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Studies on Iridoid-Related Compounds. IV. Antitumor Activity of Iridoid Aglycones

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An extensive study has been made on the antitumor activity of several iridoid glycosides and their aglycones in mice bearing the experimental tumor leukemia P388. Most of the aglycones were found to be active. Among them, scandoside methyl ester aglycone (SMEH) showed the most potent activity, and it was further tested against several kinds of experimental tumor. None of the glucosides showed any activity. Our observations suggest that the hemiacetal structure of iridoid aglycones plays a significant role in the antitumor activity, as in the case of the antimicrobial activity reported previously.

Keywords——antitumor activity; leukemia P388; iridoid glycoside; iridoid aglycone; scandoside methyl ester aglycone

Introduction

Iridoid glycosides have many pharmacological activities, such as antimicrobial, hypotensive, analgetic, antiphlogistic, sedative, laxative and various other effects.[1] Aucubin, an iridoid glucoside, isolated from *Aucuba japonica* THUNB., shows antimicrobial activity against *Staphylococcus aureus* in the presence of β-glucosidase (an effect equivalent to that of 600 I.U. of penicillin).[1,2] Our previous paper demonstrated that its unstable aglycone, aucubigenin, is the active form exhibiting antimicrobial activity. We also confirmed that the hemiacetal structure of aglycones is essential for the manifestation of antimicrobial activities, by testing 21 kinds of iridoid aglycones obtained by treatment with β-glucosidase.[3] These findings led us to investigate the antitumor activity of iridoid aglycones, since it has been suggested that there could be a relationship between antimicrobial and antitumor activities.

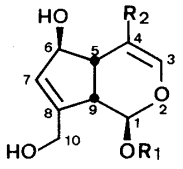
Here we wish to report on the antitumor activities of iridoid glucosides and their aglycones in mice bearing the experimental tumor leukemia P388. More detailed studies were carried out on the most active compound, scandoside methyl ester aglycone (SMEH).

Materials and Methods

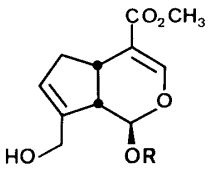
Isolation of Iridoid Glucosides——Aucubin (**1**) was isolated from *Aucuba japonica* THUNB, scandoside methyl ester (**2**) from *Hedyotis colymbosa* LAM., geniposide (**3**) from *Gardenia jasminoides* ELLIS, loganin (**4**) from *Cornus officinalis* SIEBOLD *et* ZUCCARINI, sweroside (**5**) from *Gentiana scabra* BUNGE, gardenoside (**6**) from *Gardenia jasminoides* ELLIS and gentiopicroside (**7**) from *Gentiana scabra* BUNGE. Each compound was confirmed to be identical with an authentic specimen.

Preparation of Aglycones——Each glucoside (**1**—**7**) was dissolved in H_2O. The solution was treated with 2% β-glucosidase (Miles) for about 2 h at 37 °C until the spot the of starting material had disappeared on thin layer

chromatography (TLC). The solution was then subjected to chromatography on a charcoal column using H_2O and EtOH as eluents. The EtOH eluate was concentrated and the residue was purified by chromatography on silica gel. The physical data for each aglycone (**8**—**14**) were consistent with the reported values.[3,4]



1 : R_1 = glc, R_2 = H
2 : R_1 = glc, R_2 = CO_2CH_3
8 : R_1 = H, R_2 = H
9 : R_1 = H, R_2 = CO_2CH_3



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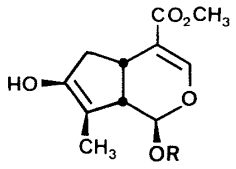

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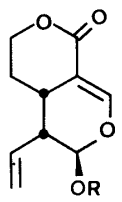

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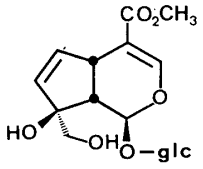

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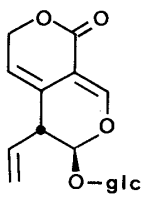

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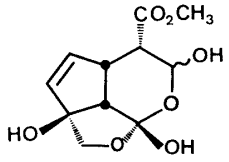

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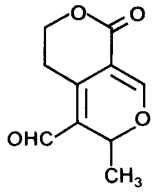

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Chart 1

Animal——Male BDF_1, ddY, BALB/C and DBA/2 mice (5 weeks old) were used in the present study. All mice were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animal (Hamamatsu, Shizuoka), and were given pellet diet CE-2 (CLEA Japan, Inc., Tokyo) and water *ad libitum*.

Test Compounds——Test compounds were suspended in 0.5% carboxymethylcellulose sodium salt (CMC, Wako Pure Chemical Industries Ltd., Osaka) and administered intraperitoneally at a dose of 0.1 ml/10 g body weight (chemical structures of the test compounds are shown in Table I).

