In these reduction reactions, the benzene extracts obtained by the reduction of the morpholino-, piperidino-, and dimethylamino-enamine of the basic 6-oxo-compounds were treated with NH_2OH-HCl (0.02 molar equivalents) in warm water for 30 min. in order to remove contaminated 6-oxo-compound. Then, the mixture was made alkaline with NH_4OH, and extracted with benzene. The benzene solution was washed with water, dried over Na_2SO_4, and chromatographed on active Al_2O_3 (ten times of the solute). The benzene eluate was collected and evaporated to give a pure sample of the 6-amino compound.

General Method for Catalytic Reductive Amination of 6-Oxo-compound——A mixture of 6-oxo-compound (0.01 mole), secondary amine (0.01 mole), 10% Pd-C (3 g.), and MeOH (100 ml.) was treated as described in the case of catalytic reduction of enamines. This method is particularly suitable for preparation of the pyrrolidino-derivatives.

The author is grateful to Prof. K. Tsuda of the University of Tokyo, Mr. M. Matsui, Director of this laboratories, and Dr. I. Iwai, Assistant Director of this laboratories for their advice and encouragement throughout this work. Thanks are also due to the members of analytical and physical measuring section in this laboratories for the micro-analysis and measuring of IR spectra.

Summary

Preparation of 6-amino-hydrophenanthrene compounds from the 6-oxo-hydrophenanthrene compounds derived by the Hofmann degradation reaction of the 6-oxo-morphine alkaloids was described. There have been used catalytic reductive amination of the 6-oxo-compounds and catalytic, sodium borohydride, and formic acid reductions of their enamine derivatives. Behaviors of the 6-oxo-compounds in the described reactions were quite similar to those of the 6-oxo-morphines reported previously.

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[Chem. Pharm. Bull. 14(5) 461~466 (1966)] UDC 615.771.7 : 547.722.5.09

62. Toshimitsu Ujiie : Experimental Anticancer Studies. XXVIII.*1 Anticancer Activity of Some Nitrofuran Derivatives.*2

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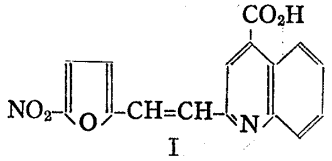
It was reported in the previous paper[1] that 2-[2-(5-nitrofuryl)vinyl]-4-quinoline-carboxylic acid (I) showed moderate prolongation of life span of mice implanted with Ehrlich ascites carcinoma cells. Meanwhile, Miura, *et al.*, who examined a variety of analogous nitrofuran derivatives, found that 2-[2-(5-nitrofuryl)vinyl] quinoline was similarly effective.[2]



I

The results of anticancer experiments with some of the Schiff base compounds of 2,4-dihydroxy-5-*n*-hexylbenzaldehyde were already reported elswhere.[3]

*1 Part XXVII. Japan. J. Exptl. Med., **35**, 249 (1965)

*2 A part of this paper was presented at the 44th Meeting of Juzen Medical Society (Kanazawa) on February 27, 1965.

*3 Takara-machi, Kanazawa (氏家俊光).

1) T. Ujiie : Ann. Rep. Tbc. Kanazawa, **21**, (3), 363 (1964).

2) K. Miura, M. Ikeda, T. Oohashi, I. Okada, Y. Igarashi : Yakugaku Zasshi, **84**, 341 (1964); *Idem* : *Ibid.*, **84**, 537 (1964).

3) R. Koshiura, Y. Kagotani, T. Ujiie : This Bulletin, **10**, 528 (1962); T. Ujiie, Y. Kagotani, S. Koshimura : Presented at the 82nd Annual Meeting of the Pharmaceutical Society of Japan (Shizuoka), 1962.

