spectropolarimeter. Dr. Walter Gensler and Miss Rawji, of the Department of Chemistry, Boston University, helped us in obtaining the NMR spectra. This investigation was supported by Research Grants CA 26281 from the National Cancer Institute, DHEW, and CH-34 from the American Cancer Society. Thanks to Drs. Sati C. Chattoraj and Thomas F. Brennan, Department of Obstetrics and Gynecology, Boston University Medical Center, for assistance in manuscript writing and for their continued interest in the work.

Studies on the Synthesis of Chemotherapeutics. 12.[1] Synthesis and Antitumor Activity of *N*-Phthalidyl-5-fluorouracil Derivatives[2]

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Several *N*-phthalidyl-5-fluorouracil derivatives were synthesized for preliminary antitumor evaluation. N_1-Phthalidyl- and N_3-phthalidyl-5-fluorouracils (**6** and **10**) showed a significant antitumor activity against experimental solid tumors with a good blood level of 5-fluorouracil in mice, even for oral administration.

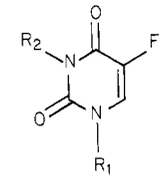
5-Fluorouracil (5-FU, **1**)[3] and its masked form, N_1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur, **2**)[4] have been widely used in cancer chemotherapy, but they have been shown to possess a high toxicity and poor tumor affinity. Chemical modification of **1** and **2** has been extensively studied to develop more effective antitumor substances with fewer toxic side effects.[5]

In connection with our synthetic work on chemotherapeutics, we previously reported a novel synthesis of **2**[6] and a synthesis of numerous *N*-acyl- and *N*-(alkoxycarbonyl)-5-fluorouracil derivatives (**3**).[7] Of these, N_1-acetyl-N_3-*o*-toluyl-5-fluorouracil (**4**) appeared to be the most promising antitumor agent. Incidentally, in recent advances of prodrug formation of therapeutic agents, talampicillin, a phthalidyl ester of ampicillin, was found to show an improved bioavailability with an approximately twice as high serum concentration of ampicillin as the lead compound.[8]

These findings stimulated us to intend the further development of new 5-fluorouracil derivatives by the introduction of a phthalidyl group into the N_1 and/or N_3 position of **1**, **2**, and *N*-acyl-5-fluorouracils (**9** and **13**). In this paper, we report the synthesis and preliminary evaluation of the antitumor activity of novel *N*-phthalidyl-5-fluorouracil derivatives (**5**) (Chart I). Among these compounds, N_1- and N_3-phthalidyl-5-fluorouracil (**6** and **10**) appeared to retain the same level of antitumor activity toward solid experimental tumors as that of **2** following oral administration.

Chemistry. For the preparation of N_1-phthalidyl-5-fluorouracil (**6**), N_3-benzoyl-5-fluorouracil (**7**)[7] was reacted with 3-bromophthalide[9] in the presence of sodium hydride in DMF to give N_3-benzoyl-N_1-phthalidyl-5-fluorouracil (**8**). Solvolysis of **8** under a protic condition (AcOH–EtOH) afforded the desired compound **6**. In a similar way, the reaction of N_1-acetyl-5-fluorouracil (**9**)[5m] with 3-bromophthalide, followed by hydrolysis of the resulting N_1-acetyl-N_3-phthalidyl-5-fluorouracil (**11**) under an acidic condition (0.05 N HCl-EtOH), gave N_3-phthalidyl-5-fluorouracil (**10**). Compound **11** was alternatively prepared by acetylation of **10** with acetic anhydride.

