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Cytotoxic activity of gypenosides and gynogenin against non-small cell lung carcinoma A549 cells

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ABSTRACT

The activity of gypenosides and gynogenin of *Gynostemma pentaphyllum* against non-small cell lung carcinoma (NSCLC) A549 cells was investigated to identify the structural characteristics of gypenosides and gynogenin to have anti-NSCLC activity. Of the tested dammarane-type compounds, 20S-dammar-24-en-2α,3β,12β,20-tetrol showed the strongest activity against A549 cells. Based on the structure and cytotoxic activity relationships of gypenosides and gynogenin, the OH group in C-2, the connected sugar number and the configuration in C-20 were important for cytotoxic activity against A549 cells.

Lung cancer has been identified as the most common cancer and the leading cause of cancer-related mortality, with an estimated 1.61 million new cases and 1.38 million deaths worldwide in 2008.[1] According to the current histological lung cancer classification proposed by the WHO in 1981, lung cancers can be divided into two broad types: small cell lung carcinoma (SCLC), accounting for 20–25% of bronchogenic carcinomas, and non-small cell lung carcinoma (NSCLC), accounting for almost all of the remaining cases. NSCLC is further divided histologically into three main sub types: adenocarcinoma, squamous cell lung carcinoma and large cell lung carcinoma.[2] Despite recent improvements in surgery, radiation and medical treatments, the prognosis of lung cancer patients is generally poor, with less than 15% of patients surviving 5 years or more.[3] So NSCLC has become a key research project. A large number of studies confirm that many effective components of traditional Chinese medicine and folk medicine show good anti-tumor activity by regulating lung cancer cell signal transduction, inhibiting cell proliferation and promoting apoptosis.[4–6]

Folk drug *Gynostemma pentaphyllum* (Thunb.) Makino belongs to the Cucurbitaceae family. It contains many biologically active phytochemicals which has been demonstrated to be effective against chronic diseases, such as cancer.[7] *G. pentaphyllum* is a wild perennial liana commonly grown in Southern China, Japan, India and Korea. In China, it is also called 'Jiaogulan' and has been used in traditional medicine to treat bronchitis. The most abundant phytochemicals in *G. pentaphyllum* include saponins, flavonoids, carotenoids and chlorophylls,[8,9] all of which were considered to be responsible for the health-enhancing effect. As the dammarane-type saponins in *G. pentaphyllum* were reported structurally closely similar to ginseng saponins, *G. pentaphyllum* is also called 'southern ginseng' or 'cheaper ginseng'.[10]

The increase in cytotoxic activity against A549 cell of ethanol extract from heat processed and alkaline hydrolyzed *G. pentaphyllum* has been identified by us.[11] Their structures were changed by heat processing and alkaline hydrolyzing. Such structural change might be closely related to their cytotoxic activity. Previous studies of the structure–activity relationship between the ginsenosides and antitumor activity showed that the aglycones were more effective than the glycosides, and that the presence of sugar moieties reduced the antitumor activities.[12] Because of the similar structure with ginsenosides, we predicted that the gypenosides show the similar structure–activity relationship.

In the present study, five dammarane-type triterpenoids were isolated from 95% ethanol fraction of the raw, heat processed and alkaline hydrolyzed[13] *G. pentaphyllum* by chromatography, respectively. They were identified with ^{1}H-nuclear magnetic resonance (^{1}H NMR) spectrum, ^{13}C-nuclear magnetic resonance (^{13}C NMR) spectrum and liquid chromatography-ion trap time of flight tandem mass spectrometry (LCMS-IT-TOF). To evaluate the efficacy of these five constituents, the MTT cytotoxicity assay was performed using A549 cells.[11]

We have isolated five dammarane-type triterpenoids by resin HP-20, silica gel and reversed octadecyl silane (ODS) column chromatography and further purification with semi-preparative HPLC.

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Compound **1** was isolated as a white powder.[14] The negative ion ESI-MS of **1** displayed a molecular ion peak at *m*/*z* 961.5346 $[M-H]^-$. Combined with ^1H and ^{13}C NMR of **1**, its molecular formula was determined as $C_{48}H_{82}O_{19}$. The ^1H NMR (CD_3OD, 300 MHz) spectrum showed 8 methyl signals in the high-field region, with δ 0.92(6H, s), 0.97(3H, s), 1.00(3H, s), 1.12 (3H, s), 1.13(3H, s), 1.61(3H, s) and 1.68(3H, s). In addition, three sugar terminal proton signals δ 4.45(1H, d, J = 7.6 Hz), 4.60(1H, d, J = 7.8 Hz), 4.75(1H, d, J = 7.6 Hz) and one trisubstituted alkenyl proton signal δ 5.10 (1H, m) were also given. According to the sugar terminal proton coupling constant values (7.6, 7.6, 7.8 Hz), the configurations of three sugars could be identified as β-type (Table 1). The ^{13}C NMR (CD_3OD, 75 MHz) spectrum showed 48 carbon signals. Among them, δ 132.3, 125.9 showed the presence of two olefinic carbons, which were significantly similar to characterized C-24, C-25 olefinic carbon signals of dammarane-type triterpenoid saponins. Combined dammarane-type triterpene carbon spectrum rule with the carbon skeleton signal δ 96.7 (C-3), 68.1 (C-2), two hydroxyl groups could be speculated in C-3 and C-2 (Table 2). Moreover, the coupling constant (9.3 Hz) in C-2 showed the configuration is α-type. In comparison with the reported data,[15] the chemical structure of compound **1** was determined to be gypenoside XLVI as shown in Figure 1.

