UDC 615.771.7[547.854.5]-092 : 616.381-003.217-006.3

171. Tyunosin Ukita, Yoshio Kato, Makoto Hori, and Hideyuki Nishizawa : On the Anti-tumor Activity of Nitrogenous Cyclic β-Diketones.

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Recently, several reports have been published on the anti-tumor activity of the analogs of naturally occurring purines and pyrimidines as well as their nucleosides. The purpose of these works is mainly focussed on the disturbance or inhibition effect of these compounds in the synthesis of nucleic acids in tumor cells. Among these compounds, derivatives of 6-azapyrimidines,[1~3] 8-azapurines,[4~12] 5-halogenated pyrimidines,[13,14] and 6-halogenated purines[15,16] have been reported on their anti-tumor activity in several test systems.

In the present series of work, some barbituric acids having acyl, halogen, or benzylidene substituted in the 5-position were examined for their anti-tumor activity by using several kinds of screening methods. These compounds are pyrimidine derivatives commonly containing a cyclic β-diketone group in their ring system and their anti-tumor properties have not hitherto been reported.

The test compounds synthesized this time can be classified into three groups from their structural point of view; (A) barbituric acids having tricarbonylmethane group, (B) halogen derivatives of barbituric acid, and (C) barbituric acids condensed with aromatic aldehydes. It has been reported by Ukita, *et al.*[17~23] that several compounds containing a tricarbonylmethane group, i.e. 2-acyl-1,3-cyclohexanediones and 3-acyl-4-hydroxycoumarins, showed potent *in vitro* antibacterial activities against several pathogenic bacteria. Furthermore, their *in vitro* anti-tumor activities were also tested and some active ones were found.[24] The new test compounds in the group A contain tricarbonylmethane grouping combined with cyclic ureido structure, a structural moiety which should have

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increased affinity to components in the tumor cells, and they are subsequently expected to reveal a larger anti-tumor activity.

The compounds included in group B commonly involve 2-bromo substituted 1,3-diketone group. The similar derivatives of 1,3-cyclohexanedione were previously tested to find their antidehydrogenase activity on ascites tumor cells.[24]

In group C, several derivatives of barbituric acid containing substituent in their 5-position containing a double bond are included. These compounds are structurally common with 1,3-diketone group in having a cross-conjugated double bond.

Experimental

Biological Tests—Several methods and techniques have usually been applied for the screening of anti-tumor activity. In the present series of work, three kinds of methods were adopted to examine their anti-tumor activity, namely, cylinder agar plate (CAP) method, cell-culture method, and *in vivo* method.

The newly synthesized compounds were tested for their antidehydrogenase activities against three kinds of ascites tumor cells, Ehrlich, Yoshida, and S-180, *in vitro* by the CAP method according to Umezawa, *et al.*[25] The activity is represented by diameter in mm. of inhibition zone and the test compound which gave larger diameter than 20 mm. against at least one of the three strains or 15 mm. against at least two of these strains were taken arbitrarily as active.

Effect of cell-growth inhibition of these compounds was examined by using the cell-culture of rat ascites hepatoma (AH-130) according to the method of Katsuta, *et al.*[26]

The *in vivo* test for Ehrlich ascites carcinoma in mice was performed, unless otherwise mentioned, according to the method of Egashira and Mizuno,[27] and the compound to be tested was given to the experimental group intraperitoneally 24 hr. after transplantation and the administration repeated each day for following 7 days. The effect of the compound was estimated by the difference in the increase of body weight between the control and experimental group, and of the survival time of the two groups.