Chart I



1, $R_1 = R_2 = H$
2, R_1 = i, $R_2 = H$
3, R_1, R_2 = H, acyl, i, or alkoxycarbonyl
4, $R_1 = COCH_3$, R_2 = *o*-toluyl
5, R_1, R_2 = H, acyl, i, or phthalidyl
phthalidyl = 1,3-dihydro-3-oxo-1-isobenzofuranyl (ii)

i

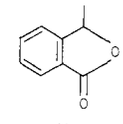


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Table I. *N*-Phthalidyl-5-fluorouracil Derivatives[a]

no.	method[b]	yield %	mp, °C (recrystn solvent)	formula[c]
6	C	10	292–296 dec	$C_{12}H_7FN_2O_4$
	D	54	(MeOH–AcOEt)	
8	A	59	207–210 (AcOEt)	$C_{19}H_{11}FN_2O_5$
10	E	50[d]	234–237 (MeOH)	$C_{12}H_7FN_2O_4$
11	A	72	187–190 (AcOEt)	$C_{14}H_9FN_2O_5$
12a	C	77[e]	255–258 (AcOEt)	$C_{20}H_{11}FN_2O_6$
12b			249–252 (AcOEt)	$C_{20}H_{11}FN_2O_6$
14	A	74	154–157 (AcOEt)	$C_{16}H_{13}FN_2O_5$
15	B	75	199–202 (AcOEt)	$C_{20}H_{13}FN_2O_5$

[a] All compounds were obtained as colorless crystals. [b] Capital letters refer to synthetic method A–E under Experimental Section. [c] Analyzed values for C, H, and N; analytical results were within ±0.4% of the theoretical values. [d] Yield from compound **9**. [e] Total yield of diastereomers (**12a** and **12b**).

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(1) Part 11: T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, S. Haga, H. Sugi, K. Tanigawa, Y. Suzuki, K. Fukawa, O. Irino, O. Saita, and S. Yamabe, *Heterocycles*, 16, 1205 (1981).

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Table II. Antitumor Activity against P-388 Leukemia [a]

compd	% T/C at dose of 200 mg/kg	100 mg/kg	50 mg/kg	25 mg/kg
tegafur (2)	106 [b]	136	104	115
6	124 [b]	129	116	118
10	102 [b]	120	104	111
11	111	104	97	106
12 [c]	102	102	104	97
14	111	115	109	104
15	109	102	99	113

[a] Test solution[14] was administered orally once daily for 9 days, starting 1 day after P-388 implantation. The antitumor activity of the compounds was expressed by the ratio of the median survival time of the treated mice (T) to that of the control mice (C). [b] Decrease of body weight was observed. [c] See ref 12.

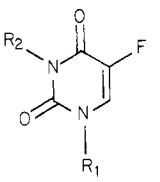
Table III. Antitumor Activity of Compounds 6 and 10 and Tegafur (2) against MH 134 and Meth A Tumors [a]

tumor	compd	% T/C at dose of 200 mg/kg	200 mg/kg	150 mg/kg	50 mg/kg	25 mg/kg
MH 134	tegafur (2)		32	50	64	100
	6		8 [b]	22	34	50
	10		17	37	45	77
Meth A	tegafur (2)	death	23	44	65	
	6	death	7 [b]	26	50	
	10	7 [b]	19	37	67	

[a] Test solution[14] was administered orally twice daily for 20 days, starting 1 day after MH 134 or Meth A implantation. On the 21st day, the mice were killed and the tumor weight was assessed. The antitumor activity of compounds was expressed by the ratio of the median weight of tumors of the treated mice (T) to that of the control mice (C). [b] Decrease of body weight was observed.

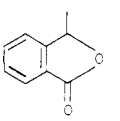
In the alkylation of 1 with 2 molar equiv of 3-bromophthalide, a diastereomeric mixture of N_1,N_3-diphthalidyl-5-fluorouracil (12, 77%) was obtained, together with 6 (10%). A similar reaction of tegafur (2) and N_3-*o*-toluyl-5-fluorouracil[7] (13), a major metabolite of 4,[7] with 3-bromophthalide afforded N_3-phthalidyl-N_1-(2-tetrahydrofuryl)-5-fluorouracil (14) and N_1-phthalidyl-N_3-*o*-toluyl-5-fluorouracil (15), respectively (Chart II).

