱ	3-Bis(hydroxymethyl)amino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine	Panfuran-methylol	A, C
Ⅲ	3-(N-hydroxymethyl)methylamino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine	M-ran-methylol	B
Ⅳ	2-[2-(5-Nitro-2-furyl)vinyl]-4,6-(bis[bis(hydroxymethyl)amino]-1,3,5-triazine	G-ran-methylol	A
Ⅴ	2-Bis(hydroxymethyl)amino-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-thiadiazole	T-ran-methylol	A
Ⅵ	2-Bis(hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl]-quinoline	2A-4Q-ran-methylol	B

Especially, 2-bis (hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl]-pyrimidine (A) and 2-bis (hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl] quinoline (B) were ranked as the most active antitumor agents to save most of the mice from death by tumor. 3-Bis (hydroxymethyl)amino-6-[2-(5-nitro-2-furyl)vinyl]-1, 2, 4-triazine[2] and 2-bis (hydroxymethyl)amino-5-[2-(5-nitro-2-furyl)vinyl]-1, 3, 4-thiadiazole were ranked second. From this fact, the presence of methylol groups in molecules seemed to give an antitumor activity, because 3-amino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine (Panfuran) and 2-amino-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-thiadiazole (T-ran) were proved not to have the activity.



(A)



(B)

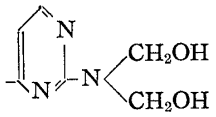
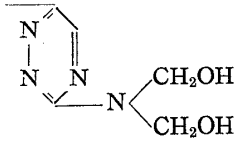
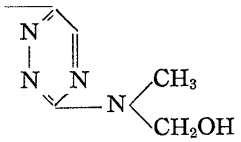
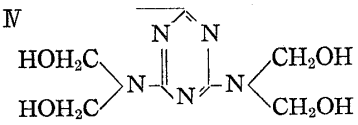
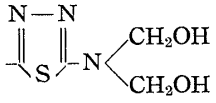
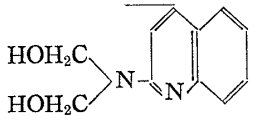
*1 Part XXXVII. K. Miura, *et al.* : This Bulletin, **13**, 525 (1965).
*2 Takaramachi, Kanazawa (三浦孝次，池田政男，大橋富次，五十嵐良子，岡田いく子).
1) K. Miura, *et al.* : Yakugaku Zasshi, **84**, 537 (1964).
2) A. Takai, *et al.* : *Ibid.*, **84**, 23 (1964).

These papers deal with their synthesis, antitumor activity, acute toxicity and antibacterial activity.

I. Chemical Items

The methylol derivatives of nitrofurylvinyl-aminoheterocyclics were prepared in the following ways (ref. Table I). (A) To a parent compound, was added 10 to 30 times of formalin in weight and in cases when it was needed, it was boiled for 1 hour. (B) To a parent compound dissolved in a certain amount of an organic solvent, was added about 5 moles of formalin and when needed, it was boiled for 1 hour. (C) To a parent compund dissolved in a certain organic solvent, was added 5 moles of paraformaldehyde and when needed, it was heated for 1 hour. Their chemical properties together with their elemental analtical data are listed in Table II. Parent compounds of these methlol derivatives were already reported.[3~5]

Table II. Chemical Properties of tested Compounds

Compd. No.	Chemical structure R	m.p. (°C) ( ) : decomp.	Appearance	UV λ_{max}^{EtOH} (mμ)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
I		(188)	yellow crystal	240	$C_{12}H_{12}O_5N_4$	49.31	4.14	19.17	49.88	4.32	18.58
II[a]		161	yellow crystal	296	$C_{11}H_{11}O_5N_5$	45.05	3.78	23.88	45.22	3.94	23.83
III		155	orange crystal	300	$C_{11}H_{11}O_4N_5$	47.65	4.00	25.26	47.35	4.23	24.99
IV		295	orange yellow crystal	296	$C_{13}H_{16}O_7N_6$	42.39	4.38	22.82	42.43	4.58	22.53
V		(224)	orange yellow crystal	231, 316	$C_{10}H_{10}O_5N_4S$	40.27	3.38	18.79	40.62	3.56	18.45
VI		(238)	orange crystal	235	$C_{17}H_{15}O_5N_3$	59.82	4.43	12.31	60.22	4.51	12.72

a) All the compounds except II listed in this table were prepared newly in our laboratory.

