Anti-cancer Constituents of Liliaceae Plants

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Introduction

Liliaceac is one of the largest families of flowering plants, consisting of about 220-240 genera with 3500-4000 species. According to A. Englcr' s classification(1964)[1], it is divided into 13 subfamilies(Table I).

Table I. Subfamilies and main genera of Liliaceae by Engler(I964)

Alstromerioideae: *Alstromeria*
Allioideae: *Agapanthus, Allium, Brodiaea, Ipheion, Triteleia*
Asparagoideae: *Asparagus, Convallaria, Paris, Polygonatum, Rhodea, Ruscus, Trillium*
Asphodelideae: *Aloe, Chlorophytum, Hemerocallis, Hosta*
Aletroideae: *Aletris*
Lilioideae: *Erithronium, Fritillaria, Lilium, Nomocaris, Tulipa*
Herrerioideae: *Herreria*
Luzuriagoideae: *Luzuriaga*
Melanthioideae: *Narthecium, Tricyrtis, Veratrum, Zygadenum*
Ophiopogonioideae: *Liriope, Ophiopogon*
Scilloideae: *Camassia, Chionodoxa, Eucomis, Hyacinthus, Ornithogalum, Scilla, Urginea*
Smilacoldeae: *Smilax*
Wurmbaeoldeae: *Colchicum, Gloriosa*

Some Liliaceae plants are well known as important sources of medicines. For example, Rhizoma Anemarrhenae(rhizome of *Anemarrhena asphodeloides*), Radix Ophiopogonis (tuber of *Ophiopogon japonicus*) and Rhizoma Smilacis Glabrae(rhizome of *Smilax*

glabra) are listed in Japanese Pharmacopoeia. Sap of *Aloe* spp is used for a laxative, and colchicine, a medicine against gout, is obtained from *Colchicum autumnale.*

Liliaceae is a rich source of steroidal glycosides, and some plants, such as *Urginea scilla, Convallaria* spp., *Rhodea japonica,* etc. contain cardiac glycosides, but no systematic study of the chemical constituents of Liliaceae plants had been carried out until we started our research.

There are two procedures for isolating compounds with medicinal potential from plants (Chart 1). For crude drugs and medicinal plants possessing potent activity, the method indicated on the left in Chart 1 is recommended, but in the case of the plants whose medical properties are undocumented, the right-hand method is suitable. Using this method, about 500 new steroidal glycosides including cholestane glycosides and steroidal alkaloids were isolated from about 50 species of Liliaceae[2].

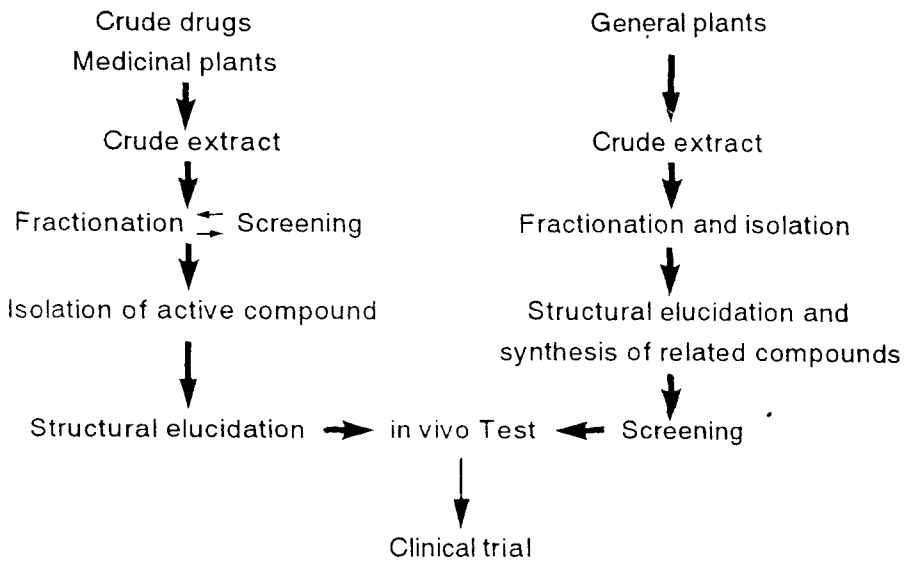


Chart 1. Procedure to identify naturally occurring compounds with medicinal potential