Chart II



	R_1	R_2
6	phthalidyl	H
7	H	benzoyl
8	phthalidyl	benzoyl
9	$COCH_3$	H
10	H	phthalidyl
11	$COCH_3$	phthalidyl
12	phthalidyl	phthalidyl
13	H	*o*-toluyl
14		phthalidyl
15	phthalidyl	*o*-toluyl

phthalidyl = 1,3-dihydro-3-oxo-1-isobenzofuranyl (ii)



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Biological Results and Discussion

Antitumor Activity against P-388 Leukemia (Table II). In vivo preliminary screening of compounds 6, 10–12, 14, and 15 was carried out against the P-388 leukemia system in female BDF_1 mice. The available results, together with those for tegafur (2), are summarized in Table II. None of our compounds showed an activity surpassing the criterion of T/C = 130. However, 6 and 10 were slightly inhibitory at a dose of 100 mg/kg and selected for further examination.

Antitumor Activity of Compounds 6 and 10 against MH 134 and Meth A Tumors (Table III). Against MH 134, compounds 6 and 10 appeared to be more effective than tegafur (2), but 6 caused loss in body weight at dose of 150 mg/kg.

Against Meth A, the antitumor activity of 6 seemed to be higher than that of tegafur (2) at lower doses, while 10 showed almost equal activity to that of 2. At higher doses, death of mice and a decrease in body weight were observed.

Acute Toxicity of Compounds 6 and 10 and Tegafur in Mice. An LD_{50} value for tegafur was found to be ~1650 mg/kg. Four out of ten mice treated with compound 10 died following the highest dose, 2500 mg/kg, while mice treated with 6 were alive at the same dose. Tegafur produced a suppression of spontaneous motor activity and respiratory rate at doses over 795 mg/kg, a relaxation of

(2) This constitutes Part 978 of "Studies on the Syntheses of Heterocyclic Compounds and Natural Products" by T. Kametani.
(3) R. Dushinsky and E. Pleven, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
(4) (a) "Tegafur" has been adopted as the recommended INN: *WHO Chron.*, **33**, 8 (1979). (b) S. A. Hiller, R. A. Zhuk, and M. Yu. Lidak, *Dokl. Acad. Nauk SSSR*, **176** 332 (1967); *Chem. Abstr.*, **68**, 29664*j* (1968). (c) S. A. Hiller, R. A. Zhuk, M. Yu. Lidak, and A. A. Ziermane, British Patent, 1 168 391 (1969).
(5) (a) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1(1965). (b) M. Yasumoto, I. Yamawaki, T. Marunaka, and S. Hashimoto, *J. Med. Chem.*, **21**, 738 (1978). (c) W. M. Odijk, M. J. Wanner, G. J. Coomen, and U. K. Pandit, *Heterocycles*, **9**, 1403 (1978). (d) G. J. Coomen, F. Alewijk, D. Blok, and U. K. Pandit, *ibid.*, **12**, 1535 (1979). (e) S. Ozaki, Y. Ike, H. Mizuno, K. Ishikawa, and H. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2406 (1977). (f) M. Tada, *ibid.*, 48, 3427 (1975). (g) T. Seita, M. Minoshita, and M. Imoto, *ibid.*, **46**, 1572 (1973). (h) W. Klotzer and M. Herberg, *Monatsh. Chem.*, **99**, 847 (1968). (i) S. A. Hiller, R. A. Zhuk, and G. Ya. Nashatyr, *Khim. Geterotsikl. Soedin.*, **3**, 577 (1968); *Chem. Abstr.*, **69**, 96641*h* (1968). (j) M. Yu. Lidak, R. Paegle, M. Plata, K. Ya. Pets, and Yu. P. Shvachkin, *ibid.*, **2**, 379 (1968); *Chem. Abstr.*, **69**, 96650*g* (1968). (k) R. Paegle, M. Yu. Lidak, and Yu., P. Shvachkin, *Khim. Geterotsikl. Soedin., Sb.*, **2**, 316 (1966). (l) T. T. Sakai, A. L. Pogolotti, and D. V. Santi, *J. Heterocycl. Chem.*, **5**, 849 (1968). (m) M. Tada, *Chem. Lett.*, 129 (1975). (n) W. Nagata and S. Kamata, *Japan Kokai*, **57**, 16 881, 32 270 and 32 271 (1982).
(6) T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, O. Kusama, H. Sugi, and K. Kawasaki, *Heterocycles*, **6**, 529 (1977); *J. Heterocycl. Chem.*, **14**, 473 (1977).
(7) T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, S. Haga, Y. Nagamatsu, H. Sugi, K. Fukawa, O. Irino, Y. Yamamoto, N. Nishimura, A. Taguchi, T. Okada, and M. Nakayama, *J. Med. Chem.*, **23**, 1324 (1980).
(8) J. P. Cayton, *Antimicrob. Agents Chemother.*, **5**, 670 (1974).
(9) I. A. Koten and R. J. Sauer, "Organic Syntheses", Collect. Vol. V, Wiley, New York, 1973, p 145.