Materials used—Barbituric acid (No. 1) and 1-methyl- (No. 2),[28] 1-phenyl- (No. 3),[28] 5-chloro- (No. 4),[29] 5,5-dichloro- (N0. 5),[30] 1-phenyl-5,5-dichloro- (No. 6), and 5-bromo-barbituric acid (No. 7),[29] sodium 5-bromo- (No. 8),[29] potassium 1-methyl-5-bromobarbiturate (No. 9),[29] 1-phenyl-5-bromobarbituric acid (No. 10),[31] sodium phenyl-5-bromobarbiturate (No. 11), 5,5-dibromo- (No. 12),[29] 1-methyl-5,5-dibromo- (No. 13),[29] 1-phenyl-5,5-dibromo- (No. 14),[31] 5-acetyl- (No. 15),[32] 1-methyl-5-acetyl- (No. 16),[32] 1-phenyl-5-acetyl- (No. 17), 1-phenyl-5-butyryl- (No. 18), 5-benzoyl- (No. 19),[33] 1-methyl-5-benzoyl- (No. 20),[33] 1-phenyl-5-benzoyl- (No. 21), 5-phenylacetyl- (No. 22), 1-methyl-5-phenylacetyl- (No. 23), 1-phenyl-5-phenylacetyl- (No. 24), 5-phenylcarbamoyl-barbituric acid (No. 25), sodium 5-phenylcarbamoylbarbiturate (No. 26), 1-methyl-5-phenylcarbamoylbarbituric acid (No. 27), sodium 1-methyl-5-phenylcarbamoylbarbiturate (No. 28), 1-phenyl-5-phenylcarbamoylbarbituric acid (No. 29), sodium 1-phenyl-5-phenylcarbamoylbarbiturate (No. 30), 5-benzylidene- (No. 31),[34] 1-methyl-5-benzylidene (No. 32), 1-phenyl-5-benzylidene- (No. 33), 5-cinnamylidene- (No. 34),[35] 1-methyl-5-cinnamylidene- (No. 35), 1-phenyl-5-cinnamylidene- (No. 36),[36] and 5-nitro-barbituric acid (No. 37).[37]

General Method for Synthesis of Compounds Nos. 17, 18, 21, 22, 23, and 24—To a solution of 0.005 mole of barbituric acid or 1-substituted barbituric acid in 8 cc. of dehyd. pyridine containing

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2 drops of piperidine, equimolar amount of acyl chloride was added dropwise with cooling to below 0°. After standing at 37° for 2, 3, or 5 hr. for Nos. 18, 24, and 22, and overnight for Nos. 17 and 23, the reaction mixture was acidified with HCl. The precipitate occurred was dissolved in EtOH, decolorized with charcoal, and recrystallized from the same solvent.

No. 17 : 1-Phenyl-5-acetylbarbituric acid. *Anal.* Calcd. for $C_{12}H_{10}O_4N_2$: N, 11.38. Found : N, 11.73.

No. 18 : 1-Phenyl-5-butyrylbarbituric acid. *Anal.* Calcd. for $C_{14}H_{14}O_4N_2$: N, 10.21. Found : N, 10.02.

No. 21 : 1-Phenyl-5-benzoylbarbituric acid. *Anal.* Calcd. for $C_{17}H_{12}O_4N_2$: N, 9.09. Found : N, 9.26.

No. 22 : 5-Phenylacetylbarbituric acid. *Anal.* Calcd. for $C_{12}H_{10}O_4N_2$: C, 58.53; H, 4.09; N, 11.38. Found : C, 58.86; H, 3.83; N, 11.99.

No. 23 : 1-Methyl-5-phenylacetylbarbituric acid. *Anal.* Calcd. for $C_{13}H_{12}O_4N_2$: C, 59.99; H, 4.65; N, 10.77. Found : C, 59.89; H, 4.65; N, 11.20.

The properties of these products are summarized in Table I.

Table I. Reaction Conditions in Syntheses, Yield, and Physical Properties of Compounds Nos. 17, 18, 21, 22, 23, and 24

No.	Product (barbituric acid)	Interval for keeping the reaction mixture at 37°	m.p. (°C)	Yield (%)	Coloration to $FeCl_3$	Crystal form
17	1-Phenyl-5-acetyl-	overnight	224～225	40	orange-brown	colorless leaflets
18	1-Phenyl-5-butyryl-	2 hr.	199	—	brown	colorless needles
21	1-Phenyl-5-benzoyl-	overnight	232.5 (decomp.)	44	yellowish-brown	slightly brownish needles
22	5-Phenylacetyl-	5 hr.	246 (decomp.)	21	〃	colorless needles
23	1-Methyl-5-phenylacetyl-	overnight	182～183	34	orange	colorless prisms
24	1-Phenyl-5-phenylacetyl-	3 hr.	195～196 (decomp.)	30	〃	slightly yellowish powder

General Method for Synthesis of Compounds Nos. 25～30—To a solution of 0.005 mole of barbituric acid or 1-substituted barbituric acid in 10 cc. of pyridine, equimolar amount of phenyl isocyanate was added. The mixture was refluxed in an oil bath for 3 hr. and reddish-colored solution was acidified with 10% HCl. The crystals that separated were collected, dissolved in dioxane, and filtered to remove a trace of insoluble matter. To the filtrate was added water to precipitate the product which was recrystallized from dioxane-water mixture.