Tumor——Sarcoma 180 and Ehrlich ascites carcinoma were maintained in ddY mice. Leukemia P388, leukemia L1210 and Meth A were maintained in DBA/2, BDF_1, and BALB/C mice, respectively, by intraperitoneal inoculation at weekly intervals.

Evaluation of Antitumor Activity——Antitumor activity was evaluated in terms of life prolongation. Leukemia P388 and L1210 were inoculated intraperitoneally into BDF_1 mice at 1×10^6 and 1×10^5 cells/mouse, respectively. Meth A was inoculated intraperitoneally into BALB/C mice at 1×10^6 cells/mouse. Sarcoma 180 and Ehrlich ascites carcinoma were inoculated intraperitoneally into ddY mice at 1×10^6 cells/mouse.

***In Vitro* Growth Inhibition Assay**——P388 and Meth A cells were cultured in RPMI-1640 supplemented with 10% calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) in a CO_2 incubator at 37 °C. The cells (1×10^5/ml) were cultured in multiwell plates (Falcon; 3047) for 48 h with a test compound at various concentrations. After the incubation, the viable cell number was determined by the trypan blue dye exclusion method.

Results

Antitumor Activities of Iridoid Glucosides and Their Aglycones

The antitumor activities of iridoid glucosides and aglycones toward leukemia P388 are shown in Table I. None of the glucosides had any activity, but almost all of the aglycones showed activity. Maximum total/control (T/C) values of the aglycones were: **8** 162%, **9** 160%, **10** 117% and **11** 129% (at 100 mg/kg), and **12** 134% (at 50 mg/kg). The artifactual aglycones **13** and **14** gave values of 122% at 100 mg/kg and 110% at 10 mg/kg, respectively.

The aglycones of aucubin (**8**) and of scandoside methyl ester (SMEH; **9**) both showed the most potent antitumor activities. We tested the effects of **9** on Ehrlich ascites carcinoma, Meth A, sarcoma 180 and L1210, since **9** was more stable than **8**.

TABLE I. Antitumor Activities of Iridoid Glucosides and Their Aglycones

Glucosides			Aglycones				
Compound	T/C%		Compound	T/C%			
	100	300 (mg/kg)		10	50	100	200 (mg/kg)
Aucubin (**1**)	103	100	**8**	103	117	130	147
						162	33
Scandoside methylester (**2**)	103	91	**9**	109	131	160	44
						158	53
Geniposide (**3**)	96	96	**10**	96	106	117	90
Loganin (**4**)	100	100	**11**	100	126	129	65
Sweroside (**5**)	103	97	**12**	109	134	44	33
Gardenoside (**6**)	106	103	**13**	97	122	122	122
Gentiopicroside (**7**)	108	116	**14**	110	55		

Tumor: P388 (1×10^6, i.p.). Administration: day 1, i.p. ($n=3$).

TABLE II. Antitumor Activity of SMEH on P388

Compound	Dose (mg/kg, i.p.)	Antitumor activity (T/C%)		
		Day 1	Day 1, 5, 9	Day 1—9
SMEH	0.5			108
SMEH	1.0			106
SMEH	2.5			110
SMEH	5.0		110	126
SMEH	10.0	109	124	126
SMEH	25.0	118	132	134
SMEH	50.0	131	136	82
SMEH	100.0	160	118	
SMEH	150.0	118	62	
5Fu	5			154
5Fu	10		132	188
5Fu	20		142	245
5Fu	50	141		
5Fu	100	147		

i.p. ($n=3$).

Activity of SMEH against Leukemia P388

The activity of SMEH against leukemia P388 was examined in three different treatment schedules, as shown in Table II. SMEH was showed a maximum T/C of 160% at the dose of 100 mg/kg, administered on day 1. Administration on days 1, 5 and 9 or on day 1 to 9 was less effective (leukemia P388 was implanted on day 0). Thus, the antitumor activity of SMEH against other tumor cells was examined with the schedule of one dose on day 1.

Antitumor Activities of SMEH against Leukemia L1210, Ehrlich Ascites Carcinoma, Meth A and Sarcoma 180

The antitumor activities of SMEH against leukemia L1210, Ehrlich ascites carcinoma, Meth A and sarcoma 180 were examined, and the results are shown in Tables III—V. As shown in Table III, SMEH was moderately active against L1210 leukemia and the maximum

TABLE III. Antitumor Activity of SMEH on L1210

Compound[a]	Dose (mg/kg, i.p.)	Av. life span (d) (Mean±S.E.)	T/C (%)	30-d survivors
Control[b]		9.0±0.2	100.0	0/5
SMEH	10	9.2±0.2	102.2	0/5
SMEH	25	9.4±0.2	104.4	0/5
SMEH	50	10.8±0.4	120.0	0/5
SMEH	75	12.2±0.8	135.6	0/5
SMEH	100	11.8±0.4	131.1	0/5
SMEH	150	8.8±1.9	97.8	0/5
SMEH	200	5.6±1.1	62.2	0/5

a) The compounds were administered intraperitoneally on day 1 after tumor inoculation ($n=5$). *b*) 0.5% CMC solution was used as the control.

