formation of meprobamate. In recent years, a number of N-glucuronides was found in various biological origins administered sulfonamide,[11] or other amino compounds,[12,13] but no examples had been reported about N-glucuronide formation of such a carbamate groups as meprobamate.

From the results of paper chromatography, metabolite (V) should be considered as ether-type glucuronide of hydroxy-meprobamate. No evidence was obtained about the structure of last glucuronide (metabolite VI), at present time.

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Summary

The urinary metabolic end-products of meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) in rabbits and dogs were studied. They were isolated and characterized as keto-, hydroxy-, carboxy-meprobamate and three glucuronides containing carbamate group, but in dogs keto- and carboxy-derivatives were not isolated. Of glucuronides, one was N-glucuronide conjugated with meprobamaet itself and another was ether-type glucuronide of hydroxy-meprobamate.

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11) S. Ogiya: Yakugaku Zasshi, **80**, 1538 (1960).
12) R. M. Bushby, A. J. Woiwod: Biochem. J., **63**, 406 (1956).
13) E. Boyland, D. Manson, S. F. D. Orr: *Ibid.*, **65**, 417 (1957).

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84. Ryozo Koshiura (Hirata), Yukiko Kagotani, and Toshimitsu Ujiie: Experimental Anticancer Studies. XVI.[*1] Preparation and Anticancer Activity of 4-Amino-6-hexylresorcinol on Ehrlich Carcinoma in Mice.

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As reported previously,[1] 4-hexyl-6-(2-hydroxy-3,5-dibromophenylazo)resorcinol (AZO-36)[2~4] and 2,6-bis-(2-hydroxy-3,5-dibromophenylazo)-4-propylphloroglucinol (AZO-106)[5~7] are the effective antitumor compounds among members of 2,2′-dihydroxyazobenzene derivatives so far tested in this laboratory.

Meanwhile, it became interest to test for the anticancer activity of compounds of Schiff's base type, which have chemical constitution of either R-CH=N-R′ or R—N=CH—R′ against that of R—N=N—R′ of AZO-36. The present communication describes the results of such experiments.

*1 Part XV: Ann. Rep. Tbc. Kanazawa, **19**, 225 (1961).
*2 Tsuchitoribanaga-machi, Kanazawa (越浦良三, 籠谷由紀子, 氏家俊光).
1) H. Okamoto, *et al.*: Zeitschrift für Krebsforschung, **62**, 408 (1958).
2) R. Hirata: This Bulletin, **4**, 60 (1956).
3) S. Koshimura, *et al.*: GANN, **47**, 393 (1956).
4) T. Masusaki: Juzen Igaku Zasshi, **60**, 1512 (1958).
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7) T. Miyaji: Juzen Igaku Zasshi, **63**, 278 (1959).

