ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5): 463 - 468
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Structure-activity relationship of tubeimosides in anti-inflammatory, antitumor, and antitumor-promoting effects

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KEY WORDS *Bolbostemma paniculatum*; tubeimosides; saponins; nonsteroidal anti-inflammatory agents; phytogenic antineoplastic agents; skin neoplasms; toxicity; topical administration; structure-activity relationship

ABSTRACT

AIM: To study structure-activity relationship of tubeimosides isolated from *Bolbostemma paniculatum* for their anti-inflammatory, antitumor, and antitumor-promoting effects. **METHODS**: Tubeimosides Ⅰ, Ⅱ, and Ⅲ were isolated from tubers of *Bolbostemma paniculatum* (Maxim) Franquet (Cucurbitaceae), a Chinese folk medicine, "Tubeimu", and their anti-inflammatory, antitumor, anti-tumorigenic activities, and acute toxicity were studied *in vivo*. **RESULTS**: Tubeimosides Ⅰ, Ⅱ, and Ⅲ are all natural analogues of oleanane type of triterpenoid saponins from the same medicinal plant, and all show anti-inflammatory, antitumor, and antitumor-promoting effects. However, the anti-inflammatory, anti-tumor, and anti-tumorigenic activities of tubeimoside Ⅱ are stronger than those of tubeimoside Ⅰ, and the acute toxicity of tubeimoside Ⅱ is lower than that of tubeimoside Ⅰ; the anti-inflammatory, anti-tumor, and anti-tumorigenic activities of tubeimoside Ⅲ are stronger than those of tubeimoside Ⅱ, and the acute toxicity of tubeimoside Ⅲ is also stronger than that of tubeimoside Ⅱ. **CONCLUSION**: C-16 hydroxyl group of tubeimoside Ⅱ plays an important role in enhancing biological activity of tubeimoside Ⅱ and in decreasing its toxicity. The difference of chemical structure in B and/or C position between tubeimosides Ⅲ and Ⅱ plays an important role in enhancing biological activity and toxicity of tubeimoside Ⅲ. Therefore tubeimosidre Ⅱ may be the most promising agent for cancer chemoprevention and chemotherapy among tubeimosides Ⅰ, Ⅱ, and Ⅲ.

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Received 2000-09-18 Accepted 2001-02-08

INTRODUCTION

The tuber of *Bolbostemma paniculatum* (Maxim) Franquet (Cucurbitaceae), a traditional Chinese folk medicinal plant, was listed in the Supplement to the Compendium of Materia Medica, compiled in the Qing Dynasty[1]. Tubeimosides Ⅰ, Ⅱ, and Ⅲ are all natural analogous oleanane type of triterpennoid saponins from this plant(Fig 1). As tubeimosides Ⅰ and Ⅲ have been shown to have anti-inflammatory, antitumor, and antitumor-promoting effects[2-5], and the differences between tubeimoside Ⅰ and Ⅲ have been demonstrated[5], it is of great interest to study whether tubeimoside Ⅱ, the natural analog of tubeimosides Ⅰ and Ⅲ, has the same effects, and the structure-activity relationship of tubeimosides Ⅰ, Ⅱ, and Ⅲ in their anti-inflammatory, antitumor, and antitumor-promoting effects.

MATERIALS AND METHODS

Plant materials The plant was collected at Shaanxi Province (China) and identified by Prof QUAN Y (Department of Botany, Shaanxi Provincial Academy of Traditional Chinese Medicine and Pharmacology, China). A voucher specimen (№ 097) has been deposited in the Department of Marine Pharmacy, Institute of Marine Biology, Zhanjiang Ocean University, Zhanjiang 524025, China.