Compound **2** was isolated as a white powder.[14] The negative ion ESI-MS of **2** displayed a molecular ion peak at *m*/*z* 1093.5826 $[M-H]^-$. Combined with ^1H and ^{13}C NMR of **2**, its molecular formula was determined as $C_{53}H_{90}O_{23}$. The ^1H NMR spectrum also showed 8 methyl signals in the high-field region, with δ 0.92(6H, s), 0.98(3H, s), 1.00(3H, s), 1.12(3H, s), 1.36(3H, s), 1.62(3H, s) and 1.68(3H, s). In addition, four sugar terminal proton signals δ 4.29(1H, d, J = 7.3 Hz), 4.44(1H, d, J = 7.5 Hz), 4.56(1H, d, J = 7.8 Hz), 4.75(1H, d, J = 7.6 Hz) and one trisubstituted alkenyl proton signal δ 5.13 (1H, m) were also given. According to the sugar terminal proton coupling constant values (7.3, 7.5, 7.8, 7.6 Hz),

Table 1
^1H NMR chemical Shift values of active constituents from the raw, heat processed and alkaline hydrolyzed *G. pentaphyllum*

No.	Gypenoside XLVI	Gypenoside LVI	Gypenoside LI	Gypenoside L	20S-dammar-24-en-2α,3β,12β,20-tetrol
1	2.12 (1H, m)	2.13 (1H, m)	2.14 (1H, m)	2.13 (1H, m)	2.13 (1H, m)
	0.89 (1H, m)	0.88 (1H, m)	0.96 (1H, m)	0.95 (1H, m)	0.96 (1H, m)
2	3.75 (1H, m)	3.75 (1H, m)	3.76 (1H, m)	3.76 (1H, m)	3.76 (1H, m)
3	3.01 (1H, d, J = 9.3 Hz)	3.01 (1H, d, J = 9.3 Hz)	3.01 (1H, d, J = 9.3 Hz)	3.01 (1H, d, J = 9.3 Hz)	3.01 (1H, d, J = 9.3 Hz)
4					
5	0.86 (1H, m)	0.85 (1H, m)	0.86 (1H, m)	0.85 (1H, m)	0.85 (1H, m)
6	1.62 (1H, m), 1.58 (1H, m)	1.62 (1H, m), 1.57 (1H, m)	1.58 (1H, m), 1.56 (1H, m)	1.57 (1H, m), 1.53 (1H, m)	1.58 (1H, m), 1.53 (1H, m)
7	1.58 (1H, m), 1.31 (1H, m)	1.57 (1H, m), 1.31 (1H, m)	1.56 (1H, m), 1.36 (1H, m)	1.53 (1H, m), 1.33 (1H, m)	1.53 (1H, m), 1.33 (1H, m)
8					
9	1.55 (1H, m)	1.51 (1H, m)	1.52 (1H, m)	1.33 (1H, m)	1.34 (1H, m)
10					
11	1.65 (1H, m), 1.06 (1H, m)	1.65 (1H, m), 1.04 (1H, m)	1.89 (1H, m), 1.32(1H, m)	1.86 (1H, m), 1.32 (1H, m)	1.86 (1H, m), 1.32 (1H, m)
12	3.69 (1H, m)	3.74 (1H, m)	3.58 (1H, m)	3.58 (1H, m)	3.58 (1H, m)
13	1.74 (1H, m)	1.75 (1H,m)	1.75 (1H, m)	1.74 (1H, m)	1.75 (1H, m)
14					
15	1.88 (1H, m), 1.29 (1H, m)	1.85 (1H, m), 1.28 (1H, m)	1.56 (1H, m), 1.07 (1H, m)	1.53 (1H, m), 1.05 (1H, m)	1.53 (1H, m), 1.05 (1H, m)
16	1.93 (1H, m), 1.39 (1H, m)	1.93 (1H, m), 1.34 (1H, m)	1.86 (1H, m), 1.29 (1H, m)	1.86 (1H, m), 1.30 (1H, m)	1.86 (1H, m), 1.30 (1H, m)