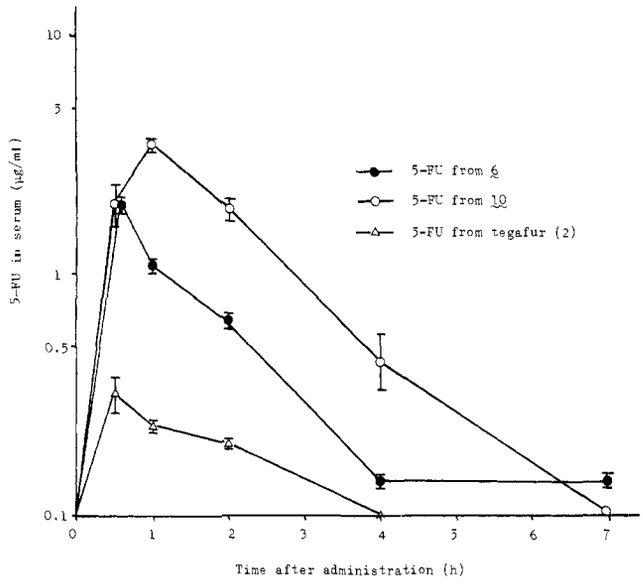

Figure 1. Serum concentration of 5-FU in mice after oral administration of compounds **6** and **10** and tegafur (**2**).

muscle tone, hypothermia, and abnormal gait at doses over 1000 mg/kg, and anemia, clonic convulsion, touch response, staggering gait, and a loss of grip strength and righting reflex at a dose of 2000 mg/kg.

Compound **10** caused a suppression of spontaneous motor activity and respiratory rate at doses over 1000 mg/kg, an abnormal gait at doses over 1260 mg/kg, and hypothermia and a relaxation of muscle tone at a dose of 2500 mg/kg. With compound **6**, no abnormal behavior was observed following the highest dose, 2500 mg/kg.

Thus, the acute toxicity of these compounds in mice would be in the sequence of tegafur (**2**) > **10** > **6**. This may be a reflection of the available serum concentrations of the three compounds (Figure 2).

Serum Concentration of 5-FU after Administration of Compounds 6 and 10 and Tegafur to Mice. The activity of compounds **6** and **10** and tegafur was assumed to be due to their biotransformation to 5-FU in serum and tissue. The serum concentration of 5-FU and the unchanged compounds (**2**, **6**, and **10**) was measured after the oral administration of **2**, **6**, and **10** (Figures 1 and 2). The serum concentration of 5-FU (**1**) in mice treated with tegafur (**2**) was comparatively low (0.3 μg/mL at maximum), while the concentration of the unchanged **2** was high (245.0 μg/mL at maximum). For mice treated with compounds **6** and **10**, the serum concentration of 5-FU reached a maximum of 2.0 μg/mL at 0.5 h and 3.6 μg/mL at 1 h, respectively, and decreased rapidly to about 0.1 mg/mL at 7 h. Although the tegafur concentration at any time was higher than the concentrations of compounds **6** and **10** (Figure 2), the serum level of 5-FU for compounds **6** and **10** was at all times higher than that for tegafur (**2**) (Figure 1). The above results revealed that compounds **6** and **10** were biotransformed to 5-FU much more effectively than was tegafur (**2**) in mice.