For the preparation of respective sodium salts of Nos. 25, 27, and 29 (Nos. 26, 28 and 30) the EtOH solution of each free compound was titrated with 0.05% NaOH to equimolar point, the salt precipitated was collected, and washed with EtOH.

No. 25 : 5-Phenylcarbamoylbarbituric acid. *Anal.* Calcd. for $C_{11}H_9O_4N_3$: C, 53.44; H, 3.67; N, 17.00. Found : C, 53.70; H, 3.42; N, 17.44.

No. 27 : 1-Methyl-5-phenylcarbamoylbarbituric acid. *Anal.* Calcd. for $C_{12}H_{11}O_4N_3$: C, 55.17; H, 4.24; N, 16.01. Found : C, 55.83; H, 4.37; N, 15.58.

No. 29 : 1-Phenyl-5-phenylcarbamoylbarbituric acid. *Anal.* Calcd. for $C_{17}H_{13}O_4N_3$: C, 63.16; H, 4.03; N, 13.00. Found : C, 63.15; H, 4.25; N, 13.22.

The properties of these products are summarized in Table II.

Table II. Physical Properties and Yield of Compounds Nos. 25, 27, and 29

No.	Product (barbituric acid)	m.p. (°C)	Yield (%)	$FeCl_3$ coloration	Crystal form
25	5-Phenylcarbamoyl-	293～294 (partial decomp.)	90	reddish-brown	colorless scales
27	1-Methyl-5-phenylcarbamoyl-	228～229.5 (partial decomp.)	85	orange-brown	
29	1-Phenyl-5-phenylcarbamoyl-	289～290 (partial decomp.)	85	orange-yellow	colorless needles

General Method for Synthesis of Compounds Nos. 32, 33, and 35—Equimolar amounts of 1-substituted barbituric acid and aromatic aldehyde were warmed in dioxane on a boiling water bath for 1 hr. After cooling and addition of water the precipitate formed was collected and recrystallized from aqueous dioxane or AcOH.

No. 32 : 1-Methyl-5-benzylidenebarbituric acid. *Anal.* Calcd. for $C_{12}H_{10}O_3N_2$: N, 12.17. Found : N, 11.76.

No. 33 : 1-Phenyl-5-benzylidenebarbituric acid. *Anal.* Calcd. for $C_{17}H_{12}O_3N_2$: N, 9.59. Found : N, 9.13.

No. 35 : 1-Methyl-5-cinnamylidenebarbituric acid. *Anal.* Calcd. for $C_{14}H_{12}O_3N_2$: N, 10.93. Found : N, 11.09.

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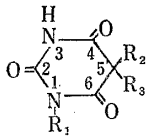
The properties of these products are summarized in Table III.

Table III. Physical Properties and Yield of Compounds Nos. 32, 33, and 35

No.	Product (barbituric acid)	m.p. (°C)	Yield (%)	$FeCl_3$ coloration	Crystal form
32	1-Methyl-5-benzylidene-	218	90	yellowish-brown	colorless needles
33	1-Phenyl-5-benzylidene-	231～232	90	dark brown	colorless powder
35	1-Methyl-5-cinnamylidene-	245～248 (decomp.)	72	yellowish-brown	yellow needles

Table IV. Antidehydrogenase Activity of Derivatives of Barbituric Acid against Ascites Tumor Cells by Cylinder Agar Plate (CAP) Method