17	2.30 (1H, m)	2.06 (1H, m)	2.06 (1H, m)	2.04 (1H, m)	2.04 (1H, m)
18	1.02 (3H, s)	1.00 (3H, s)	1.02 (3H, s)	1.00 (3H, s)	1.00 (3H, s)
19	0.98 (3H, s)	0.98 (3H, s)	0.99 (3H, s)	0.98 (3H, s)	0.97 (3H, s)
20					
21	1.34 (3H, s)	1.36 (3H, s)	1.11 (3H, s)	1.14 (3H, s)	1.13 (3H, s)
22	1.79 (1H, m), 1.65 (1H, m)	1.79 (1H, m), 1.65 (1H, m)	1.44 (1H, m), 1.42 (1H, m)	1.57 (1H, m), 1.36 (1H, m)	1.57 (1H, m), 1.36 (1H, m)
23	2.05 (2H, m)	2.09 (2H, m)	2.09 (1H, m), 2.06 (1H, m)	2.13 (1H, m), 1.98 (1H, m)	2.13 (1H, m), 1.98 (1H, m)
24	5.10 (1H, bt)	5.13 (1H, bt)	5.10 (1H, bt)	5.13 (1H, bt)	5.11 (1H, bt)
25					
26	1.68 (3H, s)	1.68 (3H, s)	1.67 (3H, s)	1.68 (3H, s)	1.68 (3H, s)
27	1.62 (3H, s)	1.62 (3H, s)	1.62 (3H, s)	1.61 (3H, s)	1.62 (3H, s)
28	0.92 (3H, s)	1.92 (3H, s)	1.13 (3H, s)	1.13 (3H, s)	1.12 (3H, s)
29	1.13 (3H, s)	0.12 (3H, s)	0.92 (3H, s)	0.92 (3H, s)	0.92 (3H, s)
30	0.93 (3H, s)	0.92 (3H, s)	0.92 (3H, s)	0.92 (3H, s)	0.92 (3H, s)
1′	4.45 (1H, d, J = 7.6 Hz)	4.44 (1H, d, J = 7.5 Hz)	4.45 (1H, d, J = 7.5 Hz)	4.45 (1H, d, J = 7.5 Hz)	
2′	3.69 (1H, m)	3.68 (1H, m)	3.70 (1H, m)	3.69 (1H, m)	
3′	3.26 (1H, m)	3.26 (1H, m)	3.26 (1H, m)	3.26 (1H, m)	
4′	3.21 (1H, m)	3.20 (1H, m)	3.20 (1H, m)	3.21 (1H, m)	
5′	3.38 (1H, m)	3.37 (1H, m)	3.37 (1H, m)	3.38 (1H, m)	
6′	3.84 (1H, m), 3.64 (1H, m)	3.84 (1H, m), 3.64 (1H, m)	3.84 (1H, m), 3.64 (1H, m)	3.84 (1H, m), 3.64 (1H, m)	
1″	4.75 (1H, d, J = 7.6 Hz)	4.75 (1H, d, J = 7.6 Hz)	4.75 (1H, d, J = 7.5 Hz)	4.75 (1H, d, J = 7.5 Hz)	
2″	3.23 (1H, m)	3.23 (1H,m)	3.23 (1H, m)	3.23 (1H, m)	
3″	3.62 (1H, m)	3.62 (1H, m)	3.62 (1H, m)	3.62 (1H, m)	
4″	3.36 (1H, m)	3.36 (1H, m)	3.36 (1H, m)	3.36 (1H, m)	
5″	3.38 (1H, m)	3.37 (1H, m)	3.37 (1H, m)	3.38 (1H, m)	
6″	3.87 (1H, m), 3.67 (1H, m)	3.88 (1H, m), 3.66 (1H, m)	3.88 (1H, m), 3.66 (1H, m)	3.87 (1H, m), 3.67 (1H, m)	
1‴	4.60 (1H, d, J = 7.8 Hz)	4.56 (1H, d, J = 7.8 Hz)			
2‴	3.08 (1H, m)	3.14 (1H, m)			
3‴	3.35 (1H, m)	3.32 (1H, m)			
4‴	3.31 (1H, m)	3.31 (1H, m)			
5‴	3.31 (1H, m)	3.38 (1H, m)			
6‴	3.80 (1H, m), 3.62 (1H, m)	4.00 (1H, m), 3.74 (1H, m)			
1⁗		4.29 (1H, d, J = 7.3 Hz)			
2⁗		3.19 (1H, m)			
3⁗		3.30 (1H, m)			
4⁗		3.45 (1H, m)			
5⁗		3.85 (1H, m), 3.18 (1H, m)			