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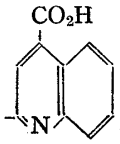
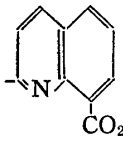
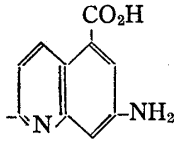
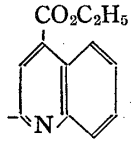
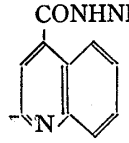
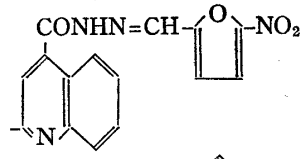
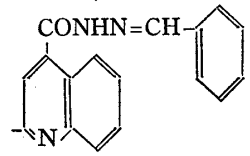
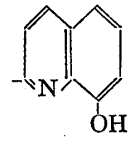
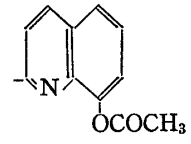
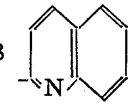
TABLE I. Quinoline Derivatives NO_2-(furan)-CH=CH-R

No.	Compound R	m.p.[a] (°C) ( )=decomp.	Recryst. solvent	Formula	Elemental analysis (%) Calcd. C	H	N	Found C	H	N
256		>290	nitrobenzene	$C_{16}H_{10}O_5N_2$	61.94	3.25	9.03	61.82	3.71	8.73
260		>290	〃	$C_{16}H_{10}O_5N_2$	61.94	3.25	9.03	61.96	3.39	9.30
261		>290	dimethylformamide	$C_{16}H_{11}O_5N_3$	59.08	3.41	12.92	59.02	3.56	13.01
262		187	ethanol	$C_{18}H_{14}O_5N_2$	63.90	4.17	8.28	63.83	4.31	7.81
263		(231)	isopropanol	$C_{16}H_{12}O_4N_4$	59.26	3.73	17.28	59.42	4.04	—
264		(289)	methylcellosolve	$C_{21}H_{13}O_7N_5$	56.38	2.93	15.66	55.98	2.96	—
265		(289)	〃	$C_{23}H_{16}O_4N_4$	66.98	3.91	13.59	66.59	4.00	—
266		192	dil. ethanol	$C_{15}H_{10}O_4N_2$	63.83	3.57	9.93	64.14	3.44	9.54
267		173	benzene, petr. benzine	$C_{17}H_{12}O_5N_2$	62.96	3.73	8.64	62.84	3.80	8.45
268		180[b]	ethanol	—	—	—	—	—	—	—

a) Uncorrected
b) m.p. 180°; K. Miura, T. Oohashi, S. Matsuda, Y. Igarashi: Yakugaku Zasshi, **83**, 771 (1963).

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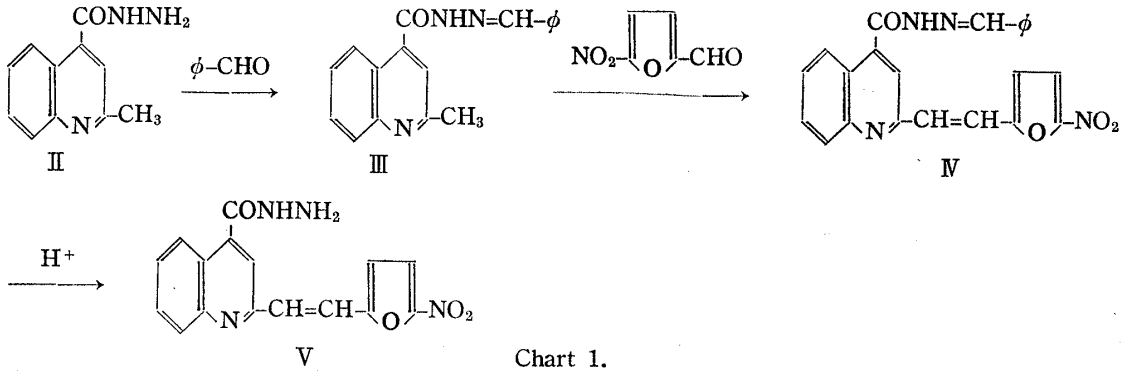

Chart 1.