No.	R_1	R_2	R_3	Ehrlich	Yoshida	S-180
1	H	H	H	13	C	C
2	CH_3	H	H	22	C	C
3		H	H	12	C	C
4	H	Cl	H	0	C	0
5	H	Cl	Cl	0	C	0
6		Cl	Cl	0	C	0
7	H	Br	H	C	C	C
8*	H	Br	H	14	C	C
9**	CH_3	Br	H	14	C	C
10		Br	H	14	C	C
11*		Br	H	14	C	C
12	H	Br	Br	45	44	45
13	CH_3	Br	Br	44	30	43
14		Br	Br	28	14	21
15	H	$COCH_3$	H	0	C	0
16	CH_3	$COCH_3$	H	0	C	C
17		$COCH_3$	H	0	C	C
18		COC_3H_7	H	C	C	C
19	H	CO- 	H	0	C	C
20	CH_3	CO- 	H	0	C	C
21		CO- 	H	0	C	0
22	H	$COCH_2$- 	H	C	0	0
23	CH_3	$COCH_2$- 	H	0	19	12
24		$COCH_2$- 	H	0	15	15
25	H	CONH- 	H	C	11	0
27	CH_3	CONH- 	H	11	22	C
29		CONH- 	H	C	12	0
31	H	=CH- 	–	0	C	C
32	CH_3	=CH- 	–	14	C	C
33		=CH- 	–	0	C	C
34	H	=CH-CH=CH- 	–	C	C	0
35	CH_3	=CH-CH=CH- 	–	C	12	C
36		=CH-CH=CH- 	–	0	C	0
37	H	NO_2	H	0	0	0

* Sodium-salt ** Potassium-salt

C: Diameter of cup (8mm.)

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Results and Discussions

The results obtained by CAP method are summarized in Table IV. Among the derivatives of barbituric acid in group (B), Nos. 4~14, 5,5-dibromobarbituric acid, and 1-methyl- and 1-phenyl-5,5-dibromobarbituric acid (Nos. 12, 13, and 14) showed distinguished inhibition on dehydrogenase activity of the three strains of ascites tumor cells and each test compound showed inhibitory effect commonly for all three kinds of the strains used. Thus, the substitution of a methyl or a phenyl group in one of the two NH groups did not cause any essential effect on the activity of the parent 5,5-dibromobarbituric acid. In contrast to these dibromo compounds, the monobromo-substituted analogs, Nos. 7~11, were practically inactive in this test, and moreover, it is interesting to note that chlorine-substituted derivatives were ineffective even in the cases of dichloro substitution (Nos. 5 and 6). In a series of compounds in the group (A), Nos. 15~29, only a few of them, Nos. 23, 24, and 27, showed a slight activity in this test, and any kind of relationship between the effectiveness and chemical structure could not be seen. None of the compounds Nos. 31~36 included in group (C) was effective, indicating that the cross-conjugation system is dispensable for the antidehydrogenase activity.

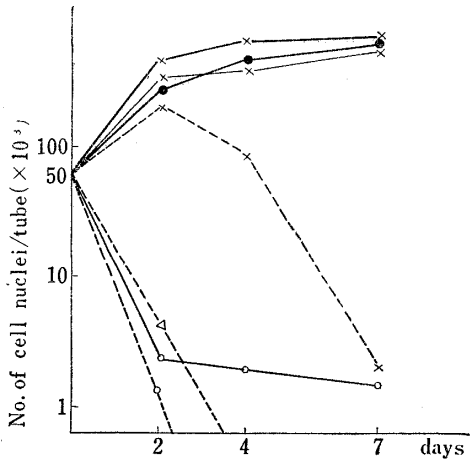

Fig. 1. Effect of Barbituric Acid Derivatives on the Multiplication of AH-130 Cells

Concn. of test compd. : 50 γ/cc. (75 γ/tube)

—×— Control
—•— Barbituric acid (No. 1)
—×— 5-Bromobarbituric acid (No. 7)
—○— 5,5-Dibromobarbituric acid (No. 12)
---×--- 5-Phenylcarbamoylbarbituric acid (No. 25)
---△--- 1-Methyl-5-phenylcarbamoylbarbituric acid (No. 27)
---○--- 1-Phenyl-5-phenylcarbamoylbarbituric acid (No. 29)

In Fig. 1, the results of *in vitro* cell growth inhibition test by several derivatives of barbituric acid are given. Here, the activities of 5-bromo- (No. 7), 5,5-dibromo- (No. 12), 5-phenylcarbamoyl- (No. 25), 1-methyl- (No. 27), and 1-phenyl-5-phenylcarbamoyl-barbituric acid (No. 29) were compared with that of barbituric acid (No. 1). In the case of bromo derivatives tested this time, the dibromo derivative (No. 12) showed a remarkable inhibition after first 2 days' incubation, while the monobromo-substituted analog (No. 7) was as inactive as barbituric acid, revealing the same tendency as was observed in the CAP test.