All methylol derivatives of nitrofurylvinylogues did not dissolve in water and were pretty unstable. Blowing, heating, recrystallization in organic solvents were liable to release formaldehyde to let them return to their parent compounds. They were also sensitive to alkali and acid. Accordingly, they were recrystallized from formalin.

3) K. Miura : Antimicrobial Agent and Chemotherapy-2-277 (1962).
4) K. Miura, *et al.* : Yakugaku Zasshi, 83, 771 (1963).
5) *Idem* : *Ibid.*, 83, 778 (1963).

There is another method to prepare panfuran-methylol, that is the treatment of acetylpanfuran with formalin. In this reaction at first the acetyl group is left from the amino group of panfuran and then the methylol group is added to this position of the unoccupied amine group and the final product is obtained. Infrared spectrum of these six methylol compounds revealed the disappearance or fading of amino groups, as compared with their parent compounds.

As for Ⅳ, the parent compound, G-ran was prepared from 2-[2-(5-nitro-2-furyl)-vinyl]-4,6-bis(acetamido)-1,3,5-triazine through deacetylation, which was synthesized from 5-nitrofurfural and 2-methyl-4,6-bis(acetamido)-1,3,5-triazine.[6] Ⅳ was prepared from G-ran through methylol-formation. G-ran : orange yellow powder, m.p. 307° (darkened). *Anal.* Calcd. for $C_9H_8O_3N_6$: C, 43.55; H, 3.25; N, 33.86. Found : C, 43.79; H, 3.45; N, 33.61. Diacety-G-ran : yellow needles, m.p. 247~248° (decomp.). *Anal.* Calcd. for $C_{13}H_{12}O_5N_6$: C, 46.99; H, 3.64; N, 25.30. Found : C, 46.75; H, 3.51; N, 25.12.

II. Curative Effect on Ehrlich Ascites Carcinoma in Mice

To mice transplanted with Ehrlich ascites carcinoma EY-33 test compounds were injected intraperitoneally. The states of the mice were observed for 50 days. 50 DPA was finally decided (50 days prolongation activity). As shown in Fig. 1, when 150 mg./kg. was administered intraperitoneally once after 24 hours of transplantation, four mice out of five survived by Ⅰ and Ⅵ, three mice by Ⅱ and one mouse by Ⅴ, while all control mice died of tumor rupture. With the administration of 250 mg./kg. after 4 days of transplantation, Ⅰ saved all the test mice. The results of comparative experiments are in Table Ⅲ.

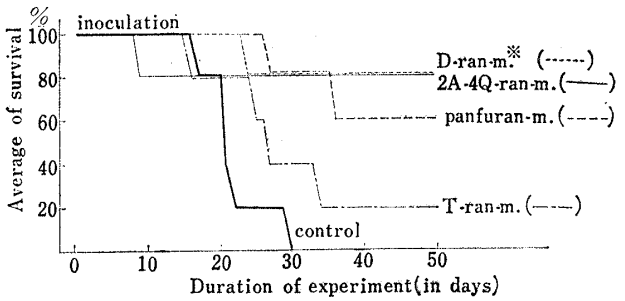


Fig. 1. Comparison of Curative Action of Methylol Derivatives of Nitrofurylvinyl-Aminoheterocyclics on Ehrlich Ascites Carcinoma in Mice

※ m : methylol
150 mg./kg. single dose (i.p.) in 24 hr. after transplantation.