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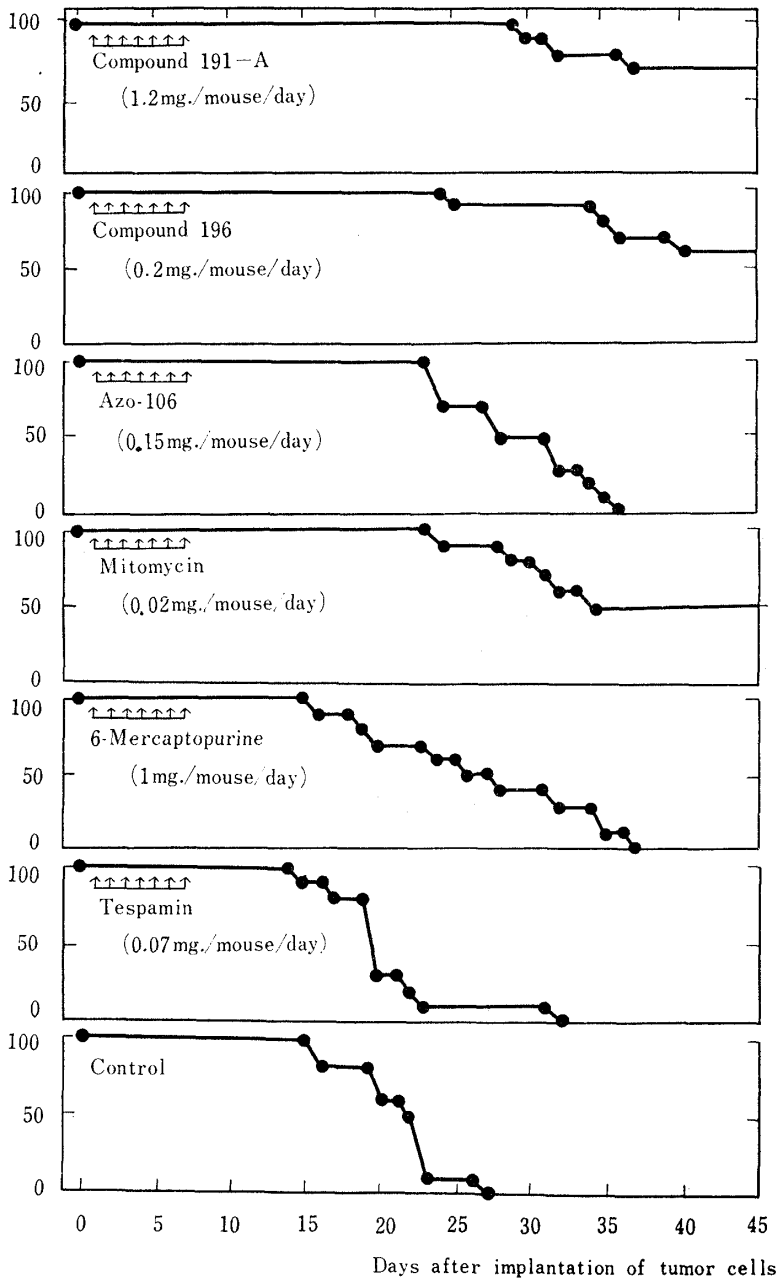

Fig. 1. Comparative Anticancer Experiment with Six Compounds on Ehrlich Ascites Carcinoma in Mice

Experimental

Preparation of Schiff's Base Compounds

4-Hexyl-6-(2-hydroxy-3,5-dibromophenyliminomethyl)resorcinol (No. 191-A)——To a suspension of 2-amino-4,6-dibromophenol hydrochloride in 99% EtOH (20 cc.), AcONa (0.3 g.) was first added, and then 2,4-dihydroxy-5-hexylbenzaldehyde (0.6 g.). After refluxing the mixture for about 20 min., the reddish solution resulted was acidified with 10% H_2SO_4 to yield yellowish-orange crystalline substance. Recrystallization from hydr. EtOH, m.p. 135°. Yield, 0.5 g. *Anal.* Calcd. for $C_{19}H_{21}O_3Br_2N$: N, 2.97. Found : N, 2.94.

This compound was dissolved in H_2O with the aid of *N* NaOH, and used for anticancer experiment.

4-Hexyl-6-(2-hydroxy-3,5-dibromobenzylideneamino)resorcinol (No. 191-B)

a) 4-Hexyl-6-nitrosoresorcinol——To a solution of 4-hexylresorcinol (5 g.) in dehyd. EtOH (20 cc.), a solution of KOH (1.8 g.) in dehyd. EtOH (10 cc.) was first added, and then amylnitrite (3 g.) dropwise with stirring and cooling. After continuous stirring for 10 min., the reaction mixture was

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allowed to stand for 5 hr. in an ice box. The reddish precipitate was collected, washed with dehyd. EtOH. The crystal thus obtained was dissolved in H_2O, and acidified with dil. HCl to produce yellowish crystal, which was further recrystallized from 50% EtOH, m.p. 150°. Yield, 4.6 g. *Anal.* Calcd. for $C_{12}H_{17}O_3N$: N, 6.27. Found: N, 5.88.

b) 4-Amino-6-hexylresorcinol hydrochloride (No. 196)——To a solution of $SnCl_2 \cdot 2H_2O$ (14 g.) in conc. HCl (100 cc.), 4-hexyl-6-nitrosoresorcinol (4 g.) was added in small portions, with vigorous stirring, and then the mixture was heated at 60~70° for 5 hr. The reaction mixture was concentrated *in vacuo* to remove excess HCl, the residue was diluted 10 fold with H_2O, and H_2S gas was introduced to the solution. The brownish-black precipitate was filtered off, and the clear filtrate obtained was concentrated in a reduced pressure in H_2. After cooling, fine colorless needles formed was collected. Yield. 1.8 g. *Anal.* Calcd. for $C_{12}H_{20}O_2NCl$: N, 5.70. Found: N, 5.38.