Isolation of tubeimosides Ⅰ, Ⅱ, and Ⅲ Tubeimosides Ⅰ, Ⅱ, and Ⅲ were isolated from the tuber of *Bolbostemma paniculatum* by a modification of the method reported previously[2,6,7]. Their chemical structure was established by Kong *et al*[8] and Kasai *et al*[7]. Tubeimosides Ⅰ, Ⅱ, and Ⅲ are all saponins having a

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
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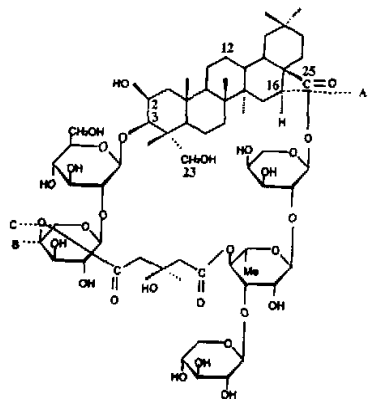
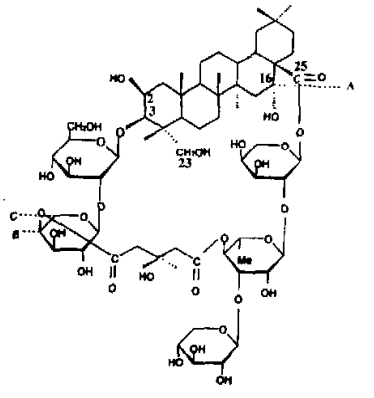
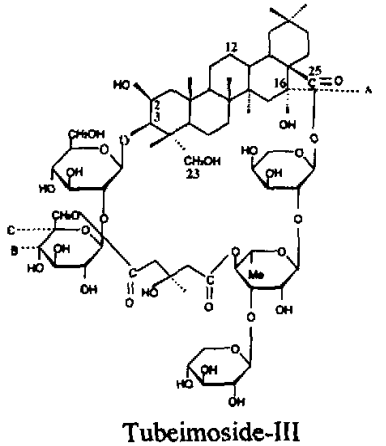


Tubeimoside-I



Tubeimoside-II



Tubeimoside-III

Structure of tubeimosides Ⅰ, Ⅱ, and Ⅲ

novel cyclic structure with a 3-hydroxy-3-methylglutarate bridge and the name "cyclic bisdesmoside" was proposed for saponins of this type. Tubeimosides Ⅰ and Ⅱ (purity > 98.5%) were dissolved in distilled water, and tubeimoside Ⅲ (purity > 99%) was initially dissolved in dimethylsulfoxide (Me_2SO).

Chemicals 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was obtained from Pharmacia (Uppsala, Sweden). 7,12-Dimethylbenz[α]anthracene (DMBA) was purchased from Wako (Osaka, Japan). All other chemicals used were of analytical grade.

Animals and tumor ICR and inbred BALB/c mice, aged about 8 weeks, both male and female, were purchased from Shizuoka Laboratory Animal Center (Japan) and the Animal Laboratory Center in Zhongshan Medical University and Guangdong Medical College (China). Transplantable mouse sarcoma 180(S_{180}) was maintained by weekly passage in inbred BALB/c mice.

Anti-inflammatory experiments Male ICR mice were treated with TPA (2 μg in 20 μL acetone) on both surfaces of each ear. The test compound dissolved in acetone was applied topically 30 min before the irritant treatment. Control mice received the vehicle alone. Ear edema, expressed in terms of increase in ear thickness, was measured with a thickness gauge 5 h after TPA treatment. Under these experimental conditions application of TPA resulted in a maximum swelling 5 h after the treatment.

***In vivo* antitumor test** Groups of BALB/c mice were inoculated subcutaneously in the left groin on d 0. Each test component was injected im on the days indicated in Tab 2. Control mice inoculated with tumor cells were given im the same amount of the vehicle at the same time. Tumors developed within a few days after inoculation. Mortality was recorded each day. Twenty days after tumor inoculation, all the tumors were removed, and the tumor weight was determined and the inhibition rate (%) was calculated.

Mouse skin two-stage carcinogenesis The backs of female ICR mice at 7 weeks of age were shaved with electric clippers 2 d before the start of the experiment. Initiation was carried out with a single topical application of 100 μg of DMBA in acetone (100 μL) on the shaved area. Promotion of 1 μg of TPA in acetone (100 μL) twice a week by topical painting was started in 1st week after initiation. Tubeimoside Ⅱ was applied topically (0.5 mg per painting applied simultaneously with TPA) or given *po* (dissolved in drinking water at a concentration of 0.1 g/L) *ad libitum* throughout the promoting stage.