Table II. Schiff Base Derivatives of 5-Nitrofuradehyde NO_2-(furan)-CH=N-R

No.	Compound	m.p.[a] (°C) ( )=decomp.	Recryst. solvent	Formula	Elemental analysis (%) Calcd. C	H	N	Found C	H	N
269	-(OH)-phenyl	(124)[b]	ethanol	$C_{11}H_8O_3N_2$	61.11	3.73	12.96	61.21	3.65	12.99
270	-(OH, Br)-phenyl	(178)[c]	〃	$C_{11}H_8O_4N_2$	56.90	3.47	12.07	57.06	3.70	11.95
271	-(Br)-phenyl	(187)	〃	$C_{11}H_6O_4N_2Br_2$	33.85	1.54	7.17	34.00	1.68	7.21
272	-phenyl-CO_2H	(234)[d]	—	$C_{12}H_8O_5N_2$	55.39	3.10	10.77	55.38	3.08	10.72
273	-phenyl-OCH_2CO_2H	(187)	—	$C_{13}H_{10}O_6N_2$	53.80	3.47	9.65	54.05	3.69	9.48
274	-NHCO-(CH3, N)-phenyl	(256)	ethanol	$C_{16}H_{12}O_4N_4$	59.26	3.73	17.28	59.21	3.77	17.14

a) Uncorrected.
b) m.p. 127.5°; R.W. Drisko, H. McKennis, Jr.: J. Am. Chem. Soc., **74**, 2626 (1952).
c) m.p. 167~169° (decomp.); O. Dann, E.F. Moeller: Chem. Ber., **82**, 76 (1949).
d) m.p. 227° (decomp.); T. Takahashi, H. Saikachi, S. Yoshina, C. Mizuno: Yakugaku Zasshi, **69**, 284 (1949).

Further efforts to obtain more potent carcinostatics among compounds of these types have been independently made in this laboratory.

This paper deals with anticancer activity of some nitrofuran derivatives related to I and of some Schiff bases of 5-nitrofuraldehyde. Ethylenic bridge linkage was effected by heating (160~170°) a mixture of an active methyl compound and 5-nitrofuraldehyde in the presence of acetic anhydride. Schiff base compound was prepared by mixing an amine and 5-nitrofuraldehyde in alcoholic solution at room temperature, if necessary under refluxing.

It has been known that in some organic compounds, sucn as INH (isonicotinic acid hydrazide) and apresoline, the introduction of hydrazino group into a certain position of the chemicals produced substances having an interesting biological activity. Therefore, it was of interest to prepare and examine hydrazide of I, which was

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prepared as illustrated in Chart 1; 2-methyl-4-quinolinecarboxylic acid hydrazide (II), after protecting by benzaldehyde, was reacted with 5-nitrofuraldehyde to produce IV, which was then converted by boiling with 10% hydrochloric acid to V,

Physical properties and analytical data of the quinoline compounds and those of Schiff base derivatives of 5-nitrofuraldehyde are shown in Table I and Table II, respectively.

Biological Results——All nitrofurans listed in Table I and II were tested for their anticancer activity. The results were recorded in Table III and IV.

Judging from both the number of survivors per test animals after 40 days of implantation and toxicity to mice of the compound, some of the compounds were shown to be very effective in inhibiting the cancer cells growth; 2-[2-(5-nitrofuryl)

Table III. Effect of Nitrofurans Listed in Table I on Ehrlich Ascites Carcinoma in Mice

No.	Toxicity LD_{50} in mice mg./kg. (i.p.)	Anticancer experiment[a] Dose[b] (mg./kg.)×(day) (i.p.)		No. of Survivors/Test animals	Remark[c]
256	125	25	7	6/8	+
		12.5	7	2/8	
260	150	25	7	8/8	++
		12.5	7	2/8	
261	—	25	7	0/8	−
262	>500	250	1	0/8	−
263	125	25	7	8/8	+++
		12.5	7	8/8	
		6.3	7	3/8	
264	>300	150	7	0/8	−
265	>300	150	7	0/8	−
266	>300	150	7	0/8	−
267	1500	500	1	8/8	++++
		250	1	8/8	
		125	1	8/8	
		62.5	1	8/8	
		31.3	1	8/8	
		15.6	1	2/8	
268	600	500	1	6/8	++++
		250	1	8/8	
		125	1	8/8	
		62.5	1	8/8	
		31.3	1	8/8	
		15.6	1	4/8	
Mitomycin C	5.2	1.0	1	5/8	++++
		0.5	1	2/8	
		0.25	1	0/8	
		0.25	7	8/8	
		0.13	7	0/8	
Control[d]		(CMC)	7	0/8	−

a) Each animal was implanted with 4×10^6 cells of Ehrlich ascites carcinoma intraperitoneally. Treatment with chemicals was initiated 24 hr. after implantation. Number of survivors per 8 test animals on the 40th day of implantation.
b) Suspended in CMC (carboxymethylcellulose) solution in the cases of Nos. 262, 264, 265, 266, 267 and 268.
c) Arbitrary grading of anticancer effect.
d) Animals were given CMC solution only.