The serum concentration of **6** was lower than that of **10** (Figure 2), and the acute toxicity of **6** was lower than that of **10**. However, the toxicity of **6** in tumor-bearing mice seemed to be higher than that of **10**, and **6** showed a higher antitumor activity in comparison with **10** (Table III). The above results of the activity and toxicity suggested that compound **6** might have a higher distribution to tissues and tumors than that of **10** in mice. On this question, investigations including chronic toxicity are in progress.

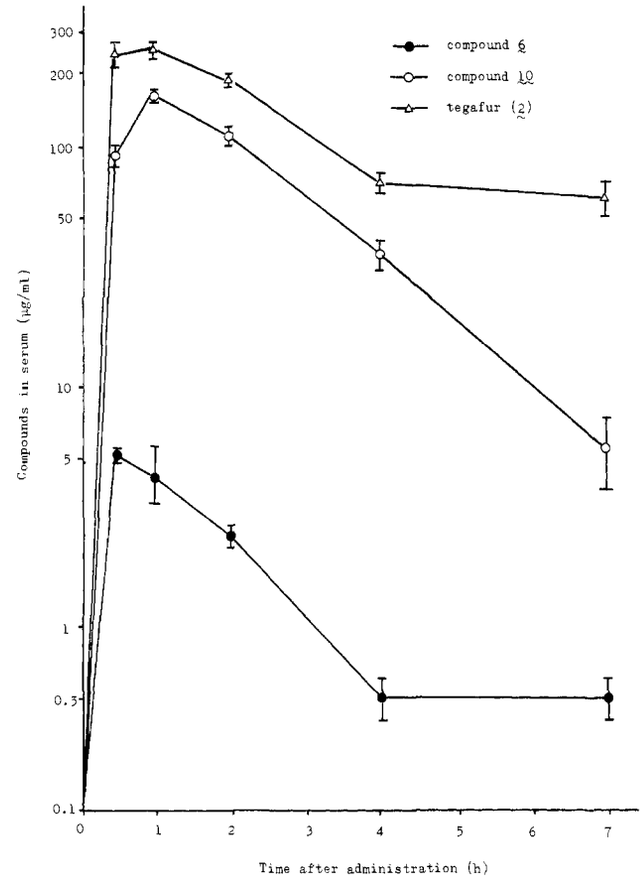

Figure 2. Serum concentration of compounds **6** and **10** and tegafur (**2**) in mice after oral administration.

Experimental Section

All melting points were measured on a Yanagimoto micro melting point apparatus and uncorrected. IR spectra were measured with a Hitachi 215 spectrophotometer; NMR spectra were measured on a JEOL PMX-60 spectrometer, with tetramethylsilane as an internal standard.

Reaction of N-Substituted 5-Fluorouracils with 3-Bromophthalide. (a) Reaction in the Presence of Sodium Hydride (Method A). Sodium hydride[10] (0.80 g, 20 mmol) was added to a stirred solution of N_3-benzoyl-5-fluorouracil (**7**;[7] 4.68 g, 20 mmol) in DMF (10 mL) under ice–water cooling. After the mixture was stirred for 15 min, a solution of 3-bromophthalide (4.69 g, 22 mmol) in DMF (5 mL) was added, and the mixture was stirred at 0 °C for 15 min and at room temperature (rt) for 1 h. The mixture was poured into water (100 mL) and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residual solid was washed with *n*-hexane (50 mL × 2) and recrystallized from AcOEt to give N_3-benzoyl-N_1-phthalidyl-5-fluorouracil (**8**). Using **9** and **2**, this method provided **11**[11] and **14**, respectively.

8: IR (KBr) 1780, 1750, 1710, 1680, 1660 cm^{-1}; NMR ($CDCl_3$) δ 6.85 (1 H, d, J = 6 Hz, C_6 H).