Table Ⅲ. Antitumor Effect of Methylol Derivative of Nitrofurylvinyl-Aminoheterocyclics on Ehrlich Ascites Carcinoma in Mice

Compd. No.	Initials	Dose × times (day)[a] mg./kg. i.p.	Toxicity[b] (S/T)	Antitumor effect[c] (S/T)	Survivor (%)
Ⅰ	D-ran-methylol	250 × 1 (4)	5/5	5/5	100
		150 × 1 (1)	5/5	4/5	80
		150 × 1 (4)	5/5	4/5	80
Ⅱ	Panfuran-methylol	250 × 1 (1)	4/5 (toxic)	2/4	50
		150 × 1 (1)	5/5	3/5	60
		50 × 3 (1, 2, 3)	5/5	2/5	40
		150 × 1 (4)	5/5	1/5	20
		15 × 5 (1, 2, 3, 4, 5)	5/5	1/5	20
Ⅳ	G-ran-methylol	150 × 3 (1, 2, 3)	5/5	0/5	0
Ⅴ	T-ran-methylol	150 × 1 (1)	5/5	1/5	20
		50 × 3 (1, 2, 3)	5/5	1/5	20
Ⅵ	2A-4Q-ran-methylol	150 × 1 (1)	5/5	4/5	80
		150 × 1 (4)	5/5	4/5	80
		50 × 2 (1, 2)	5/5	2/5	40

a) first treatment was in 24 hours after transplantation.
b) died by drug administration in tumor bearing mice.
c) 50 DPA : 50 days prolongation activity all 20 control mice died with tumor invasion in 10 to 30 days, most of them 16th to the 19th day after transplantation.

6) A. Ostrogorich : Chem. Zentr., **76II**, 1359 (1905).

Previously, Panfuran hydrochloride and T-ran were reported not to have any antitumor effect and now Panfuran-methylol and T-ran-methylol were proved to have one. This indicated methylol formation connected with the antitumor activity.

III. *in vitro* Antibacterial Activity

The results of the *in vitro* antibacterial activities against gram-positive bacteria of *Diplococcus pneumoniae* type I, *Streptococcus pyogenes*, *Staphylococcus aureus* and gram-negative bacteria of *Escherichia coli*, *Shigella flexneri* 2a are shown in Table IV.

Table IV. Antibacterial Activity of tested Compounds *in vitro*

Compd. No.	Initials	MIC[a]				
		D. pneumoniae type I	*Str. pyogenes* (haemolyticus)	*Staph. aureus* (Terashima)	*E. coli* (Gakusei)	*Sh. flexneri* 2a
I	D-ran-methylol	6.25	3.13	1.56	1.56	1.56
II	Panfuran-methylol	1.56	1.56	0.39	0.098	0.195
III	M-ran-methylol	1.56	3.13	0.78	0.195	0.195
IV	G-ran-methylol	12.5	6.25	0.78	50.0	12.5
V	T-ran-methylol	6.25	1.56	0.39	0.39	0.39
VI	2A-4Q-ran-methylol	0.195	0.78	0.195	12.5	3.13

a) minimum inhibitory concentration : γ/ml.
Result : after 48 hr. incubation at 37°

Against gram-positive bacteria, VI was the strongest. Then II and V were next in order. Against gram-negative bacteria, II, III, and V inhibited growth of the bacteria at γ/ml. levels. Compared with their parent compounds, methylol derivatives were generally weak.

IV. Acute Toxicity

With the four compounds, acute toxicities mice were examined. The results are in Table V.

Table V. Acute Toxicity in Mice by Intraperitoneal Injection

Compd. No.	Initials	Single dose mg./kg. i.p.	Survivor/Total	LD_{50} mg./kg. i.p. (estimate)
I	D-ran-methylol	1000	5/5	>1000
		500	5/5	
II	Panfuran-methylol	1000	0/5	500
		500	2/5	
		250	5/5	
V	T-ran-methylol	1000	0/5	500
		500	2/5	
		250	5/5	
VI	2A-4Q-ran-methylol	1000	5/5	>1000
		500	5/5	

