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69. Koji Miura and Ikuko Okada : Chemical and Chemotherapeutical Studies on the Furan Derivatives. XXXVII.*1 Studies on the Phage Inducing Activity on *Escherichia coli* K12 (λ) of Nitrofuranquinoline Compounds.

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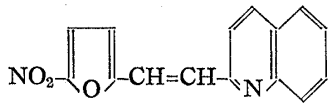
2-[2-(5-Nitro-2-furyl)vinyl]quinoline (2Q-ran) was reported[1] to have an antitumor effect of one hundred percent on Ehrlich ascites carcinoma in mice in a suitable dose. In 2Q-ran, are joined a nitrofuran nucleus and a quinoline nucleus by one double bond. It was supposed that the antitumor activity of nitrofuran derivatives were resulted from the inhibition of DNA synthesis and the inhibition of dehydrogenase connected with glycolysis.[2]



2Q-ran

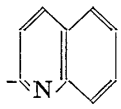
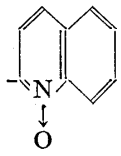
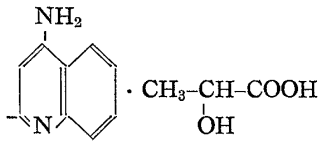
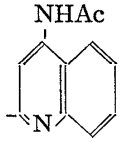
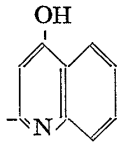
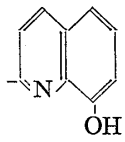
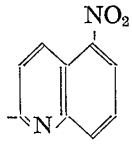
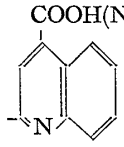
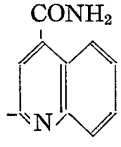
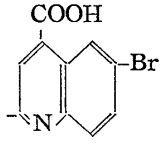
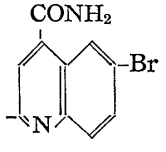
Previously, in 1960, Sekiguchi, *et al.*, reported[3] that Mitomycin C, known as a drug for cancer, had the phage inducing activity in lysogenic bacteria and it was due to the inhibition of DNA synthesis. In 1963, Endo, *et al.*, found[4] 3-amino-6-[2-(5-nitro-2-furyl)-

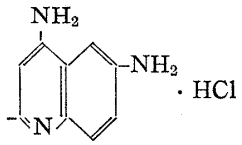
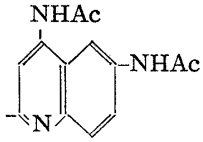
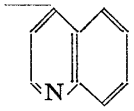
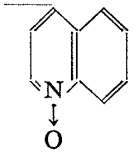
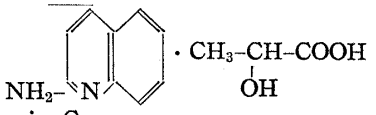
Table I.

Compounds tested	No.
2-[2-(5-Nitro-2-furyl)vinyl]quinoline	I
2-[2-(5-Nitro-2-furyl)vinyl]quinoline 1-oxide	II
2-[2-(5-Nitro-2-furyl)vinyl]-4-aminoquinoline lactate	III
2-[2-(5-Nitro-2-furyl)vinyl]-4-acetamidoquinoline	IV
2-[2-(5-Nitro-2-furyl)vinyl]-4-quinolinol	V
2-[2-(5-Nitro-2-furyl)vinyl]-8-quinolinol	VI
2-[2-(5-Nitro-2-furyl)vinyl]-5-nitroquinoline	VII
2-[2-(5-Nitro-2-furyl)vinyl]cinchoninic acid (Na)	VIII
2-[2-(5-Nitro-2-furyl)vinyl]cinchoninamide	IX
2-[2-(5-Nitro-2-furyl)vinyl]-6-bromocinchoninic acid	X
2-[2-(5-Nitro-2-furyl)vinyl]-6-bromocinchoninamide	XI
2-[2-(5-Nitro-2-furyl)vinyl]-4,6-diaminoquinoline hydrochloride	XII
2-[2-(5-Nitro-2-furyl)vinyl]-4,6-bis(acetamido)quinoline	XIII
4-[2-(5-Nitro-2-furyl)vinyl]quinoline	XIV
4-[2-(5-Nitro-2-furyl)vinyl]quinoline 1-oxide	XV
4-[2-(5-Nitro-2-furyl)vinyl]-2-aminoquinoline lactate	XVI