Evaluation of toxicity ICR mice (n = 20)

were used for acute toxicity testing (intraperitoneal route). Mortality was recorded each day during 1 week. The data were calculated according to the Bliss' method.

RESULTS

Anti-inflammatory activity of tubeimosides As shown in Tab 1, tubeimosides Ⅰ, Ⅱ, and Ⅲ greatly inhibited TPA-induced ear edema in a dose-dependent manner, but anti-inflammatory activity of tubeimosides Ⅱ, and Ⅲ was much stronger than that of tubeimoside Ⅰ, and anti-inflammatory activity of tubeimoside Ⅲ (0.037 μmol per ear) was stronger than that of tubeimoside Ⅱ ($P<0.01$). It is noteworthy that no difference of anti-inflammatory activity between tubeimosides Ⅱ and Ⅲ was observed at the dose of 0.0075, 0.075, and 0.11 μmol per ear.

Tab 1. Inhibition of tubeimosides Ⅰ, Ⅱ, and Ⅲ applied topically on TPA-induced mouse ear edema. $n=6$. $\bar{x}\pm s$. Average ear thickness of untreated mice was 0.19 mm. Tubeimosides or vehicles alone without irritants caused no change in ear thickness. $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* tubeimoside Ⅰ; $^gP>0.05$, $^hP<0.05$ *vs* tubeimoside Ⅱ.

Conditions	$10^{-2}\times$ Increase in ear thickness/mm	Inhibition/%
Control (TPA 2 μg per ear)	10.6±0.8	
+ tubeimoside Ⅰ/μmol per ear		
0.0075	7.8±0.5^b	26.4
0.037	6.8±0.5^c	35.8
0.075	5.6±0.6^c	47.2
0.11	1.80±0.22^c	83.0
+ tubeimoside Ⅱ/μmol per ear		
0.0075	6.8±0.5^{cd}	37.7
0.037	4.6±0.4^{cf}	56.6
0.075	1.6±0.3^{cf}	84.9
0.11	0.00±0.23^{ce}	100.0
+ tubeimoside Ⅲ/μmol per ear		
0.0075	6.0±0.4^{cfg}	41.5
0.037	1.8±0.4^{cfh}	83.0
0.075	1.6±0.5^{cfg}	84.9
0.11	0.60±0.29^{cfg}	92.5

Antitumor activity of tubeimosides As shown in Tab 2, tubeimosides Ⅰ, Ⅱ, and Ⅲ greatly inhibited the growth of transplantable mouse S_{180} in a dose-dependent manner, but anti-tumor activity of tubeimosides Ⅱ and Ⅲ was stronger than that of tubeimoside Ⅰ. Furthermore, antitumor activity of tubeimoside Ⅲ was stronger than that of tubeimoside Ⅱ at the dose of 12 $mg\cdot kg^{-1}\cdot d^{-1}$ for 2 and 3 d.

Antitumor-promoting activity of tubeimosides Fig 1 illustrates the time course of skin tumor formation in the groups of mice treated with DMBA and TPA, with or without topical application of tubeimoside Ⅰ (1.0 mg per painting), Ⅱ (0.5 mg per painting), or Ⅲ (0.5 mg per painting), applied simultaneously with TPA. In the group treated with DMBA and TPA, the first tumor appeared in 6th week; at the end of the experiment (the 18th week) the percentage of tumor-

Tab 2. Anti-tumor activity of tubeimosides Ⅰ, Ⅱ, and Ⅲ on transplantable mouse S_{180} in BALB/c mice. $n=10$ mice. $\bar{x}\pm s$. Inhibition rate = (1 - A/B) × 100 %, where A is the average weight of tumors in the test group, and B is the average weight of tumors in the control group and no animal died throughout the experimental course.

