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The Effects of Substituted Phenyl Glycosides and Corresponding Thioglycosies on the Growth of Transplanted Tumor in Mice

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Forty substituted phenyl glycosides and corresponding thioglycosides were synthesized and tested the tumor growth effect against sarcoma 180 with these compounds. Most of the aromatic thioglycosides markedly increased the tumor growth in weight. It seems that compound XIV or XVII possessed the highest activity among the compounds examined. One of most active substances accelerate a growth of the body weight in young mice and a regeneration of the partial hepatectomy in rats. On the other hand, these compounds have no immunosuppressive or activating effect.

Although a great many of studies have indicated on substances having the tumor inhibiting effect, not many studies have been reported on substances having the tumor growth promoting effect. The works of Akagi, *et al.*[2] and Hasegawa, *et al.*[3] reported that some sugars containing sulfur showed an antitumor effect *in vivo* and *in vitro*. The present paper reports the syntheses of several substituted phenyl glycosides and corresponding thioglycosides, and the test for the tumor growth effect of these compounds against sarcoma 180. Influences to the body weight of young mice and to the regeneration of a partial hepatectomy of rats are also examined with the compound of which have a tumor growth promoting effect.

Table I summarizes our screening results. A, B, C, and D, in the table, represent D-glucopyranosides, D-galactopyranosides, D-xylopyranosides and D-glucose derivatives, respectively. Most of the substances markedly increased the growth rate of tumor in mice. It is difficult to correlate the structure of compounds, but these results demonstrate that these thioglycosides possess a very aggressive effect to the tumor growth. Especially, groups treated with compounds XIV, XVII, and XVIII gave about 50% increase in the growth rate as compared with an each control group. The tumor growth promoting effect is always observed in our re-examinations in this screening system. On the other hand, the tumor growth was not enhanced by the sugars or aglycons as shown in Table II. We used glucose and inositol as referential materials. Tanino, *et al.*[4] demonstrated that myo-inositol had a growth promoting activity against MM_2-C_3H/He ascites tumor *in vivo*. On the contrary, inositol has not been shown an activity against sarcoma 180 as far as our test system used. As a reason of differences of tumor growth between O- and S-glycosides, the authors speculate

1) Location: *Ukima 1-3-32, Kita-ku, Tokyo, 115, Japan.*
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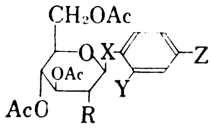
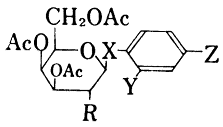
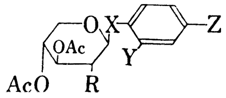
TABLE I. Screening Results of Substituted Phenyl Glycosides and Corresponding Thioglycosides