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Table IV. Effect of Schiff Base Derivatives of 5-Nitrofuraldehyde Listed in Table II on Ehrlich Ascites Carcinoma in Mice

No.	Toxicity LD_{50} in mice mg./kg. (i.p.)	Anticancer experiment[a] Dose[b] (mg./kg.)×(day) (i.p.)		No. of Survivors/Test animals	Remark[c]
269	300	150	5	7/10	±
		50	5	0/10	
270	>300	150	5	9/10	+
		50	5	3/10	
271	>300	200	3	9/10	+
		50	5	0/10	
272	>300	150	5	7/10	+
		50	5	3/10	
273	>300	150	5	5/10	±
		50	5	0/10	
274	>300	150	7	0/10	−
Mitomycin C	5.2	0.25	7	10/10	++
		0.13	7	0/10	
Control	—	(CMC)	7	0/10	−

a) Same conditions were employed as in Table III.
b) Mitomycin C was dissolved in physiological saline. The solvent employed as vehicle for other compounds was CMC.
c) Arbitrary grading of anticancer effect.

vinyl]-4-quinolinecarboxylic acid hydrazide, No. 263, exhibited greater anticancer activity than did its parent acid (I). Although 2-[2-(5-nitrofuryl)vinyl]-8-quinolinol, No. 266, was not effective, its O-acetate, No. 267, was tested to be as effective as No. 268, the effectiveness of which was reported by Miura, *et al.*[2] in 1964.

As may be seen from Table IV, some of compounds having both ethylenic (-CH=CH-) bridge linkage and azomethine (-CH=N-) linkage showed moderate activity against the cancer.

It was thus interested to make further investigation on this line of derivatives.

Experimental

Preparation of Chemicals :

No. 256, 2-[2-(5-Nitrofuryl)vinyl]-4-quinolinecarboxlic Acid——After gently refluxing a mixture of 0.2 mole of isatine in 200 ml. of acetone and 225 ml. of 15% NaOH for 8 hr., excess solvent was evaporated. Neutralization of the residues gave 2-methyl-4-quinolinecarboxylic acid in semiquantitative yield. m.p. 243~245° (from water). 2.0 g. of 2-methyl-4-quinolinecarboxylic acid and 2.0 g. of 5-nitrofuraldehyde were heated at 160~170° for 30 min. in the presence of 5.0 ml. of acetic anhydride. The deposited material, after cooling, was recrystalized from nitrobenzene. Yield, 2.5 g.

2-Methyl-8-quinolinol,[4] 2-methyl-7-amino-5-quinolinecarboxylic acid,[5] 2-methyl-4-quinolinecarboxylic acid ethylester, 2-methyl-4-quinolinecarboxylic acid hydrazide and 2-methyl-8-quinolinecarboxylic acid[6] all were prepared according to the methods described in literature with some modifications. 2-Methylquinoline was purchased from commercial source. Nos. 260, 261, 262, 266 and 268 were also prepared by the same way as described in the case of No. 256.

No. 264, 2-[2-(5-Nitrofuryl)vinyl]-4-quinolinecarboxylic Acid(5-Nitrofurylidene)hydrazide, and No. 265, 2-[2-(5-Nitrofuryl)vinyl]-4-quinolinecarboxylic Acid (Benzylidene)hydrazide——2.0 g. of 2-methyl-4-quinolinecarboxylic acid hydrazide and 2.0 g. of 5-nitrofuraldehyde were dissolved in 30 ml. of alcohol and refluxed for 5 hr., the reaction mixture was condensed under reduced pressure, the deposited material then

4) O. Doebner, W. v. Miller : Chem. Ber., **17**, 1698 (1884).
5) L. Velluz, G. Amiard, M. Pesez : Bull. soc. chim. France, **1948**, 678.
6) O. Doebner, W. v. Miller : Chem. Ber., **17**, 939 (1884).