The biological activities of the compounds isolated were examined using several methods[2]. For anti-cancer activities, the following two tests were carried Out:

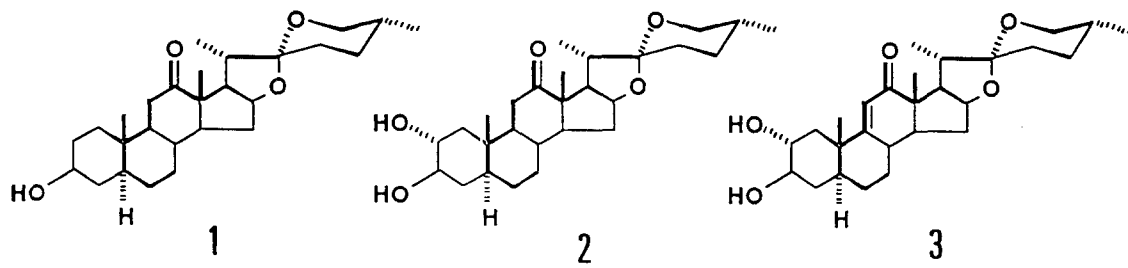
1. *in vitro* Anti tumor promoter activity test

2. Antitumor activity test:cytotoxicity towards HL-60 and MOLT-4 leukemia cells

1. *In vitro* Antitumor Promoter Activity

Some foods originating from Liliaceae plants, such as *Allium sativum*(garlic) and *A. wakegi* are effective in the prevention of malignant tumors. Sterol, laxogenin, was identified as the active compound[3]. *in vitro* Antitumor promoter test of the steroidal glycosides isolated by our studies was carried out, and the activity was estimated from the rate of incorporation of phospholipid into HeLa cells stimulated with TPA(12-O-tetradecanoylphorbol 13-acetate).

The steroids(1-3) isolated from *Hosta longipes* have a carbonyl group as well as the laxogenin. They showed relatively strong activities.



A sterroidal glycoside(4) bearing HMG methyl ester at C-27 hydroxyl position[4] isolated from the bulbs of *Lilium* spp. showed prominent activity which was ten times higher than that of the laxogenin. It also showed comparative strength in inhibiting the proliferation of several human malignant tum or cells[5] (Table II).

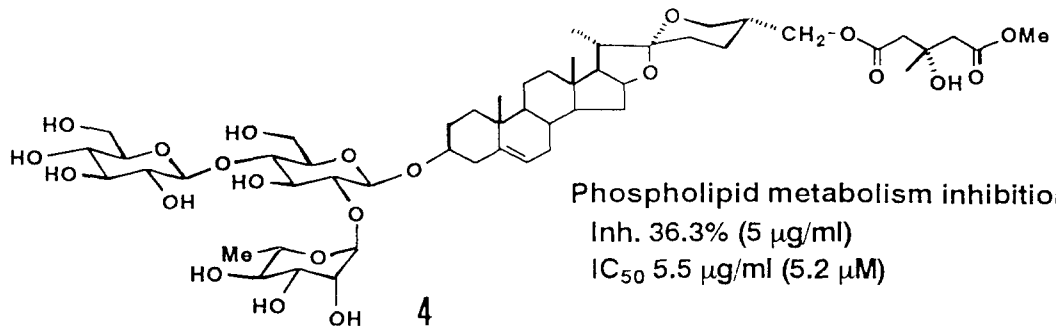


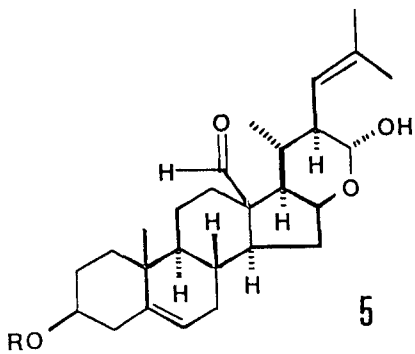
Table Ⅱ. Inhibition of human malignant tumor cell proliferation with 4

cells	Inhibition(%)
pancreatic cancer(PANC-1)	95.5
Osteosarcoma (OST)	72.2
Human gastric cancer(HGC-27)	70.8
Pheochromocytoma(PC-12)	24.8

4:IC_{50} 4μg/ml(3.8μM) Sample concentration:5μg/ml

2. Antitumor Activity

The series of cholestane glycosides isolated from *Ornithogalum saundersiae*, a bulbous plant native to South Africa, exhibited very strong cytostatic activity towards several malignant tumor cells. The activities of two rearranged cholestane glycosides(5[6], 6[7]) against human leukemia cells, HL-60 and MOLT-4 were almost equal or more potent than those of the clinically applied anticancer agents, etoposide, methotrexate, adriamycin(ADM) and vincristine[8](TabIe III).