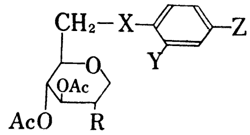
A



B



C



D

Compound	R	X	Y	Z	n[a]	ΔG (T/C)	Tumor weight[b] (T/C, g)	Growth rate (T/C, %)	Reference for preparation
A									
I	OAc	O	H	CHO	10	+4.2/+4.3	1.20±0.51/0.91±0.36	131.9	5)
II	OAc	O	NH_2	H	10	+5.5/+4.0	1.76±0.80/1.11±0.64	158.6	6)
III	OAc	O	HNAc	H	8	+4.0/+3.7	0.64±0.43/0.59±0.36	108.5	7)
IV	OAc	O	NO_2	H	8	+4.6/+3.7	0.41±0.16/0.59±0.36	69.5	6)
V	OAc	O	CHO	Br	10	+4.1/+4.3	1.02±0.54/0.91±0.36	112.1	c)
VI	OMs	O	NO_2	H	8	+4.0/+3.7	0.66±0.35/0.59±0.36	111.9	c)
VII	OMs	O	NH_2	H	8	+4.0/+3.7	0.51±0.22/0.59±0.37	86.4	c)
VIII	OMs	O	NHAc	H	8	+4.7/+3.7	0.47±0.23/0.59±0.36	79.7	c)
IX	OAc	S	H	NH_2	8	+5.5/+4.3	1.19±0.71/0.91±0.36	130.8	8)
X	OAc	S	H	Cl	10	+5.2/+4.0	1.78±0.50/1.18±0.50	150.8	9)
XI	OAc	S	NH_2	H	10	+4.3/+5.9	0.89±0.22/0.73±0.39	121.9	8)
XII	OAc	S	NH_2	Cl	10	+4.4/+5.6	1.44±0.36/1.22±0.34	117.4	c)
XIII	OAc	S	NHAc	H	9	−2.0/+5.9	0.72±0.39/0.73±0.39	98.6	8)
XIV	OAc	S	NHMs	H	11	+3.7/+5.9	1.53±0.87/0.73±0.39	209.5	c)
					10	+3.1/+3.4	2.14±0.45/1.51±0.44	141.7	
XV	OAc	S	NHBz	H	10	+4.6/+5.9	1.12±0.52/0.73±0.39	153.4	c)
XVI	OMs	S	NH_2	H	10	+5.6/+5.9	0.77±0.37/0.73±0.39	105.4	c)
					10	+5.0/+4.0	1.59±0.55/1.18±0.50	134.7	c)
XVII	OMs	S	NHAc	H	10	+4.0/+5.9	1.26±0.41/0.73±0.39	172.6	c)
					10	±0 /+4.0	1.79±0.45/1.18±0.50	151.7	
XVIII	OMs	S	NHMs	H	10	+4.0/+5.9	1.28±0.56/0.73±0.39	175.3	c)
					10	+5.5/+4.0	1.68±0.92/1.11±0.64	151.4	
XIX	OMs	S	NHBz	H	10	+5.9/+5.9	1.22±0.58/0.73±0.39	167.1	c)
XX	NHAc	S	NH_3	H	10	+8.0/+5.9	1.57±0.60/1.11±0.64	141.4	c)
XXI	NHAc	S	NHAc	H	10	+4.1/+4.0	1.27±0.86/1.11±0.64	114.4	c)
XXII	NHAc	S	NHMs	H	10	+6.4/+4.0	1.49±0.54/1.11±0.64	134.2	c)
XXIII	NHAc	S	H	Cl	10	+4.0/+4.0	1.03±0.36/1.11±0.64	92.8	c)
XXIV	OMs	S	H	NHAc	9	+4.5/+4.0	1.72±0.66/1.18±0.50	145.8	c)
XXV	OMs	S	H	NHMs	9	+3.8/+4.0	1.52±0.51/1.18±0.50	128.8	c)
XXVI	OMs	S	H	NHBz	10	+5.0/+4.0	1.64±0.52/1.18±0.50	139.0	c)
B									
XXVII	OAc	O	NO_2	H	10	+3.0/+3.7	0.59±0.29/0.59±0.36	100	10)
XXVIII	OMs	O	NO_2	H	10	+3.8/+3.7	0.54±0.27/0.59±0.36	91.5	c)
XXIX	OMs	O	NH_2	H	10	+3.3/+3.7	0.53±0.35/0.59±0.36	89.8	c)
					10	+4.1/+4.3	1.15±0.77/0.91±0.36	126.4	
XXX	OAc	S	H	Cl	10	+4.0/+4.0	1.46±0.39/1.18±0.50	123.7	c)
XXXI	OAc	S	NH_2	H	10	+5.8/+2.9	1.64±0.56/1.42±0.73	115.5	c)
XXXII	OAc	S	NHAc	H	10	+5.1/+5.6	2.04±0.40/1.22±0.34	159.0	c)
XXXIII	OAc	S	HNMs	H	10	+4.2/+4.0	1.12±0.43/1.11±0.64	100.9	c)
XXXIV	OMs	S	NH_2	H	10	+5.7/+5.6	1.35±0.65/1.37±0.56	98.5	c)
C									
XXXV	OAc	S	NH_2	H	10	+7.2/+6.0	2.04±1.08/1.08±0.62	188.9	c)
XXXVI	OAc	S	NHAc	H	10	+3.9/+4.0	1.59±0.93/1.11±0.64	143.2	c)
XXXVII	OAc	S	NHBz	H	10	+7.0/+4.0	1.82±1.19/1.11±0.64	164.0	c)
XXXVIII	OAc	S	H	Cl	10	+7.8/+4.0	1.53±0.75/1.18±0.50	129.7	c)
D									
XXXIX	OAc	S	NH_2	H	8	+3.9/+4.3	1.11±0.28/0.91±0.36	122.0	c)
XL	OTs	S	NH_2	H	8	+5.5/+4.3	1.62±0.55/0.91±0.36	178.0	c)