c) Under the introduction of H_2, first AcONa (0.2 g.), and next 2-hydroxy-3,5-dibromobenzaldehyde (0.7 g.) were added to a solution of 4-amino-6-hexylresorcinol hydrochloride (0.6 g.) in 99% EtOH (20 cc.). The mixture, after refluxing for 1 hr. under the same condition, was cooled, and filtered to obtain the crude product, which was recrystallized from benzene. Reddish crystalline (No. 191-B), m.p. 190°. Yield, 0.3 g. *Anal.* Calcd. for $C_{19}H_{21}O_3Br_2N$: N, 2.97. Found: N, 2.93.

The No. 191-B compound is rapidly decomposed in alkaline solution, giving brownish precipitates. For this reason, the compound was omitted in the biological test.

Biological Test——a) Experimental animal: Inbred mice (18~20 g. body weight) of "dd"-strain were used throughout.

b) Preparation of carcinoma cell suspension: A mouse was transplanted intraperitoneally with Ehrlich ascites carcinoma cells. After 9 days, ascitic fluid was withdrawn from the tumor bearing animal, and diluted with physiologic saline to the concentration of 30×10^6 tumor cells per cc. Therefore, 0.1 cc. of the suspension contained 3×10^6 of the tumor cells.

c) Compounds tested: 4-Hexyl-6-(2-hydroxy-3,5-dibromophenyliminomethyl)resorcinol (No. 191-A), 2-amino-4,6-dibromophenenol hydrochloride (No. 195),[8] 4-amino-6-hexylresorcinol hydrochloride (No. 196), 2-hydroxy-3,5-dibromobenzaldehyde (No. 197),[9] 2,4-dihydroxy-5-hexylresorcinol (No. 198),[10] mitomycin (MC, Kyowa Fermentation Ind. Co, Ltd.), 6-mercaptopurine (6-MP, Takeda Chem. Ind, Ltd.), tespamin (thio-TEPA, Sumitomo Chem. Co.) and AZO-106.

d) Anticancer experiment: i) Experiment on Ehrlich ascites carcinoma in mice: Animals were implanted intraperitoneally with 0.1 cc. of the ascites carcinoma cell suspension. Treatment was started 24 hr. after transplantation. A daily dose of the compound to be tested was successively injected intraperitoneally into each animal for 7 days. The effect of the compound was judged by the difference in the survival time between treated and untreated (control) animal groups.

ii) Experiment on solid tumor of Ehrlich carcinoma in mice: Animals were implanted subcutaneously in the left groin with 0.1 cc. of the ascites carcinoma cell suspension. The treatment with compound to be tested was made as mentioned above. The effect of the compound was estimated by the difference in average weight of tumors between treated and untreated animal groups on the 14th day of transplantation.

Results

Firstly, six compounds, Nos. 191-A, 195~198 and AZO-36, were tested for their effect on the Ehrlich ascites carcinoma in mice. The results are summarized in Table I.

As may be seen from the Table I, a marked prolongation of the life-span of mice bearing Ehrlich ascites carcinoma was found by the treatment with No. 191-A compound at the level of a daily dose of 60 mg./kg. (which corresponds to 1/5 the LD_{50}). In 30 mg./kg./day, the compound was slightly effective.

The mother substances of No. 191-A compound, Nos. 195, 198, and 197 compound were all entirely ineffective.

The No. 196 compound was found to be effective in a daily dose of 10~15 mg./kg. (which corrsponds to 1/3~1/5 the LD_{50}).

