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ANTITUMOR AGENTS 171. CYTOTOXICITIES OF LOBATOSIDES B, C, D, AND E, CYCLIC BISDESMOSIDES ISOLATED FROM *ACTINOSTEMMA LOBATUM* MAXIM[1]

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Abstract: Cytotoxicities for lobatosides B–E and actinostemmosides B–F, isolated from *Actinostemma lobatum*, were evaluated against a panel of *ca* 60 human cancer cell lines. Lobatosides B–E (**1–4**) were cytotoxic ($GI_{50} \leq 1 \times 10^{-4}$ M) against all tumor cell lines; **4** showed potent cytotoxicity especially against A-549, SK-MEL-5, and SW-620 cell lines with GI_{50} values of 0.14, 0.14, and 0.36 μM, respectively.

Actinostemma lobatum MAXIM. (Cucurbitaceae) is a vine that grows from China to Japan. The herb has been traditionally used in China as a diuretic for treating nephrotic edema and as an antidote (applied externally) for poisonous snake bites.[2] In the course of our continuing search for novel antitumor agents from natural products, MeOH extracts of this herb were found to show significant ($ED_{50} < 20$ μg/mL) cytotoxicity.

Most plants belonging to the Cucurbitaceae family contain cucurbitacins and oleanane-type/dammarane-type triterpenes. Among these classes of compounds, cucurbitacins are well known cytotoxic triterpenes. In contrast, our group showed that *A. lobatum* accumulates triterpene saponins, actinostemmosides, and lobatosides, and does not contain cucurbitacins. This evidence prompted our cytotoxic evaluation of lobatosides B–E (**1–4**) and actinostemmosides B–F (**9–13**) against a panel of about 60 tumor cell lines.[3]

The saponins evaluated for cytotoxicity are shown in Figure 1 and are classified into three structural groups. Lobatosides B–E (**1–4**) are bayogenin bisdesmosides having a macrocyclic structure with a 3-hydroxy-3-methyl glutarate bridge between the two sugar moieties. This type of glycoside is called "cyclic bisdesmoside."[4] The actinostemmosides (**9-13**) were divided into two groups: actinostemmosides B–D (**9–11**) possessing a dammarane-type aglycone and actinostemmosides E (**12**) and F (**13**) with a baccharane-type aglycone.

Lobatosides B–E were cytotoxic against all human tumor cell lines tested; representative data are shown in Table 1. Lobatoside B (**1**) exhibited potent cytotoxicities with GI_{50} values ranging from 1.15 to 4.79 μM, but was not selective, which is reflected by the small Delta value (0.14). In contrast, lobatoside E (**4**) showed selective cytotoxicities against several tumor cell lines. The A549/ATCC (non-small cell lung cancer), SW-620 (colon cancer) and SK-MEL-5 (melanoma) cell lines (GI_{50} = 0.14, 0.36, and 0.14 μM, respectively) were especially sensitive to **4**, about ten times more sensitive than the other cell lines. Overall, **4** was more cytotoxic against melanoma cell lines than the other cell lines. Lobatosides C (**2**) and D (**3**) were less potent than **1** and **4**, though **2** and **3** were also relatively more selective to melanoma cell lines. The structures of **1**, **2**, **3**, and **4** are quite similar, except for the terminal sugar of the glycosyl moiety bonded to C-3. In **1** and **4**, this terminal sugar is a hexose

(glucose and galactose, respectively), while in **2** and **3**, it is arabinose. This observation suggested that the terminal hexose in the C-3 glycosyl moiety might be important to the potency of these compounds.

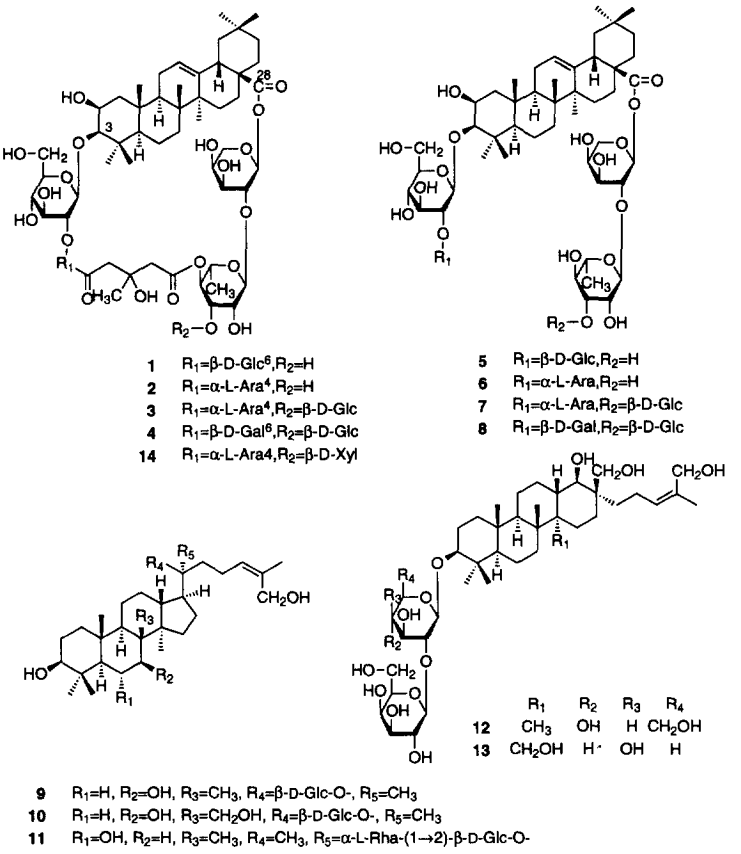

Figure 1

On the other hand, actinostemmosides C–F (**10–13**) were not cytotoxic ($GI_{50} > 1 \times 10^{-4}$M) against any cell line. Actinostemmoside B (**9**) exhibited potent or relatively potent cytotoxicities only against FXF-393 (renal cancer) and RPMI-8226 (leukemia) cell lines with GI_{50} values of 3.47 and 5.37 μM.