Administration of compound was performed intraperitoneally in a dose of 2000 μg/mouse once a day for 7 days, being started from the day following inoculation with the tumor cells.
ΔG: body weight differences of animals
T/C: ratio of the treated to the control
a) Figures refer to number of mice treated. Control group is 10 mice in every case.
b) Tumors were weighed on the 14th day after transplantation. mean tumor weight ±standard deviation.
c) new compounds, see Experimental

TABLE II. Screening Results of Sugars and Aglycones

Compound	n	ΔG (T/C)	Tumor weight (T/C, g)	Growth rate (T/C, %)
Glucose	10	$+4.2/+3.7$	$1.87\pm0.68/1.38\pm0.37$	135.5
2,3,4,6-Tetra-O-acetyl-D-glucose	10	$+4.4/+3.7$	$1.30\pm0.80/1.38\pm0.37$	94.2
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide	10	$+3.8/+3.7$	$1.12\pm0.59/1.38\pm0.37$	81.2
Inositol	10	$+3.4/+3.7$	$1.44\pm0.50/1.38\pm0.37$	104.3
o-Aminophenol	10	$+3.6/+3.7$	$1.26\pm0.30/1.38\pm0.37$	91.3
o-Aminobenzenethiol	10	Toxicity[a]		

Experimental conditions are the same as in the Table I.
a) All the mice were dead immediately after injection of the compound.

that the O-glycosides may be easily hydrolyzed by glycosies in the host, while S-glycosides may be not.

The effect of compound XIV on the body weight change of mice was examined as shown in Fig. 1. The body weight in the groups of compound XIV or inositol as compared with control was considerably rised about 5 days after the start of experiment. These two drugs show almost the same effect on the growth of body weight as the upper curve in Fig. 1, though the activity of inositol was inferior to that of the compound XIV in the tumor growth rate (Table I and II). Furthermore, the effects of these drugs on the regeneration of a partial hepatectomy of rats were studies as indicated in Table III. The slightly accelerating effect against the liver regeneration is shown by the compound XIV and inositol.

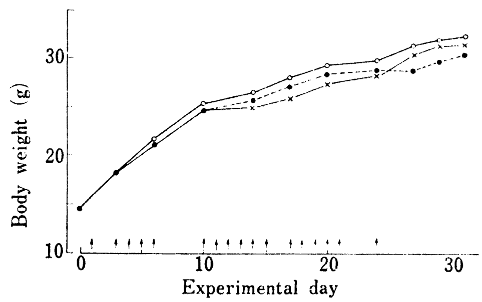
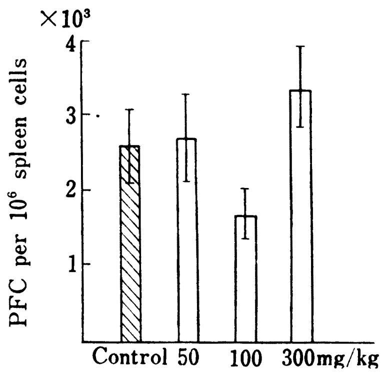


Fig. 1. The Effect of Drugs on Body Weight Changes in Mice

The data reported in the figures represent the average values of 10 mice in each group. The each arrow shows the drug injection in a dose of 2000 μg/mouse.