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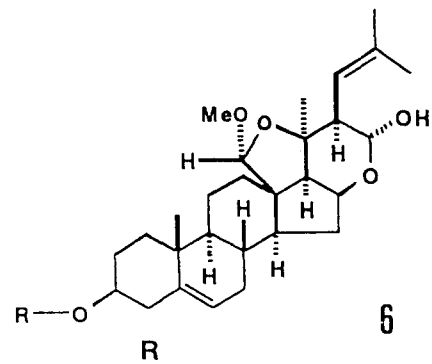

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4-*O*-*p*-methoxybenzoyl-Rha-(1→2)-Glc

Table Ⅲ. Cytostatic activities of 5 and 6 towards HL-60 and MOLT-4 cells

	HL-60	MOLT-4
5	0.0092	0.0024
6	0.025	0.018
etoposide	0.025	0.018
adriamycin	0.0072	0.035
vincristine	0.0029	0.00059
methotrexate	0.012	0.048

IC_{50} (μM)

The cytostalic activities of the cholestane glycosides partly resulted from the induction of apoptosis. The apoptosis is caused by the activation of calcium dependent endonuclease. An analysis of flow cytometry showed that glycosides stopped the cell cycle of HL-60 at S2/M phase and induced apoptosis at G0/G1phase[9].

The cholestane glycoside (7), the main constituent of the bulbs of *O. saundersiae*, showed a remarkably strong cytostatic activity, about 10-100 times stronger than those of mitomycin C(MMC), ADM, cisplatin(CDPP), camptpthecin(CPT) and taxol(TAX)[10](Table IV).

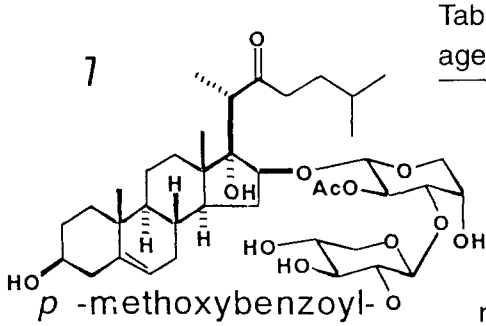


Table Ⅳ. Cytostatic activities of and some anticancer agents towards HL-60 and MOLT-4 cells

	HL-60	MOLT-4	
7	0.00025	0.00049	
etoposide	0.025	0.054	
adriamycin	0.0072	0.035	
vincristine	0.0029	0.00059	
methotrexate	0.012	0.048	IC_{50} (μM)

Table Ⅴ. Cytostatic activities of 7 and clinically applied anticancer anents on various malignant tumor cells

malignant cell	IC_{50} (μM)					
	7	MMC	ADM	CDDP	CPT	TAX
CCD-19Lu	1.5	2.0	2.0	10	2	2
P388	0.00013	0.01	0.003	0.05	0.005	0.01
P388/ADM	0.00077					
P388/CPT	0.00010					
FM3A	0.00016					
A-549	0.00068					
Lu-65	0.00020					
Lu-99	0.00020	0.01	0.002	0.001	0.001	0.002
RERF-LC-AI	0.00026					
CCRF-CEM	0.00016	0.02	0.01	0.005	0.005	0.001

CCD-CCD-19Lu(human normal pulmonary cell) P388(mouse leukemia)
P388/ADM(adriamycin-resistant P388) P388/CPT(camptothecin-resistant P388)
FM3A(mouse mastrocarcinoma) A-549(human pulmonary adenocarcinoma)
Lu-65(human pulmonary large cell carcinnoma) Lu-99(human pulmonary large cell carcinnoma)
RERF-LC-AI(human pulmonary squamous cell carcinoma)
CCRF-CEM(human leukemia)

It is worth noting that the activity of 7 towards tumor cells including carcinostatic-resistant cells is about 10,000 times more potent than that towards normal cells(CCD-19Lu)[10] (Table V).

The data of the 60 Cell Line Assay by National Cancer Institute (NCI) in America indicated that 7 has a wide anti tumor spectrum and is especially effective in proliferation of melanoma cells.