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washed with alcohol. 3.5 g. of No. 274, 2-methyl-4-quinolinecarboxylic acid (5-nitrofurylidene) hydrazide, was yielded. By the use of benzaldehyde instead of 5-nitrofuraldehyde, the corresponding benzylidene hydrazide was obtained. Yield, 92%, m.p. 204~206° (from ethanol). 2.3 g. of No. 274 was reacted with 1.0 g. of 5-nitrofuraldehyde in the presence of 3.0 ml. of acetic anhydride at 160~170° for 30 min. and the mixture, after cooling, was digested with benzene and filtered. Yield, 2.6 g. No. 265 was prepared by the same way. A suspension of 1.5 g. of No. 265 compound in 100 ml. of 10% hydrochloric acid was heated until all benzaldehyde had been distilled. After cooling, the clear solution was condensed and neutralized with 5% ammonia solution. The precipitate was filtered, and washed with ammonia and with water. Yield, 1.0 g.

No. 267, 2-[2-(5-Nitrofuryl)vinyl]-8-quinolinol Acetate, and No. 266, 2-[2-(5-Nitrofuryl)vinyl]-8-quinolinol——A mixture of 0.8 g. of 2-methyl-8-quinolinol and 5-nitrofuraldehyde in 1.5 ml. of acetic anhydride was heated for 30 min., after evaporating the solvent, residues were digested with benzene and filtered, washed with weak alkaline solution. Yield, 1.6 g. Yellowish needles. 0.6 g. of the compound obtained was suspended in concentrated hydrochloric acid and heated for 3 hr. Yield, 0.5 g. Yellowish needles.

Schiff Bases from 5-Nitrofuraldehyde——Nos. 269, 271, 272 and 273 were prepared by reacting 5-nitrofuraldehyde with the respective aromatic amine. The alcoholic solution of 0.01 mole of 5-nitrofuraldehyde was added to the solution of equivalent amount of the amine, and the mixture was refluxed for 1 hr. or allowed to stand at room temperature overnight. The precipitate was collected and recrystalized from ethanol, excepting that No. 272 and No. 273 were not recrystalized because of their instability.

Anticancer Experiment :

Experimental Animal——Male albino mice of ddN-JCL strain, weighing 20~22 g., were purchased from the Central Laboratory of Experimental Animal in Japan.

Toxicity——LD_{50} of the compounds was calculated after Behrens-Kaerber's rule from the data obtained in experiments, in which each of 5 mice in a group was received an intraperitoneal dose of a chemical to be tested.

Implantation of Carcinoma Cells——Ehrlich ascites carcinoma cells were harvested from mice bearing 7-day old ascites tumors. The milky fluid was centrifuged at 1,500 r.p.m. for 5 min. The sedimented cells were washed two times with saline and resuspended in saline to contain 4×10^7 cells per ml. To each animal, 0.1 ml. of the tumor suspension was intraperitoneally implanted.

Treatment with Compound——Treatment was started 24 hr. after implantation of cancer cells. A daily dose of a solution of the compound to be tested was given intraperitoneally into each mouse for the period stated in Table Ⅲ and Table Ⅳ. Insoluble compounds in water were, however, suspended in carboxymethylcellulose solution. For comparative purpose, an experimental series with Mitomycin C (Mitomycin "Sankyo," Sankyo Co., Ltd.) was run parallel. The average survival time of untreated mice was about 18~21 days. Animal died during experimental period were examined for the cause of death, and animals still alive on the 40th day of implantation were sacrificed and autopsied.

The author is grateful to Prof. H. Okamoto and Prof. S. Koshimura of this laboratory for their encouragement throughout this work. He is also indebted to Mr. H. Itadani of the Faculty of Pharmacy, Kanazawa University, for elemental analyses and to the Chugai Pharmaceutical Co., Tokyo, for the supply of 5-nitrofuraldehyde.

Summary

8 Nitrofurans related to 2-[2-(5-nitrofuryl)vinyl]-4-quinolinecarboxylic acid and 6 Schiff bases of 5-nitrofuraldehyde were prepared and tested for their effect upon Ehrlich ascites carcinoma in mice. As the results, 2-[2-(5-nitrofuryl)vinyl]-8-quinolinol acetate and 2-[2-(5-nitrofuryl)vinyl]-4-quinolinecarboxylic acid hydrazide were shown to be very effective in inhibiting the cancer cells growth.

(Received August 4, 1965)

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