Of the compounds in group (A), three derivatives (Nos. 25, 27, and 29) of 5-phenylcarbamoylbarbituric acid together with their sodium salts (Nos. 26, 28, and 30) were examined by this method to find a potent activity in all of them. The results obtained in this test are not consistent with the results of CAP test, in which these compounds did not show any remarkable antidehydrogenase activity.

The bromine-substituted barbituric acids and the three sodium 5-phenylcarbamoylbarbiturates were also examined for their *in vivo* anti-tumor activity against Ehrlich ascites carcinoma in mice. Two types of bromine derivatives, sodium 5-bromobarbiturate (No. 8) and 5,5-dibromobarbituric acid, did not show any influence on the increase of body weight and life-span of the mice transplanted with Ehrlich ascites carcinoma (Table V).

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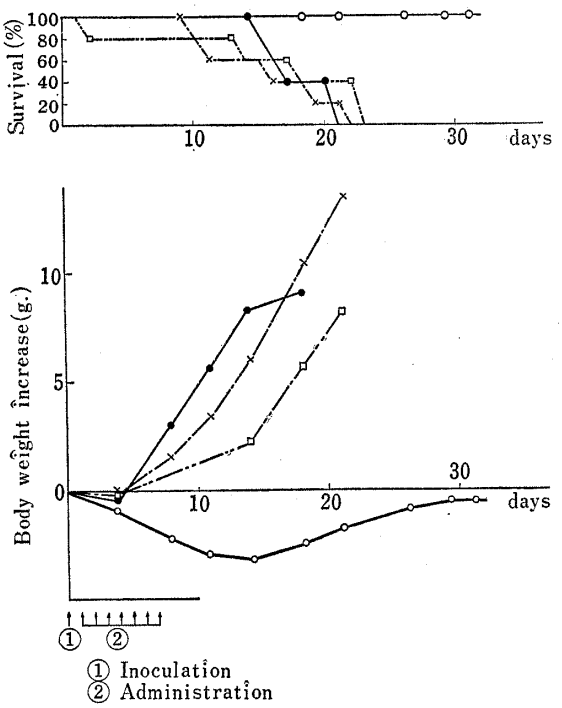

Fig. 2. Effect of Barbituric Acid Derivatives on Ehrlich Ascites Carcinoma in Mice

(1) Sodium 5-phenylcarbamoylbarbiturate

—•— Control
—○— 6-Mercaptopurine 600 γ/day/mouse
—×— Test compd. 600 γ/day/mouse
—□— 〃 1200 γ/day/mouse

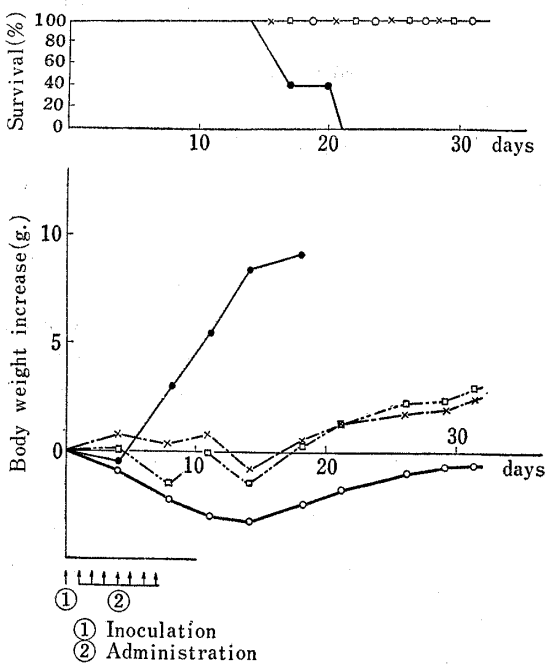

Fig. 3. Effect of Barbituric Acid Derivatives on Ehrlich Ascites Carcinoma in Mice