*1 Part XXXVI. K. Miura, *et al.* : Yakugaku Zasshi, 84, 537 (1964).
*2 Takaramachi, Kanazawa (三浦孝次, 岡田いく子).
1) K. Miura, *et al.* : Yakugaku Zasshi, **84**, 341 (1964).
2) K. Miura, *et al.* : *Ibid.*, **84**, 537 (1964).
3) M. Sekiguchi, Y. Takagi : Biochem. Biophys. Acta, **41**, 434 (1960).
4) H. Endo, M. Ishizawa, T. Kamiya, M. Kuwano : *Ibid.*, **68**, 502 (1963).

Table II.

No.	NO_2-(furan)-CH=CH-R R	M. I. C. *E. coli* K12 (λ) (γ/ml.)	Phage[a] induction (γ/ml.)	Antitumor[b] effect
I		0.78	1.0	++++
II		0.195	1.0	+++
III		0.78	0.5	+++
IV		0.78	0.5	+++
V		0.195	1.0	++
VI		0.195	1.0	—
VII		50.0	——	—
VIII		50.0	——	+++
IX		1.56	——	+++
X		50.0	——	++
XI		50.0	——	—

XII		0.049	0.25	+++
XIII		0.39	0.5	+
XIV		1.56	1.0	++
XV		1.56	1.0	+++
XVI		0.78	0.5	++++
Mitomycin C		1.56	0.5	++++

a) Minimum inducting concentration.
—: means no effect at 1.0 γ/ml.
b) at the optimal condition reported before.
++++: 90~100% cured
+++: 50~90% cured
++: 10~50% cured
+: 0% cured (prolongation)
−: no effect

vinyl]-*as*-triazine HCl (Panfuran HCl)[5] to be an inducing agent and they indicated it was caused by DNA-specific metabolic inhibition.

This paper deals with the result of the study on the antibacterial activity as well as the phage inducing activity of 2-Q-ran and its analogous against *Escherichia coli* K 12 (λ), a lysogenic strain. Their chemical structures were especially considered in relation to the phage inducing activity. Moreover, the relationship between an inducing activity and an antitumor effect was investigated on the nitrofuran-quinoline compound in comparison with Mitomycin C.

The first test was the antibacterial activity in vitro against *E. coli* K 12 (λ), a lysogenic strain, by the two fold dilution method. The results are summarized in Table II. It shows Mitomycin C inhibited the growth of the bacteria at the 1.5 γ/ml., while the other compounds were effective at the equal level or more diluted levels except VII, VIII, X, and XI. Among them, 4,6-Diamino-2Q-ran HCl (XII) was the strongest and it was 32 times stronger than Mitomycin C.

The second test was the activity to develop free phages, measuring the change of the turbidity of the bacterial culture at 30 minute intervals. Free phages were ascertained by the plaque assay with the indicator strain, *E. coli* C 600. The results are presented in Table II. The

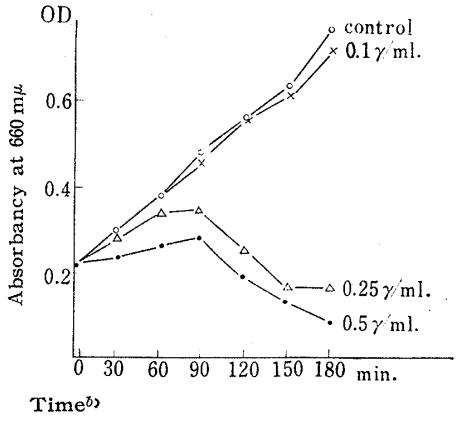


Fig. 1. Change of Turbidity by 4,6-Diamino-2Q-ran HCl (XII)[a]

a) Effect at XII for the induction of λ-phage development in *E. coli* K12 (λ).
b) Compound was contacted at 0 time.

5) K. Miura, *et al.*: Yakugaku Zasshi, 81, 1372 (1961).

change of the turbidity of the bacterial culture caused by 4,6-Diamino-2Q-ran HCl is shown in Fig. 1.