11: IR (KBr) 1775, 1750, 1725, 1690 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 2.65 (3 H, s, $COCH_3$), 8.41 (1 H, d, J = 7 Hz, C_6 H).

14: IR (KBr) 1765, 1715, 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.52–2.58 (4 H, m, 3′- and 4′-CH_2), 3.72–4.52 (2 H, m, 5′-CH_2), 5.72–6.00 (1 H, m, 2′-H).

(b) Reaction in the Presence of Potassium Carbonate (Method B). A mixture of N_3-*o*-toluyl-5-fluorouracil (**13**;[7] 1.22 g), 3-bromophthalide (1.17 g), K_2CO_3 (1.00 g), and DMF (10 mL) was stirred at rt for 1 h, and the mixture was evaporated. The resultant residue was added to water (50 mL) and then extracted

(10) Approximately 60% in mineral oil.

(11) This product in method A was not obtained as a pure compound and was used in the next reaction without further purification.

with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residual oil was solidified on trituration with ether to give N_1-phthalidyl-N_3-*o*-toluyl-5-fluorouracil (**15**): IR (KBr) 1780, 1740, 1710, 1680, 1660 cm^{-1}; NMR ($CDCl_3$) δ 2.72 (3 H, s, CH_3), 6.70 (1 H, d, J = 6 Hz, C_6H), 7.17-8.33 (9 H, m, Ar H and Ar CH).

Reaction of 5-Fluorouracil with 3-Bromophthalide (Method C). 5-FU (1.30 g, 10 mmol) was reacted with 3-bromophthalide (4.69 g, 22 mmol) in the presence of sodium hydride (0.80 g, 20 mmol) as described for the preparation of **8**. The product was crystallized from $CHCl_3$ to yield N_1-phthalidyl-5-fluorouracil (**6**) as a colorless solid (0.26 g, 10%), which showed identical spectra (IR and NMR) with those of compound **6** prepared by method D. The above $CHCl_3$ filtrate was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$ as an eluent, yielding one of the diastereomers of **12** (**12a**; 0.23 g, 6%): IR (KBr) 1785, 1730, 1690, 1670 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.00 (1 H, d, J = 6 Hz, C_6 H).

Subsequent elution with $CHCl_3$ gave **12**[12] (2.53 g, 64%) and the following elution afforded the other diastereomer of **12** (**12b**; 0.28 g, 7%): IR (KBr) 1770, 1730, 1680 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 6.95 (1 H, d, J = 6 Hz, C_6 H).

Deacylation of *N*-Acyl-*N*-phthalidyl-5-fluorouracils. (a) Solvolysis in AcOH-EtOH (Method D). A mixture of **8** (3.00 g), EtOH (450 mL), and AcOH (25 mL) was refluxed for 35 h. After the mixture was cooled, the resulting precipitate was collected and washed with ether to give **6**: IR (KBr) 1790, 1700, 1660 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.43-8.17 (6 H, m, C_6 H, Ar H and Ar CH).

(b) Hydrolysis in Dilute HCl-EtOH (Method E). N_1-Acetyl-N_3-phthalidyl-5-fluorouracil (**11**;[11] 4.38 g) was heated with a mixture of 0.05 N HCl (50 mL) and EtOH (50 mL) under reflux, and the solvent was removed. To the residue was added water, and the precipitated crystals were collected and washed with water. The dried crystals were treated with hot $CHCl_3$ (15 mL) for 3 min. The insoluble crystals in $CHCl_3$ were collected to give **10**: IR (KBr) 1780, 1725, 1675 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.38-8.05 (6 H, m, C_6 H, Ar H and Ar CH).

Antitumor Activity against P-388 Leukemia (Table II). Female BDF_1 mice (Charles River, Japan) weighing 17-23 g were used. Five mice for each test group were implanted intraperitoneally with 10^6 cells of P-388.[13] Test solution[14] was administered orally once daily for 9 days, starting 1 day after implantation. The antitumor activity of the compounds was expressed by the ratio of the median survival time of the treated mice (T) to that of the control mice (C).