(2) Sodium 1-methyl-5-phenylcarbamoylbarbiturate

—•— Control
—○— 6-Mercaptopurine 600 γ/day/mouse
—×— Test compd. 600 γ/day/mouse
—□— 〃 1200 γ/day/mouse

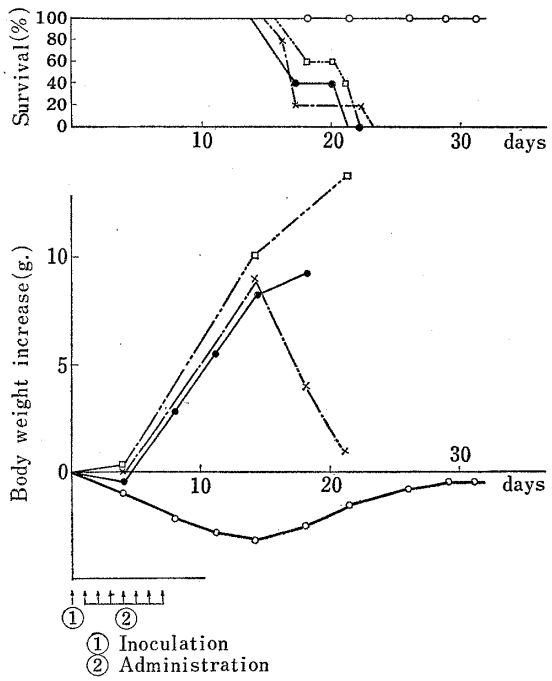

Fig. 4. Effect of Barbituric Acid Derivatives on Ehrlich Ascites Carcinoma in Mice

(3) Sodium 1-phenyl-5-phenylcarbamoyl-barbiturate

—•— Control
—○— 6-Mercaptopurine 600 γ/day/mouse
—×— Test compd. 600 γ/day/mouse
—□— 〃 1200 γ/day/mouse

On the other hand, as is shown in Table VI and Figs. 2~4, among sodium 5-phenylcarbamoyl- (No. 26), sodium 1-methyl-5-phenylcarbamoyl- (No. 28), and sodium 1-phenyl-5-phenylcarbamoyl-barbiturate (No. 30), the first one (No. 26) revealed a remarkable anti-tumor activity in this test. The effect observed was comparable with that given by

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Table V. Effect of 5-Bromobarbituric Acids on Ehrlich Ascites Carcinoma in Mice*1

No.	Compound	Dose (mg./kg. × 7)	Mortality*2	Average Increase*3 In Body Wt. (g.)	Average Viable Time (day)
8	Sodium 5-bromobarbiturate	50	0/3	7.7	14
12	5,5-Dibromobarbituric acid	50	0/3	4.0	15
—	Control	—	0/12	5.5	15

*1 This test was perfomed by Dr. K. Kajiwara of Research Laboratories, Takeda Pharmaceutical Industries, Ltd.
*2 Death occurred within 8 days after tumor inoculation (No. died/No. of mice used).
*3 8th day of inoculation.

Table VI. Effect of Barbituric Acid Derivatives on Ehrlich Ascites Carcinoma in Mice

Test Substance	Dose (γ/day/mouse) × 7	Mortality*1	Tumor type: Ascites Viability*2	Ascites Effect	Solid Average wt. of tumor*3 (g.)	Solid Effect
5-Phenylcarbamoyl-barbituric acid	600	0/5	5/5	++	——	——
〃	1200	0/10	5/5	++	0.14	±
1-Methyl-5-phenylcarbamoyl-barbituric acid	600	0/5	0/5	—	——	——
〃	1200	2/10	0/5	—	0.237	—
1-Phenyl-5-phenylcarbamoyl-barbituric acid	600	0/5	0/5	—	——	——
〃	1200	0/10	0/5	—	0.169	—
6-Mercaptopurine	600	1/10	5/5	++	0.032	++
Control	——	0/10	0/5	——	0.203	——

*1 Death occurred within 9 days after tumor inoculation (No. died/No. of mice used).
*2 30th day of inoculation (No. living/No. of mice used).
*3 14th day of inoculation.