TABLE IV. Antitumor Activities of SMEH and 5Fu on Ehrlich Ascites Carcinoma

Compound[a]	Dose (mg/kg, i.p.)	Av. life span (d) (Mean±S.E.)	T/C (%)	30-d survivors
Control[b]		11.2±1.5	100.0	0/5
SMEH	5	15.0±2.5	133.9	0/5
SMEH	10	17.8±3.4	158.9	1/5
SMEH	25	24.4±2.3[c]	217.9	1/5
SMEH	50	25.6±2.3[d]	228.6	2/5
SMEH	100	30.0±0.0[d]	267.9	5/5
Control		11.6±1.3	100.0	0/5
5Fu	50	17.0±3.3	146.6	1/5
5Fu	100	14.2±0.4	122.4	0/5

a) The compounds were administered intraperitoneally on day 1 after tumor inoculation ($n=5$). *b*) 0.5% CMC solution was used as the control. *c*) $p<0.01$, *d*) $p<0.001$, significantly different from the control.

TABLE V. Antitumor Activities of SMEH on Meth A and Sarcoma 180 (Ascites)

Compound[a]	Dose (mg/kg, i.p.)	Meth A		Sarcoma 180	
		Av. life span (Mean±S.E.)	T/C (%)	Av. life span (Mean±S.E.)	T/C (%)
Control[b]		11.0±0.3	100.0	14.2±1.6	100.0
SMEH	5	11.8±0.4	107.3	18.2±3.1	128.2
SMEH	10	12.2±0.6	110.9	15.4±2.5	108.5
SMEH	25	18.0±0.8[e]	163.9	26.8±1.9[e]	188.7
SMEH	50	20.0±1.3[d]	181.8	27.6±1.6[e]	194.4
SMEH	100	11.0±2.8	100.0	27.2±1.7[e]	191.5
Control		12.2±0.4	100.0	12.0±1.4	100.0
5Fu	50	14.8±0.7[c]	121.3	12.8±0.5	106.7
5Fu	100	15.0±0.0	123.0	23.5±3.9[c]	195.8

a) The compounds were administered intraperitoneally on day 1 after the tumor inoculation ($n=5$). *b*) 0.5% CMC solution was used as the control. *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$, significantly different from the control.

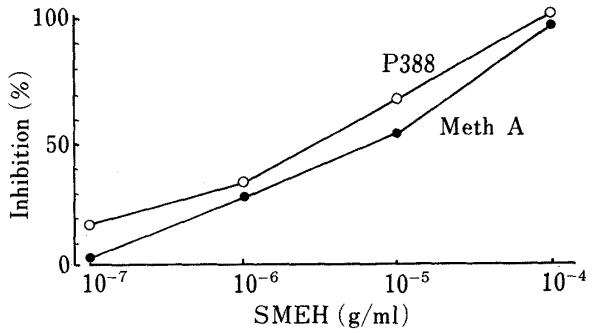

Fig. 1. Growth Inhibition of P388 and Meth A Cell Cultures by SMEH

T/C was 136% at 75 mg/kg. Antitumor activity of SMEH against Ehrlich ascites carcinoma is shown in Table IV. SMEH was remarkably active against Ehrlich ascites carcinoma in a dose-dependent manner at doses of 5 to 100 mg/kg, and all of the mice given 100 mg/kg of SMEH survived for more than 30 d. The effect of SMEH in this test was more potent than that of 5-fluorouracil (5Fu). Moreover, as shown in Table V, SMEH also showed marked antitumor activity against Meth A and Sarcoma 180 (ascites form), being more potent than 5Fu. At the optimal dosage, the lifespans of mice bearing ascitic Meth A and Sarcoma 180 were increased by 81.8 and 94.4%, respectively.

The activities of SMEH against P388 and Meth A cells *in vitro* were examined, and the results are shown in Fig. 1. SMEH inhibited the growth of P388 and Meth A cells *in vitro* in a dose-dependent manner at concentrations of 10^{-7}—10^{-4} g/ml.

Discussion

As shown in Table I, iridoid glucosides had no activity against mice bearing the experimental tumor leukemia P388, while most of the aglycones were active. Hemiacetal aglycones exhibited higher levels of antitumor activity than artifactual aglycones. It appears that hemiacetal aglycone structure is also important for the antitumor activity, as was found to be the case for antimicrobial activity. The aglycones of aucubin (**8**) and of scandoside methyl ester (SMEH; **9**) both showed highly potent antitumor activity, suggesting that the 6-hydroxyl group results in an increase of activity. SMEH was tested for activities against several experimental tumors since **8** was less stable. The activities of SMEH against Ehrlich ascites carcinoma, Meth A and Sarcoma 180 were more potent than those of 5Fu.

It is interesting that iridoid glucoside showing no activity themselves manifested antimicrobial or antitumor activity after enzymatic hydrolysis. Further studies on the antitumor activity of iridoid related compounds are in progress.

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