Methylol derivatives had comparatively weak toxicity. One thousand milligrams per kilo of I or VI did not kill animals by intraperitoneal injection. The same amount of II or V revealed toxic symptoms and killed them within 24 hours. For example, the LD_{50} calculated in the subcutaneous injection of panfuran hydrochloride, which is the parent compound of panfuranmethylol, is 300 mg. per kilo of animal body, and also its LD_{50} in the peroral route is 400 mg. per kilo. But the methylol derivative of panfuran

did not kill animals by the intraperitoneal injection of 300 mg./kg. The LD_{50} level of 2A-4Q-ran lactate is 30 mg. per kilo of body weight in a single intraperitoneal dose. It was observed that the toxicity of 2A-4Q-ran-methylol is considerably less than that of 2A-4Q-ran itself, *viz*, this methylol derivative did not cause toxic death for a mouse even in the intraperitonal injection of 1000 mg. per kilo. That is to say, methylol formation decreased toxicity.

Experimental

Synthesis

I) 2-Bis(hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl]pyrimidine : One and half grams of 2-amino-4-[2-(5-nitro-2-furyl)vinyl]pyrimidine (m.p. 226° (decomp.)) and 15 ml. of 37% formalin were weakly boiled for 1 hr. and then filtered while warm. The filtrate was poured in water. Recrystallized from 37% formalin and washed with water. yield : 0.5 g.

II) 3-Bis(hydroxymethyl)amino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine : a) One gram of 3-amino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine (m.p. 267° (decomp.)) and 1.0 g. of paraformaldehyde in the mixed solution of 10 ml. N,N-dimethylformamide (DMFA) and 10 ml. water were heated at 95° for 1 hr. and then filtered while warm. Yellow needles were washed with water. Yield : 0.7 g.

b) Three-tenth grams of 3-acetamido-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine (m.p. 278° (decomp.)) and 20 ml. of 37% formalin were heated at 95°. When dissolved, concentrated under depression, then added with 40 ml. water and heated again. It was then filtered while warm. Yellow needles, yield: 0.3 g. These needles were identified with II by analysis and by the IR spectrum.

III) 3-(N-hydroxymethyl)methylamino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine : One half grams of 3-methylamino-6-[2-(5-nitro-2-furyl)vinyl]-1,2,4-triazine (m.p. 234° (decomp.)) and 15 ml. of 37% formalin were weakly boiled and then filtered while warm. Recrystallized from 37% formalin and washed with water. Yield : 0.25 g.

IV) 2-[2-(5-Nitro-2-furyl)vinyl]-4,6-bis[bis(hydroxymethyl)amino]-1,3,5-triazine : 1) Two point one grams of 2-methyl-4,6-bis(acetamido)-1,3,5-triazine and 1.4 g. of 5-nitrofurfural dissolved in 20 ml. glacial acetic acid were weakly boiled for 3 hr. Precipitate was recrystallized from ethylene glycol monomethyl ether (GM). Yellow needles were 2-[2-(5-nitro-2-furyl)-vinyl]-4,6-bis(acetamido)-1,3,5-triazine. m.p. 247~248° (decomp.), yield : 1.3 g.

2) One gram of the diacetyl compound was heated with 100 ml. of 6% HCl under refluxing for 4 hr. After cooling, it was neutralized with NH_4OH. The precipitate was recrystallized from GM. The orange yellow crystal was 4,6-diamino-2-[2-(5-nitro-2-furyl)vinyl]-1,3,5-triazine (G-ran). m.p. 307° (darkend), yield : 0.5 g.

3) One half grams of G-ran in DMFA and 3 ml. of 37% formalin were heated at 95° for 1 hr. It was then poured in water after cooling. Recrystallized from 37% formalin and washed with water. Yield: 0.2 g.

V) 2-Bis(hydroxymethyl)amino-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-thiadiazole : Five grams of 2-amino-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-thiadiazole (m.p. 246° (decomp.)) and 150 ml. of 37% formalin were weakly boiled for 1 hr. The precipitate was recrystallized from 37% formalin and washed with water. Yield: 3.5 g.