6-mercaptopurine and the treated mice survived more than 23 days after final administration of this material. The increase in body weight of transplanted mice was also suppressed by this treatment for about 25 days. In Table VI, the inhibiting activity of this compound for the growth of solid tumor of the same strain in subcutaneous region is also included. The other two homologs of this series of compounds, Nos. 28 and 30, which have respectively methyl and phenyl in one of the nitrogen atoms in the heterocyclic system, were found not so effective as No. 26 (Figs. 3 and 4).

Conclusion

In the screening of anti-tumor compounds, a result obtained by one of the several test methods for a particular test compound is not usually parallel with that of another kind of test system. This discrepancy in the results obtained by various screening methods is due to the difference of the sites on which the test compound exerts its proper inhibition among the multiple metabolism of the tumor cells. Similar discrepancy in the results was observed in the present series of experiments. Thus 5,5-dibromobarbituric acid, which was inhibitory for both dehydrogenase systems of the ascites cells and *in vitro* growth of AH-130 cells, showed no observable inhibition in the *in vivo* test on Ehrlich ascites carcinoma. However, among 5-phenylcarbamoylbarbituric acid derivatives, which showed activity in the growth inhibition of *in vitro* culture of AH-130 cells but not in *in vitro* antidehydrogenase screening test by CAP method, No. 26, namely 5-phenylcarbamoylbarbituric acid, showed a remarkable inhibitory action in *in vivo* test of Ehrlich ascites carcinoma in mice, and this compound seems to be the most promising for further research as a chemotherapeutic. Furthermore, from the point of chemical structure,

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one of the two bromine atoms substituted at 5-position in 5,5-dibromobarbituric acid is so unstable that it is capable of a nonspecific intermolecular bromination of co-existing compounds. On the other hand, since the compounds of the 5-phenylcarbamoyl derivatives do not have such a reactive chemical group in their structure, the *in vivo* anti-tumor activity of No. 26 should be attributed to some unknown specific affinity responsible for its anti-tumor activity.

The authors are deeply indebted to Dr. D. Mizuno and his collaborators at the National Institute of Health, Tokyo, and to Dr. K. Kajiwara of Research Laboratories, Takeda Pharmaceutical Industries, Ltd., for carrying out a part of the anti-tumor tests.

Summary

5-Halo, 5-acyl, 5-benzylidene, and 5-phenylcarbamoyl derivatives of 1-hydrogen, 1-methyl, and 1-phenyl-barbituric acids were synthesized and examined for their antidehydrogenase activity against ascites tumor cells by the cylinder agar plate (CAP) method (A), for their growth inhibitory activity in cell-culture of AH-130 (B), and for their anti-tumor activity against Ehrlich ascites carcinoma in mice (C). Among the compounds tested, sodium 5-phenylcarbamoylbarbiturate showed potent anti-tumor activity, both *in vitro* and *in vivo* biological tests (B) and (C), to reveal its promising properties as a cancer chemotherapeutic.

(Received August, 13 1960)

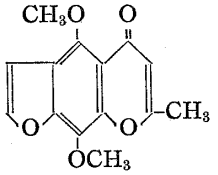
UDC 547.816.07

172. Kazutaka Yamaguchi, Seigo Fukushima, and Hiroshi Yamada: Studies on Benzochromones. I. Synthesis of 2-Methyl-5,6-dimethoxy-7,8-benzochromone.

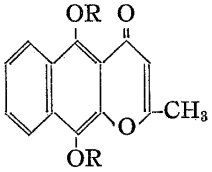
(*National Institute of Hygienic Sciences*[*1])

The natural product khellin (I) has attracted considerable attention as a potent vasodilator and an antispasmodic agent, and great many variants of khellin have been prepared in an effort to find derivatives with greater activity than that of khellin.[1,2]

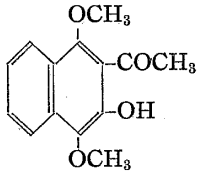
This paper deals with some investigations on the attempted synthesis of a compound in which the furan ring of khellin is replaced by a benzenoid ring, such as 2-methyl-5,8-dimethoxy-6,7-benzochromone (II).



(I)



(II) R=CH_3
(II′) R=H



(III)

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