Antitumor Activity of Compounds 6 and 10 and Tegafur (2) against MH 134 and Meth A Tumors. Five female mice (C3H for MH 134 and BALB/c for Meth A) for each group, weighing 17-23 g, were implanted with one of the tumors: 10^6 cells of MH 134 and 5×10^6 cells of Meth A were implanted subcutaneously in the side of the abdominal region of the mouse. Test solution[14] was administered orally twice daily for 20 days, starting 1 day after implantation. On 21st day, the mice were killed, and the tumor weight was assessed. The antitumor activity of compounds was expressed by the ratio of the median weight of tumors of the treated mice (T) to that of the control mice (C).

Acute Toxicity of Compounds 6 and 10 and Tegafur (2). Ten female ICR mice for each group, weighing 22-30 g, were used. Test solution[14] was administered orally. The LD_{50} value was calculated by the Litchfield and Wilcoxon method[15] from the mortality of mice for 21 days after the administration. In addition, the general behavior of the mice was observed after the administration.

Serum Concentration of 5-FU for Mice Treated with Compounds 6 and 10 and Tegafur (2). Female BDF_1 mice weighing 20-26 g (Charles River, Japan) were subjected to this study. Test solution[14] was administered orally at a dose of 1 mmol/kg (dosage of **6** and **10**, 260 mg/kg). After 0.5, 1, 2, 4, and 7 h, the mice were killed by bleeding under ether anesthesia. The collected blood was centrifuged, and the sera were decanted into tubes. The sera were diluted with saline, acidified, and extracted with $CHCl_3$. In a similar way, 5-FU (aqueous layer) was separated from **6**, **10**, and tegafur (organic layer). 5-FU and tegafur concentrations in the sera were determined biologically by the antibacterial activity against *Staphylococcus aureus* P-209,[16] while the concentrations of compounds **6** and **10** were determined by high-pressure liquid chromatography (Waters Associates, Inc.).

Acknowledgment. We thank President A. Yanagisawa and Director H. O. Takagi, Grelan Pharmaceutical Co., Ltd., for their encouragement. We also thank the Pharmaceutical Institute, Tohoku University, for microanalyses.

(12) A mixture of diastereomers of **12a** and **12b** was formed in a ratio of approximately 1:1, mp 214-217 °C (AcOEt).
(13) R. I. Geran, H. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 8 (1972).
(14) Test solution was prepared by dissolving or suspending the test compound in 0.3% sodium carboxymethylcellulose (CMC).
(15) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(16) Y. Yasuda, T. Togo, N. Unemi, S. Watanabe, K. Harima, and T. Suzue, *J. Jpn. Soc. Cancer Ther.*, **21**, 1711 (1973).

Cardenolide Analogues. 14. Synthesis and Biological Activity of Glucosides of C17β-Modified Derivatives of Digitoxigenin

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An improved method for the synthesis of cardiac glycosides was used to prepare 3β-glucosides of digitoxigenin derivatives in which the 17β side chain was CH=CHX (X = COOH, $CONH_2$, $COCH_3$, CN, or COOR). We compared the inotropic activity of the compounds with that of digitoxigenin glucoside using guinea pig left atria. All compounds were active except for the acid (**7**) and the amide (**8**). The inactivity of the amide, in spite of its favorable shape and high capacity for forming intermolecular hydrogen bonds, is incompatible with some previous structure-activity relationship theories. Of the active genins, glucosidation enhanced activity by a factor of about 2. All glucosides, including those with high potency, showed rapid onset and offset of action. The stepwise fall in potency that occurred when the ester group (CH=CHCOOR) was increased in bulk supported previous suggestions that the portion of the digitalis receptor that interacts with the C17 side chain lies within a cleft.

In 1971 we described[1] a simple route whereby the lactone of digitoxigenin could be replaced with a variety of open-chain moieties to produce compounds of the type shown in Scheme I (**4a-h**). In subsequent publications we de-

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