Treatment	Dose/ $d\times mg\cdot kg^{-1}\cdot d^{-1}$	Average body weight/g Before	After	Tumor weight/g	Inhibition/%	P
Control		18.0±1.0	19.6±1.7	2.7±0.8		
5-Fluorouracil	3×12	18.6±0.8	19±3	2.0±0.6	27.2	<0.05
Tubeimoside Ⅰ	1×12	18.5±0.6	20.0±1.9	2.1±0.6	23.3	<0.05
Tubeimoside Ⅰ	2×12	18.5±0.6	19.3±1.8	1.85±0.17	31.3	<0.05
Tubeimoside Ⅰ	3×12	18.7±1.3	18.1±1.7	1.3±1.3	52.9	<0.01
Tubeimoside Ⅱ	1×12	18.4±0.4	20.2±1.4	1.5±0.4	43.3	<0.01
Tubeimoside Ⅱ	2×12	18.5±0.6	19.2±1.6	1.35±0.29	49.8	<0.01
Tubeimoside Ⅱ	3×12	18.5±0.7	18.0±1.5	1.07±0.17	60.1	<0.01
Tubeimoside Ⅲ	1×12	18.5±1.0	19.8±2.2	1.71±0.26	36.0	<0.05
Tubeimoside Ⅲ	2×12	18.6±0.6	17.3±1.7	0.9±0.3	65.6	<0.01
Tubeimoside Ⅲ	3×12	18.4±0.7	17.3±1.6	0.73±0.17	72.8	<0.01

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
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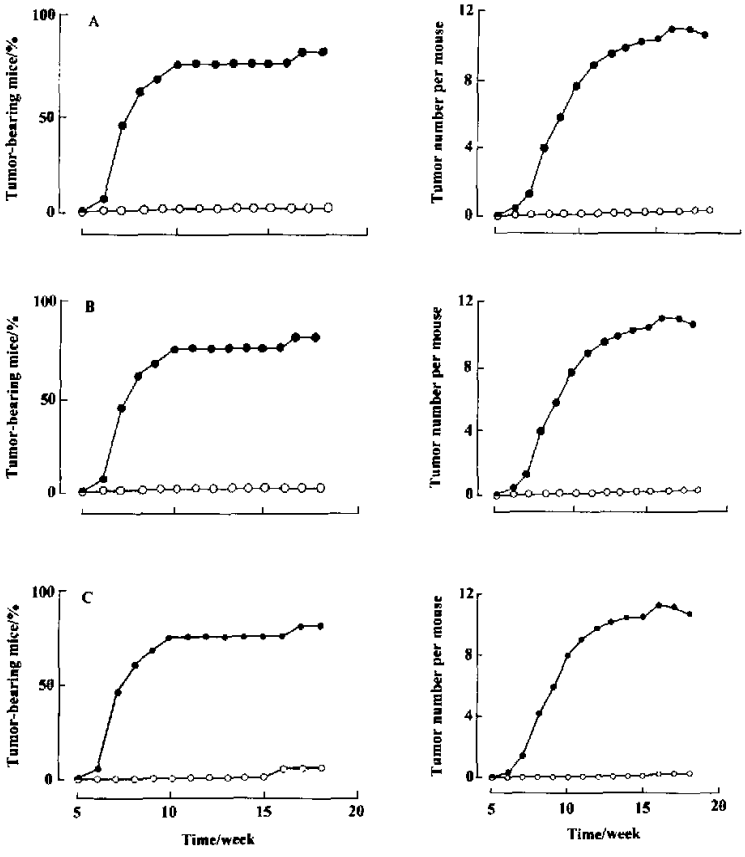

Fig 1. Effect of topically applied tubeimosides on the TPA-induced skin tumor formation in DMBA-initiated mice. Initiation was accomplished by a single topical application of DMBA (100 μg). For tumor induction, TPA (1 μg per painting) was applied twice a week from 1st week after the initiation. Tubeimoside Ⅰ 1 mg per painting (A), tubeimoside Ⅱ 0.5 mg per painting (B), tubeimoside Ⅲ 0.5 mg per painting (C), or its vehicle was applied topically simultaneously with TPA. The incidence and number of tumors were examined weekly. ●—●, DMBA plus TPA; ○—○, DMBA plus TPA and tubeimoside Ⅰ, Ⅱ or Ⅲ (n=15).