●——● control
○——○ compound XIV or inositol
×------× glucose



Fif. 2. Effect of Compound XVII on the Number of Hemolitic Plaque Forming Cells in the Spleens of Immunized Mice

Mice were immunized by intravenous inoculation of 4×10^8 sheep red cells and 4 animals of each group were sacrificed on day 4 after immunization. Drugs were given intraperitoneally 1 day before, immediately after, and 2 days after immunization in each dose.

Immuno-suppressive or activating effect was also studied with the compound XVII. The values of plaque forming cells are given in Fig. 2. These results show no effect against immunocytes.

TABLE III. The Effects of Compounds on Regeneration of the Liver after Partial Hepatectomy

Group	n	Body weight (g)		Liver weight (g)		Regeneration rate[a)]
		Immediately after operation	6 days after hepatectomy	Rejection	Recovery	
Control	4	93	101	3.4	4.4	85.0 ± 8.8
Compound XIV	4	90	104	3.3	4.9	99.2 ± 5.7
Inositol	3	90	108	3.2	5.3	106.6 ± 8.8

Drug was given subcutaneously in a dose of 250 mg/kg for 4 days, being started from the day following partial hepatectomy.
a) (recovery/rejection)$\div$0.65$\times$100
A part of partial hepatectomy considered 65% of total liver.
mean$\pm$standard deviation

It seems reasonable to conclude from these experiments that the most of phenyl thioglycosides tested have growth promoting effects against tumor and normal tissue in any case. Further studies into these problems are necessary.

Experimental

Biology

The animals used in these experiments were ddY male mice 3—5 weeks old and Donryu female rats about 95 g. They were supplied from an animal farm in Shizuoka Prefecture and Nippon Rat Co. in Tokyo, respectively. They were kept on standard diet CE-2 CLEA Japan Inc. in Tokyo with unlimited supply of water. Sarcoma 180 cells which were maintained by serial intraperitoneal transplantation into ddY male mice, were employed for the experiments. Tumor cells harvested 7 days after transplantation were used. The screening test was performed by the method of Sugiura.[11] And each mouse was inoculated subcutaneously with 2×10^6 cells of sarcoma 180. A partial hepatectomy was performed approximately 65% of total liver in rat. Sheep red blood cells were obtained from Shiihashi Co. in Tokyo. Determination of antibody production in the spleen was carried out according to the method of Cunningham and Szenberg.[12]

Chemistry

Method A——To a solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (27 g) and 5-bromosalicylaldehyde (13.4 g) in acetone (100 ml) was added a solution of sodium hydroxide (2.6 g) in water (20 ml). The mixture was allowed to stand overnight at 4°. After the solvent was evaporated to dryness, the residue was dissolved in chloroform, then poured into water. The chloroform layer was washed with 10% sodium hydroxide solution, water and dried over calcium chloride, then filtered. The filtrate was evaporated to give a syrup which crystallized from ethanol. Recrystallization from ethanol gave pure material.

Method B——A mixture of *o*-nitrophenol (13 g), anhydrous potassium carbonate (13 g) and 2-O-mesyl acetobromoglucose or 2-O-mesyl acetobromogalactose (26 g) in dry acetone (400 ml) was heated under reflux for 20 hr. After the solvent was evaporated to dryness, the residue was dissolved in chloroform, then poured into ice-water. The chloroform layer was treated similarly as the method A. Recrystallization from ethanol gave compound VI or XXVIII, respectively.

Method C——To suspension of the compound VI or XXVIII (4 g) in dry methanol (100 ml) was added Raney nickel (4 g). The mixture was agitated 4 hr at room temperature with hydrogen. After the catalyst was removed by filtration, the filtrate was concentrated to dryness to give crystalline residue. Recrystallization from ethanol gave compound VII or XXIX, respectively.