Biological Items

1) Curative Effect on Ehrlich Ascites Carcinoma in Mice——A pure strain of the ddN mice weighing 18 to 20 g. were used in this experiment and five mice were made one group. At one time 5 or 6 groups were used. 0.1 ml. of ascites containing 3 million carcinoma cells were transplanted intraperitoneally to all mice. Twenty-four hours later, or at a certain time, test compounds were injected intraperitoneally. Control mice died of tumor in about 10 to 19 days after transplantation.

Insoluble compounds in water were suspended in 1% CMC solution. Activity was determined by 50 days observation. (50 DPA) Survival mice on 50th day were examined of their ascites and nodes and judged whether they were completely cured or not.

2) **Acute Toxicity**——LD_{50} was determined by testing mice of the ddN strain weighing 18 to 20 g., using five mice as one group. It was administered intraperitoneally, as a 1% CMC suspension, and observed for 7 days. LD_{50} was calculated after Behrens Körber method with survival ratio.

3) ***in vitro* Antibacterial Activity**——Two fold dilutions of test compounds were made in 2 ml. of pH 7.0 common broth. Inoculum consisted of two drops of a 1:100,000 dilution of 24 hr. test culture except *Diplococcus pneumoniae* (its dilution was 1:1,000 ratio) and incubation was at 37° for 48 hr. Minimum inhibitory concentration was determined.

Summary

Six methylol derivatives of nitrofurylvinylogues were prepared from their parent compounds with formaldehyde or paraformaldehyde in water or in organic solvents. Of these, antitumor effect on Ehrlich ascites carcinoma in mice was investigated. 2-Bis(hydroxymethyl)amino-4-[2-(5-nitro-2-furyl) vinyl] pyrimidine (D-ran-methylol) and 2-bis(hydroxymethyl)amino-4-[2-(5-nitro-2-furyl)vinyl]quinoline (2A-4Q-ran-methylol) were revealed to save mice completely from tumor death with the suitable dose intraperitoneally. These two compounds had small toxicities and administrations of 1,000 mg. per kilo did not kill any mouse.

(Received August 6, 1964)

[Chem. Pharm. Bull. 13(5) 534~538 (1965)]

UDC 543.061.544.81:547.473.2

71. Tsutomu Momose, Yosuke Ohkura, and Kazuya Kohashi: Determination of 3-Hydroxybutyric Acid in Blood *via* Acetone with Trinitrobenzene (Organic Analysis. LX*[1]).

(*Faculty of Pharmaceutical Sciences, Kyushu University*[*2])

The current colorimetric method for the determination of 3-hydroxybutyric acid in blood is based on the oxidation of the acid with a dichromate-sulfuric acid solution. The yielded acetone is distilled[1~3] or extracted[4~6] from the reaction mixture and colored with salicylaldehyde,[1,2] furfural,[3] or 2,4-dinitrophenylhydrazine.[4~6] This method, however, is laborious and usually requires extensive equipment. Therefore it may be unsuitable for routine work in a clinical laboratory.

In the writer's laboratory, a simple piece of oxidation-distillation equipment has been designed which can treat with many samples at the same time. Thus, a simple method of determining 3-hydroxybutyric acid in blood is now presented by combining the procedure with the previously established method of determining acetone and acetoacetic acid in blood with trinitrobenzene as a color developing agent.[7]

Experimental

Reagents

1,3,5-Trinitrobenzene solution (0.1%), NaOH solution (1.8%), NaH_2PO_4 solution (18 g./dl.), Na_2WO_4 solution (7.5 g./dl.) and $KAl(SO_4)_2$ solution (7.2 g./dl.) are prepared in the same way as described in the previous paper.[7]

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*1 Part LIX: Bunseki Kagaku, **14**, 240 (1965).

*2 Katakasu, Fukuoka (百瀬 勉, 大倉洋甫, 小橋一彌).

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6) H. Schön, I. Lippach: Klin. Wochschr., **34**, 1083 (1956); P. A. Mays, W. Robson: Biochem. J., **67**, 11 (1957).

7) T. Momose, Y. Ohkura, K. Kohashi, R. Nagata: This Bulletin, **11**, 973 (1963).