bearing mice was 80.0 %, and the average number of tumors was 10.3 per mouse. In contrast, no tumors appeared in the group treated with DMBA and TPA plus tubeimoside Ⅰ or Ⅱ, meaning that application of tubeimoside Ⅰ (1.0 mg per painting) or Ⅱ (0.5 mg per painting) completely inhibited the formation of tumors up to the 18th week, and only one tumor appeared in the group treated with DMBA and TPA plus tubeimoside Ⅲ, showing that application of tubeimoside Ⅲ (0.5 mg per painting) almost completely inhibited the formation of tumors up to the 18th week. Furthermore, we observed that tubeimoside Ⅰ or Ⅱ-treated mice had a smooth skin, while those treated with DMBA and TPA or with DMBA and TPA plus tubeimoside Ⅲ had multiple inflammatory foci. Body-weight measurement during the experiment showed that tubeimoside Ⅰ or Ⅱ did not cause any growth retardation, but rather improved the growing rate of weight which was suppressed by the application of tumor promoter (data not shown). However, tubeimoside Ⅲ caused growth retardation to some measure (data not shown).

Fig 2 shows the time-course of skin tumor formation in the groups with or without oral administration of tubeimoside Ⅰ (0.2 g/L), Ⅱ (0.1 g/L), or Ⅲ (0.1 g/L), dissolved in drinking water, *ad libitum*, throughout the promotion stage. In a preliminary experiment, it

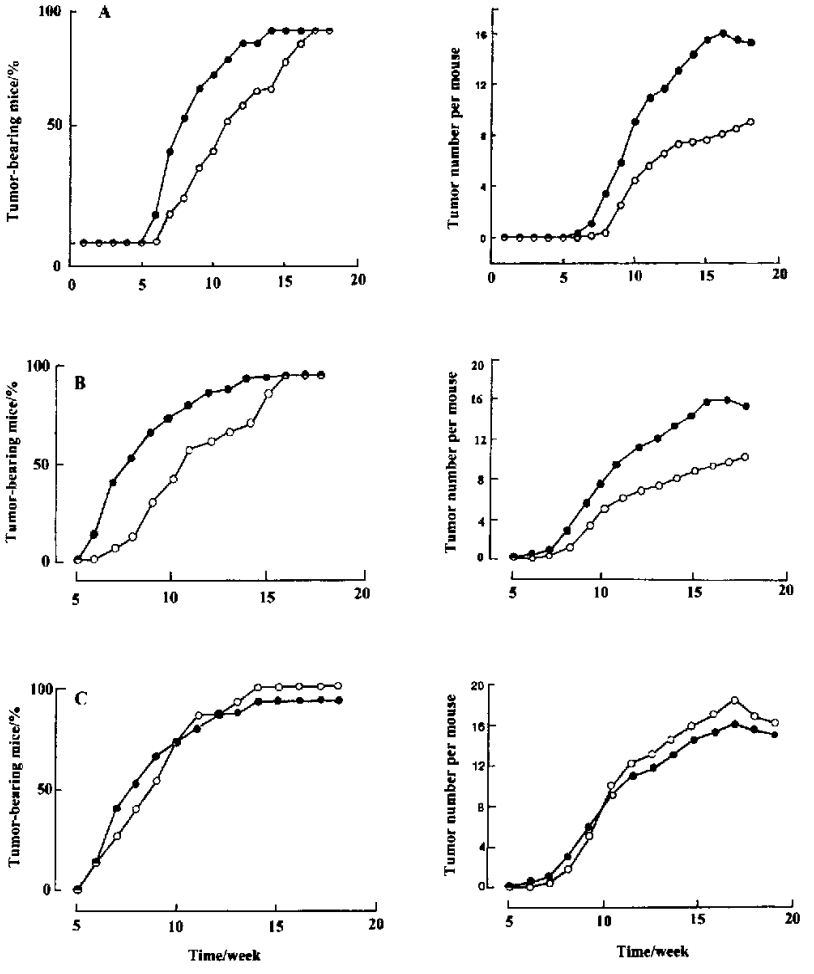

Fig 2. Effect of orally administered tubeimosides on the TPA-induced promotion of skin tumor formation in DMBA-initiated mice. Tubeimoside Ⅰ 0.2 g/L (A), tubeimoside Ⅱ 0.1 g/L (B), and tubeimoside Ⅲ 0.1 g/L (C) was administered *po* (dissolved in drinking water, *ad libitum*, throughout the stage of tumor promotion). ●—●, DMBA plus TPA, ○—○, DMBA plus TPA and tubeimoside Ⅰ, Ⅱ, or Ⅲ ($n=15$).