Method D——Compound VI, XVI, XX, XXXI, or XXXV was added to a chilled mixture of acetic anhydride and pyridine (1:1 v/v) for preparation of compound VIII, XVII, XXI, XXIV, XXXII, or XXXVI, respectively. The mixture was left overnight at room temperature, then poured into ice-water, and the product was extracted with chloroform. The chloroform layer was washed with dilute sulfuric acid, aqueous sodium bicarbonate and water dried over sodium sulfate and evapotated to give a syrup which crystallized from ethanol. Recrystallization from ethanol gave pure materials.

Method E——A solution of the appropriate glycopyranosyl halide (0.01 mole) in chloroform (50 ml) was added to a solution of the appropriate substituted benzenethiol (0.02 mole) in 50 ml of methanolic potassium

11) K. Sugiura, *Cancer Res.* (Suppl.), 3, 18 (1955).
12) A.J. Cunningham and Szenberg, *Immunology*, **14**, 599 (1968).

Table IV. Analytical Data of New Compounds of Substituted Phenyl Glycosides and Corresponding Thioglycosides

Compound	mp (°C)a)	Yield (%)	$_D^t$ b)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N	Methodc)
V	119 —121	25	-24.4^{18}	$C_{21}H_{23}O_{11}Br$	47.47	4.36		47.94	4.63		A
VI	164 —165	14	$+35^{14}$	$C_{19}H_{23}O_{13}NS$	45.33	4.58	2.77	45.76	4.55	2.80	B
VII	156 —158	92	-45^{14}	$C_{19}H_{25}O_{11}NS$	47.99	5.30	2.95	47.45	5.32	2.91	C
VIII	177 —183.5	92	-44^{14}	$C_{21}H_{27}O_{12}NS$	48.73	5.26	2.71	48.73	5.16	2.66	D
XII	153 —155	66	-7.5^{19}	$C_{19}H_{24}O_{10}NS_2Cl$	43.38	4.60	2.66	42.73	4.49	2.26	E
XIV	164 —166	64	-33.1^{23}	$C_{21}H_{27}O_{11}NS_2$	47.28	5.10	2.63	47.04	4.94	2.59	F
XV	142 —143	70	$+14.1^{23}$	$C_{27}H_{29}O_{10}NS$	57.96	5.22	2.50	57.80	5.17	2.41	G
XVI	155 —156	82	$+28^{23}$	$C_{19}H_{25}O_{10}NS_2$	46.44	5.13	2.85	46.70	5.08	2.72	E
XVII	132 —133	80	$+28^{23}$	$C_{21}H_{27}O_{11}NS_2$	47.28	5.10	2.63	47.46	4.94	2.58	D
XVIII	165 —167	87	-7.4^{23}	$C_{20}H_{27}O_{12}NS_3$	42.19	4.78	2.46	41.83	4.69	2.30	F
XIX	138 —139	91	$+94^{23}$	$C_{26}H_{29}O_{11}NS_2$	52.44	4.91	2.35	52.43	4.91	2.18	G
XX	171 —172	72	$+2.8^{18}$	$C_{20}H_{26}O_8N_2S$	52.86	5.77	6.17	53.22	5.76	6.16	E
XXI	242 —243	92	-6.4^{18}	$C_{22}H_{28}O_9N_2S$	53.22	5.68	5.57	53.12	5.59	5.57	D
XXII	220 —221	90	-36.6^{18}	$C_{21}H_{28}O_{10}N_2S_2$	47.34	5.30	5.26	47.90	5.31	4.97	F
XXIII	257	45	-4^{18}	$C_{20}H_{24}O_8NSCl$	50.68	5.10	2.95	50.77	5.16	2.83	E
XXIV	128 —138 (decomp.)	75	-35.0^{18}	$C_{21}H_{27}O_{11}NS_2$	47.28	5.10	2.63	47.49	5.15	2.41	D
XXV	154 —155 (decomp.)	58	-23.0^{18}	$C_{20}H_{27}O_{12}NS_3$	42.19	4.78	2.46	42.21	4.78	1.89	F
XXVI	150 —151 (decomp.)	60	-22.0^{18}	$C_{26}H_{29}O_{11}NS_2$	52.44	4.91	2.35	52.51	4.87	2.39	G
XXVIII	149 —150	38	$+17^{14}$	$C_{19}H_{23}O_{13}NS$	45.33	4.58	2.77	45.09	4.51	2.80	C
XXIX	126.5—128	90	-11^{14}	$C_{19}H_{25}O_{11}NS$	47.99	5.30	2.95	48.21	5.55	2.89	B
XXX	118 —119	72	-41.7^{18}	$C_{20}H_{23}O_9SCl$	50.58	4.88		50.75	4.83		E
XXXI	101 —103	55	$+26^{11}$	$C_{20}H_{25}O_9NS$	52.72	5.50	3.08	52.62	5.41	3.06	E
XXXIId)		92	$+34^{15}$	$C_{22}H_{27}O_{10}NS$							D
XXXIIId)		81	$+37^{18}$	$C_{27}H_{29}O_{10}NS$							F
XXXIVd)		91	$+11^{11}$	$C_{19}H_{25}O_{10}NS_2$							E
XXXVd)		62	-38.7^{18}	$C_{17}H_{21}O_7NS$							E
XXXVI	117	85	-51.5^{18}	$C_{19}H_{23}O_8NS$	53.65	5.45	3.29	52.99	5.54	3.25	D
XXXVII	117 —118	50	-43^{18}	$C_{24}H_{25}O_8NS$	59.13	5.17	2.87	59.61	5.14	2.87	G
XXXVIII	120 —121	65	-42.5^{18}	$C_{17}H_{19}O_7SCl$	50.30	4.72		50.57	4.69		E
XXXIX	124 —125	70	$+36^{16}$	$C_{20}H_{25}O_9NS$	52.74	5.53	3.08	52.37	5.61	2.98	H
XL	133	65	$+103$	$C_{25}H_{29}O_{10}NS_2$	52.91	5.15	2.47	52.75	4.89	2.40	H