Lobatosides contain a cyclic bisdesmoside structure with a 3-hydroxy-3-methyl glutarate bridge, while actinostemmosides are simple triterpene glycosides. Thus, either the unique macrocyclic structure or the bisdesmoside structure is important for exhibiting potent cytotoxicity. To clarify which structural feature is essential, **1–4** were converted to simple triterpene bisdesmosides (**5–8**, respectively) by treatment with 0.5% KOH, and the cytotoxicity of compounds **4-8** against HeLa (cervical cancer) cells was evaluated. Compound **4** displayed cytotoxicity against HeLa cells, whereas **5–8** did not inhibit cell growth. (Figure 2) This result suggested that the macrocyclic structure as seen in compounds **1–4** plays an important role in the demonstration of potent cytotoxicity.

Previously, L. Yu et al. reported that tubeimoside I (**14**) showed potent antitumor activity by inhibiting DNA synthesis and induced phenotypic reverse transformation of tumor cells.[5] Since tubeimoside I and lobatosides both contain a cyclic bisdesmoside structure, their mechanism(s) of action also might be correlated. Therefore, the inhibitory activity of **1–4** against topoisomerase II was examined; this enzyme is an important enzyme for DNA replication, transcription, and chromosome segregation and is a target for antitumor agents. However, **1–4** failed to inhibit topoisomerase II. Although the inhibitory activity of **14** against topoisomerase II was not tested, the inhibition of DNA synthesis by **14** might not be related to inhibition of this enzyme.

In summary, our previous chemical investigation of *A. lobatum* showed that this plant contains a series of cyclic bisdesmosides, including lobatosides B–G and tubeimoside I. This class of compounds is considered to include the cytotoxic principles of *A. lobatum*.

Table 1. Cytotoxicity (GI_{50} in μM) of Compounds 1–4 against Representative Human Cancer Cell Lines In Vitro

	1	2	3	4
Disease Type and Cell Line				
Leukemia				
HL-60(TB)	1.41	1.78	1.82	1.45
Non-small Cell Lung Cancer				
A549/ATCC	1.32	2.24	3.89	0.14
Colon Cancer				
HCC-2998	1.32	2.40	2.45	1.41
SW-620	1.15	8.91	9.33	0.36
CNS Cancer				
SF-295	1.78	3.39	4.07	1.51
Melanoma				
SK-MEL-28	1.51	1.94	1.94	1.29
SK-MEL-5	1.20	4.17	11.4	0.14
Ovarian Cancer				
OVCAR-5	1.62	1.32	8.91	2.00
Renal Cancer				
UO-31	1.23	6.03	4.47	1.62
Prostate Cancer				
PC-3	1.38	2.19	7.94	1.95
MDA-MB-435	1.58	5.89	2.45	1.45
Mean GI_{50} (μM)	1.62	5.01	7.08	2.57
MG-MID[a]	–5.79	–5.30	–5.15	–5.59
Delta[b]	0.14	0.44	0.70	1.27
Range[c]	0.58	0.91	1.08	1.95

—: Not tested

[a]Calculated mean panel $\log_{10} GI_{50}$; compounds with $\log_{10} GI_{50}$ values < –4 are considered active.

[b]The number of log units by which the delta of the most sensitive line(s) of the panel differs from the corresponding MG-MID.

[c]The number of log units by which the delta of the most sensitive line(s) of the panel differs from the delta of the least sensitive line(s).

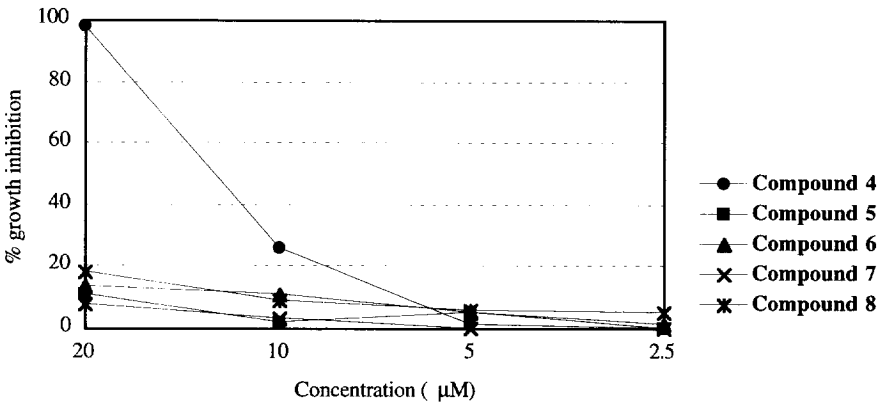

Figure 2. Dose Response Curves for Inhibitory Activities of 4–8 against HeLa Cell Growth.

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References and Notes

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3. **Cytotoxicity assay**: The in vitro cytotoxicity assay against a panel of human tumor cells was carried out in the National Cancer Institute, and the details of the assay procedures have been reported.[6,7] The in vitro cytotoxicity against HeLa cells was carried out according to a National Cancer Institute protocol.[8]
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