In *in vivo* test, 7 increased the life span of mice with P388 leukemia by 59% with one-time administration of 0.01mg/kg[10]. 7 also inhibited the proliferation of human hepatoma 134 transplanted in nude mice at 0.06mg/kg (iv)[11](Fig. 1).

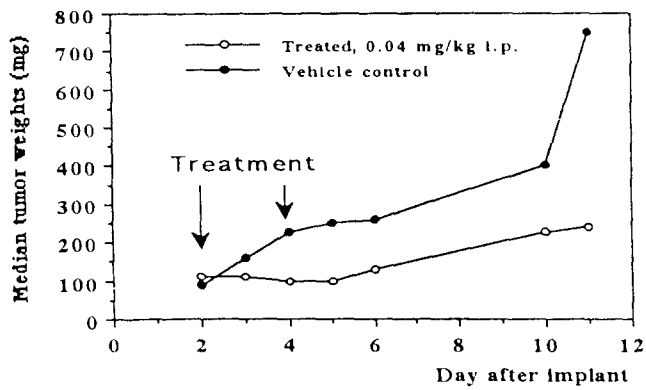
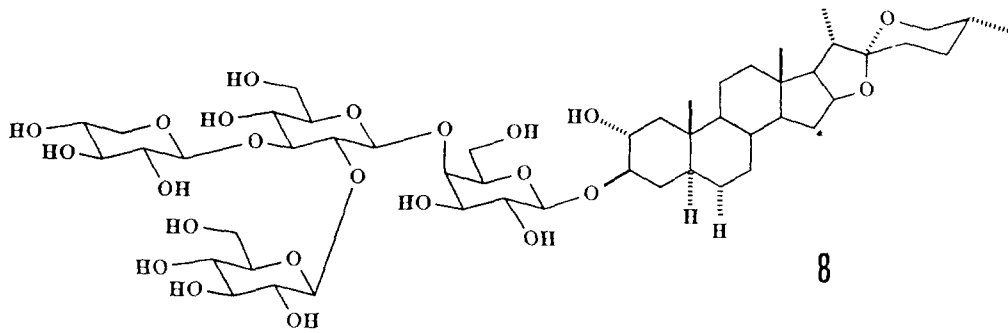


Fig. 1. Response of HEP 134 xenografts to 7

Thus, the compounds isolated from *Ornithogalum saundersiae* have extremely potent cytostatic activity, and some other steroidal glycosides isolated from Liliaceae plants also exhibited strong cytostatic activities against several malignant tumor cells. Recently a steroidal glycoside(8) was isolated from the bulb of *Allium jesdianum*. 8 exhibited considerable cytostatic activity toward HL-60 cells with an GI_{50} value of 1.5$\mu g/ml$ compared with etoposide used as positive control(GI50 0.3$\mu g/ml$). The evaluation of 8 by the National Cancer Institute 60 cell line assay showed that the mean concentrations required to achieve GI_{50}, TGI and LC_{50} levels against the panel of cells tested were 4.5μM, 18μM and 54μM, respectively, and some cell lines, such as leukemia CCRF-CEM, non-small



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cell lung cancer HOP-62 and breast cancer MCF7 were affected by 8[12].

The compounds isolated from plants have attracted special attention as sources for medicines. Our studies clarify that the steroidalglycosides isolated from Liliaceae plants are important compounds for of anti-cancer agents.

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Curriculum Vitae

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Education

Mar. 1963	Graduated from Tokyo College of Pharmacy(present name:TokyoUniversity of Pharmacy and Life Science)
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Mar. 1971	Completed the above School.
Mar. 1972	Obtained the degree of Ph.D. from the above School

Occupation

1963-1966, 1971-1972	Assistanl of Tokyo College of Pharmacy
1972-1977	Lecturer of the above College
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1985-1986	Visiting researcher of Departmcnt of Chemistry, University of British Columbia, Canada(prof. Kutney)
1989-prcsent	Professor of Tokyo College of Pharmacy(Tokyo University of Pharmacy and Life Scicnce)
1993-present	Councilor of the Medicinal and Pharmaccutical Society for WAKAN-YAKU
1998-present	Editor of the Journal of the Pharmaceutical Society of Japan
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Award

1988	Obtained the Prize from Isukura Foundation for the studies on Chinese traditional medicines