a) All melting points were uncorrected.
b) All optical rotations were measured in 0.3% solution (chloroform).
c) see synthetic procedure
d) syrup

hydroxide (0.02 mole). The mixture was heated under reflux for 15 min. During the course of the reaction, the mixture became turbid and potassium bromide precipitated. The reaction mixture was poured into ice-water. The water layer was extracted with chloroform (50 ml). The combined chloroform layers were washed with 10% potassium hydroxide solution and water, and dried over sodium sulfate, then filtered. The filtrate was evaporated to give a syrup which crystallized from a little amount of ethanol. Recrystallization from ethanol gave compound XII, XVI, XX, XXIII, XXX, XXXI, XXXIV, XXXV, or XXXVIII, respectively. *p*-Aminophenyl 3,4,6-tri-O-acetyl-2-O-mesyl-1-thio-β-D-glucopyranoside (XLI) was also prepared to use the following procedure. This product is syrup.

Method F——To a stirred chilled solution of the compound XII, XVI, XX, XLI, or XXXI in pyridine was added dropwise mesyl chloride. The mixture was treated similarly as the method D. Recrystallization from ethanol gave compound XIV, XVIII, XXII, XXV, or XXXIII, respectively.

Method G——To a stirred chilled solution of the compound XII, XVI, XLI, or XXXV in pyridine was added dropwise benzoyl chloride. The mixture was treated similarly as the method D. Recrystallization from ethanol gave compound XV, XIX, XXVI, or XXXVII, respectively.

Method H——A solution of 6-iodo-6-deoxy-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose or 6-iodo-6-deoxy-1,3,4-tri-O-acetyl-2-O-tosyl-α-D-glucopyranose (0.01 mole) in chloroform (50 ml) was added to a solution of *o*-aminothiophenol (0.02 mole) in 50 ml of methanolic potassium hydroxide (0.02 mole), and the mixture was treated similarly as the method E. Recrystallization from ethanol gave compound XXXIX or XL, respectively.

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