It is clear that at 0.25 γ/ml. and 0.5 γ/ml. levels 4,6-Diamino-2Q-ran HCl developed free phages. The minimum inducing concentrations of the test compounds are shown in Table II: 2Q-ran at 1.0 γ/ml., 4,6-Diamino-2Q-ran HCl at 0.25 γ/ml. and Mitomycin C at 0.5 γ/ml. 2Q-ran, 4Q-ran, and their derivatives having $-NH_2$, -OH, or $-NHCOCH_3$ on the quinoline nucleus induced free phages at comparable levels. The minimum inhibitory concentrations of these compounds except IX were found in general to run parallel to the activities of the phage induction. And the phage inducing activities also ran parallel to the antitumor effect except VI, VIII, IX, X.

In conclusion, Table II. shows: 1) Except six compounds (from IV to IX), tested 11 compounds had both the phage inducing activity as well as antitumor effect and they had also strong antibacterial activity against *E. coli*, lysogenic strain. 2) Compounds having each substituent of $-NO_2$, -COOH, $-CONH_2$, and two substituents of COOH and Br, $-CONH_2$ and Br, had not the phage inducing activity but VIII, IX, and X had antitumor effect. An antitumor effect of 4-carboxy-2Q-ran (VIII) was thought to result from the inhibition of dehydrogenase of tumor cell connected with glycolysis.[6] 3) It is now estimated that IX gives ammonia and VIII under hydrolytic decomposition *in vivo*. The cause will be searched for in future. 4) V is equal to VI in the grade of phage inducing activity and also antibacterial effect but VI had not antitumor effect different from V. This fact is due to difference in a position of OH-group on the quinoline-nucleus of 2Q-ran.

Experimental

1) ***in vitro* Antibacterial Activity against *E. coli* K 12 (λ), a Lysogenic Strain**——*E. coli* K 12 (λ) was cultured for 24 hr. at 37° in sarcous*3 broth and diluted to 10^{-5}. Test compounds were diluted by the two fold dilution method in the tubes containing the broth. To these dilution tubes, two drops of the diluted culture were added and then they were incubated for 48 hr. at 37° and the minimum inhibitory concentrations were determined.

2) **Inducing Activity for the Phage Developement in Lysogenic *E. coli* K 12 (λ)**——*E. coli* K 12 (λ) was grown to cell density of 5×10^8 cells/ml. in a glucose-salts synthetic medium, washed once, resuspended to the original density in a salts solution containing various concentrations of the test compounds and incubated for 10 min. at 37° under shaking. The bacteria were washed to avoid further interaction of the compounds and resuspended in λ-broth*4 and then incubated for 180 min. at 37° under shaking. Turbidities were measured at 30 min. intervals and when the cells were lysed, 0.1 ml. of the culture was taken out for the plaque assay. The 0.1 ml. was put into the first tube containing 10 ml. of salts solution and 0.5 ml. of chloroform and was mixed well, then diluted properly. The final 0.1 ml. was plated on an agar plate with the indicator strain, *E. coli* C 600. After incubation overnight, at 37°, plaques were counted. This plaque assay was to ascertain the induction of lysogenic bacteria.

The authors are indebted to Dr. H. Sawada and Dr. A. Taketo of the Medical Department of Kanazawa University for supplying us with *E. coli* K 12 (λ) and *E. coli* C 600 and for their helpful advice.

Summary

Sixteen nitrofurylvinylquinoline compounds were examined for their phage inducing activity on *E. coli* K 12 (λ), a lysogenic strain. Excepting compounds having -COOH, $-CONH_2$, $-NO_2$, or -Br on the quinoline nucleus, they had phage inducing ability. The compound, 4,6-Diamino-2Q-ran HCl was the strongest among them and its activity was twice as strong as that of Mitomycin C, a drug for cancer.

It is a notable fact that the tested nitrofurylvinylquinoline compounds which have an inducing activity in lysogenic bacteria exhibit, with one exception, an antitumor effect on bearing Ehrlich ascites carcinoma.

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*3 Meat extract 10.0 g. Polypepton 10.0 g. NaCl 2.0 g. Total (H_2O) 1000 g.

6) K. Miura, *et al.*: Yakugaku Zasshi, **84**, 344 (1964).

*4 Polypepton 10.0 g. NaCl 2.5 g. Total (H_2O) 1000 g.