Fig. 1 shows the results of comparative anticancer experiments with Nos, 191-A, 196 compounds, AZO-106, and three commercially available anticancer agents, MC, 6-MP,

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9) C. M. Brewster: J. Am. Chem. Soc., **46**, 2464 (1924).
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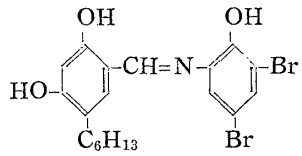
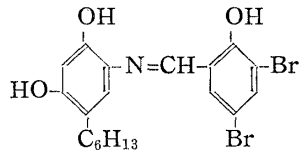
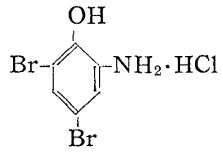
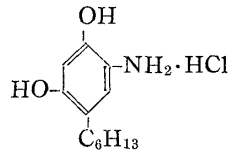
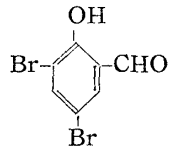
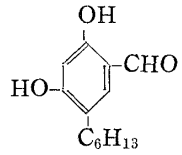
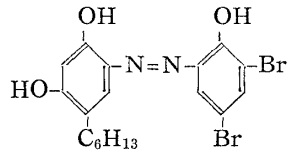
Table I. Anticancer Activities on Ehrlich Ascites Carcinoma in Mice

No.	Compound	Dose mg./kg./day	Dose days	Average survival days after implantation[b]	Anticancer activity	LD_{50} in mice (i.p.; mg./kg.)
191-A		60	7	>30/17.3	+	300
		30	7	25/17.3	±	
191-B		a)	a)	a)	a)	a)
195		40	7	15.2/17.3	−	200
196		15	7	>30/17.3	+	
		10	7	>30/17.3	+	50
		5	7	20.4/17.3	−	
197		50	7	15.9/17.3	−	250
198		10	7	16.4/17.3	−	50
AZO-36		8	7	25/17.3	±	40

a) not tested
b) treated/control

and thio-TEPA, performed on Ehrlich ascites carcinoma in the similar experimental conditions.

In these experiments, Nos. 191-A and 196 compounds were found to be as effective as MC in causing the prolongation of life-span of mice bearing Ehrlich ascites carcinoma.

On the solid form of Ehrlich carcinoma in mice, Nos. 191-A and 196 compounds were, however, less effective than MC. Table II shows the results of comparative experiment on the solid form of Ehrlich carcinoma in mice. It is seen that when compared the anticancer activity of the compounds on their 1/5 LD_{50} dose basis, 6-MP was most potent in inhibiting the solid-tumor of Ehrlich carcinoma in mice, followed by MC, thio-TEPA, No. 196 compound, AZO-106, and No. 191-A compound in that order.

The present data seems to be of interest in the light of the experiments recently made by Takano, Mizuno and Hase, *et al.*,[11] who reported that some of alkyl and haloalkyl derivatives of resorcinol were inhibitory against Ehrlich carcinoma in mice.

11) K. Takano, D. Mizuno, J. Hase, *et al.*: Japan. J. Med. Sci. & Biol., **14**, 45 (1960).

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Table II. Comparative Anticancer Activity on Solid Tumor of Ehrlich Carcinoma in Mice

Experimental groups Dose	Treated groups: Compound 191-A (1.2 mg./mouse/day) ×7	Compound 196 (0.2 mg./mouse/day) ×7	AZO-106 (0.15 mg./mouse/day) ×7	Mitomycin (0.02 mg./mouse/day) ×7	6-Mercapto-purine (1 mg./mouse/day) ×7	Tespamin (0.07 mg./mouse/day) ×7	Control group
Weight of tumor (g.)	0.87	0.59	0.92	0.40	0.27	0.58	1.19
	0.65	0.46	0.80	0.39	0.18	0.42	0.89
	0.57	0.38	0.66	0.34	0.17	0.40	0.88
	0.56	0.36	0.51	0.29	0.17	0.38	0.77
	0.55	0.34	0.45	0.29	0.14	0.37	0.71
	0.48	0.29	0.44	0.25	0.12	0.30	0.68
	0.27	0.27	0.21	0.17	0.12	0.18	0.52
	0.23	0.24	0.16	0.14	0.11	0.17	0.44
	0.20	0.20	0.15	0.14	0.08	0.17	0.40
	(Death)	(Death)	(Death)	0.07	0.05	0.16	0.39
Average weight of tumors (g.)	0.486	0.347	0.477	0.248	0.141	0.313	0.688
Average weight of tumors (%)	70.5	50.4	69.3	36.0	20.5	45.5	100

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Summary

4-Hexyl-6-(2-hydroxy-3,5-dibromophenyliminomethyl)resorcinol (No. 191-A) and 4-amino-6-hexylresorcinol (No. 196) showed antitumor activity in experimental animals.

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