was seen that tubeimosides Ⅰ and Ⅱ had no acute toxic effects, under these experimental conditions. In the 18th week, the average number of tumors per mouse in the control group was 15.5, while in the group treated with tubeimoside Ⅰ or Ⅱ, it was 10.0, in the group treated with tubeimoside Ⅲ, it was 17.0 ($P>0.05$). The percentage of tumor-bearing mice was the same in the control and tubeimoside Ⅰ or Ⅱ-treated groups (93.3 %), except in tubeimoside Ⅲ-treated group (100.0 %) (Fig 2).

Acute toxicity of tubeimosides The acute LD_{50} values of tubeimosides Ⅰ, Ⅱ, and Ⅲ in ICR mice are (18.7±2.8), (21±3), (15±5) mg/kg, respectively.

DISCUSSION

We have demonstrated previously that tubeimoside Ⅰ has potent anti-inflammatory, anti-tumor, and anti-tumorigenic activities[3,4]. The experimental results presented here indicate that the anti-inflammatory, anti-tumor, and anti-tumorigenic activities of tubeimoside Ⅱ are

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
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stronger than those of tubeimoside Ⅰ, and the acute toxicity of tubeimoside Ⅱ is lower than that of tubeimoside Ⅰ. It is evident that C-16 hydroxyl group of tubeimoside Ⅱ plays an important role in enhancing biological activity of tubeimoside Ⅱ and in decreasing its toxicity, because that is the only difference in the chemical structure between tubeimoside Ⅰ and tubeimoside Ⅱ (Fig 1).

We have also demonstrated that the anti-inflammatory and anti-tumorigenic activities of tubeimoside Ⅲ are much stronger than those of tubeimoside Ⅰ[5]. The experimental results presented here indicate that the anti-inflammatory and anti-tumor activities of tubeimoside Ⅲ are stronger than those of tubeimoside Ⅱ, and the acute toxicity of tubeimoside Ⅲ is also stronger than that of tubeimoside Ⅱ to some degree. The unexpected finding is the non-activity of tubeimoside Ⅲ as an inhibitor of tumor promotion if administered orally. It is evident that the difference in chemical structure at B and/or C position between tubeimosides Ⅲ and Ⅱ plays an important role in enhancing biological activity and toxicity of tubeimoside Ⅲ.

The above-mentioned experimental results suggest that tubeimoside Ⅱ may be be the most promising agent for cancer chemoprevention and chemotherapy among tubeimosides Ⅰ, Ⅱ, and Ⅲ.

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土贝母皂苷抗炎、抗肿瘤和抗促癌的构效关系

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关键词 土贝母; 土贝母苷类; 皂苷类; 非甾类消炎药; 植物性抗肿瘤药; 皮肤肿瘤; 毒性; 局部投药; 构效关系

目的: 研究土贝母苷Ⅰ、Ⅱ和Ⅲ的抗炎、抗瘤和抗促瘤效果的构效关系. **方法**: 从中药土贝母中分离土贝母苷Ⅰ、Ⅱ和Ⅲ, 并研究它们的抗炎、抗瘤和抗促瘤活性及急性毒性. **结果**: 土贝母苷Ⅰ、Ⅱ和Ⅲ是来自同一植物的齐墩果烷型三萜皂苷天然类似物. 实验证实, 它们都具有抗炎、抗瘤和抗促瘤效果. 然而, 土贝母苷Ⅱ的抗炎、抗瘤和抗促瘤效果比土贝母苷Ⅰ强, 其急性毒性却比土贝母苷Ⅰ低; 土贝母苷Ⅲ的抗炎、抗瘤和抗促瘤效果比土贝母苷Ⅱ高, 其急性毒性也比土贝母苷Ⅱ强. **结论**: 土贝母苷Ⅱ16位碳上的羟基对于增强土贝母苷Ⅱ的生物学活性和降低它的毒性具有重要意义; 土贝母苷Ⅲ和土贝母苷Ⅱ在B和/或C位上化学结构的差异对于增强土贝母苷Ⅲ的生物活性和毒性起着重要作用.

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