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Research Paper

Structural requirement of spirostanol glycosides for rat uterine contractility and mode of their synergism

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Abstract

Objectives Total steroidal saponins extracted from the rhizome of *Paris polyphylla* (TSSP) have been used in China for the treatment of abnormal uterine bleeding. The aim of this study was to analyse the structure–activity relationship of steroidal saponins purified from *P. polyphylla* Sm. var. *yunnanensis* on rat myometrial contractions, and investigate the synergism among themselves as well as with known inherent agonists, such as Prostaglandin $F_{2\alpha}$ (PGF-2α).

Methods In this study, 22 steroidal saponins purified from TSSP were screened for their contractile activity in isolated uterine strips from estrogen-primed rats.

Key findings It was shown that spirostanol glycosides exhibited inducible or inhibitory activity in rat uterine contraction based on the difference of their structures, which was not only attributed in part to the number, the length and the position of sugar side chains attached by a glycoside, but also related to the structure of the aglycone. Furthermore, synergistic actions were observed among pennogenin or diosgenin glycosides as well as with the known inherent agonist PGF-2α, indicating they may share, at least in part, similar pathways with PGF-2α in stimulating myometrial contractions. Finally, the contractile response of rat myometrium to spirostanol glycosides was significantly enhanced with advancing pregnancy.

Conclusions Together, these data support the possibility that some spirostanol glycosides may represent a new type of contractile agonist for the uterus and their synergism may be responsible for the therapeutic effect of TSSP on abnormal uterine bleeding.

Keywords abnormal uterine bleeding; myometrial contractility; spirostanol glycoside; structure–activity relationship

Introduction

Abnormal uterine bleeding is one of the most common disorders encountered by the gynaecologist. About one-third of gynaecological consultations are carried out for abnormal uterine bleeding and this ratio rises to 70% in women at the premenopause and postmenopause.[1] Abnormal uterine bleeding includes both dysfunctional uterine bleeding, which can be anovulatory or ovulatory, and bleeding due to structural causes, including fibroids, polyps, endometrial carcinoma and pregnancy complications.[2] In addition, abnormal uterine bleeding can also result from contraception.[3] In view of the high morbidity, treatment of abnormal uterine bleeding is quite important for women's healthcare. Several drugs have been demonstrated to decrease menstrual bleeding in patients with abnormal uterine bleeding, such as progestins, combinations of estrogen and progestin, prostaglandin synthetase inhibitors and plasminogen inhibitors, such as tranexamic acid. However, side effects often make them unsuitable for long-term use.[4,5]

GongXueNing (GXN), the total steroidal saponins extracted from the rhizome of *Paris polyphylla* Sm. var. *yunnanensis* (TSSP), ceased or remarkably reduced the amount of haemorrhage by approximately 95% in treatment of 300 cases of abnormal uterine bleeding, including 122 cases of dysfunctional uterine bleeding, 103 cases of menorrhagia and 75 cases of other causes.[6] Because of its cheapness, convenience and low incidence of

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side effects, GXN has been widely used in China for the treatment of abnormal uterine bleeding. In our previous studies, it was found that TSSP dose-dependently induced phasic myometrial contractions *in vitro,* and experiments with calcium channel blockers or kinase inhibitors demonstrated that TSSP-stimulated myometrial contraction was mediated by an increase in $[Ca^{2+}]_i$ via influx of extracellular calcium and release of intracellular calcium. It was then proposed that the strengthening of the uterine contraction by TSSP may be responsible for the therapeutic effect of GXN in abnormal uterine bleeding. Through bioassay-guided separation, we have identified pennogenin-3-*O*-α-L-arabinofuranosyl (1→4) [α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (PARG) as the active ingredient of TSSP in stimulating myometrial contractions.[7]

Steroidal saponins have been considered as the main bioactive components of many famous Chinese medicines, including *P. polyphylla* Sm. var. *yunnanensis*, possessing a broad range of biological and pharmacological properties, such as hypocholesterolaemic, anti-tumour, antidiabetic, anti-inflammatory, antifungal and platelet agonistic and inhibitory activity.[8–10] These compounds are classified as spirostanol glycosides with a sugar chain at C_3 position, and furostanol saponins with two sugar chains at both C_3 and C_{26} positions, respectively.[8,9] The uterine contractility of steroidal saponins has not been reported except in our previous study. Therefore, the aim of this study was to analyse the structure–activity relationship of steroidal saponins purified from *P. polyphylla* Sm. var. *yunnanensis* on rat myometrial contractions, and investigate the synergism among themselves as well as with the known inherent agonists, such as prostaglandin $F_{2\alpha}$ (PGF-2α).

Materials and Methods

Chemicals

Dried rhizomes of *P. polyphylla* Smith var. *yunnanensis* were obtained from the local farms in China. PGF-2α and estrostilben were obtained from Beijing Yimen Co. Ltd (Beijing, China) and dissolved in 0.9% sodium chloride. Stock solutions were prepared in dimethyl sulfoxide (DMSO). All drugs were added to the bath in volumes of 5 μl.

Purification of steroidal saponins from *Paris polyphylla* Sm. var. *yunnanensis*

The dried rhizome of *P. yunnanensis* was collected from the Lijiang region of the Yunnan Province, People's Republic of China, in November 2004. The plant was identified by Prof. Jian-Mei Huang (Beijing University of Traditional Chinese Medicine), and a voucher specimen (No. 041120) was deposited in the Herbarium of the Beijing Institute of Radiation Medicine, Beijing. The extraction and fractionation of air-dried powdered root was done as reported recently.[11] The compounds contained therein were characterized by nuclear magnetic resonance (Varian ^{UNITY}INOVA 600 spectrometer) and mass spectroscopy (by 9.4 T Q-FT-MS Apex Qe; Bruker Co.) at the Natural Center of Biomedical Analysis, Beijing, China.

The isolation and identification of compound **3** was reported in the literature.[12] Compounds **12** and **16** are new steroidal saponins and elucidated as (25R)-spirost-5-ene-3β, 12α-diol-3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-M-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (12) and 26-*O*-β-D-glucopyranosyl-(25R)-5-ene-furost-3β,17α,22α,26-tetraol-3-*O*-α-L-arabinofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**16**). Detailed information on the isolation and identification can be found elsewhere.[11]

The other compounds were identified as prosapogenin A of dioscin (**1**),[13] gracillin (**2**),[14] Pa (**4**),[15] loureiroside (**5**),[16] reclinatoside (**6**),[16] Pb (**7**),[17] Tb (**8**),[18] pennogenin-3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**9**),[19] pennogenin-3-*O*-α-L-arabinofuranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (**10**),[20] Tg (**11**),[21] diosgenin -3-*O*-α-L-arabinofuranosyl-(1→4)-β-D-glucopyranoside (**13**),[20] pennogenin-3-*O*-α-L-arabinofuranosyl-(1→4)-β-D-glucopyranoside (**14**),[20] parisvientnaside A (**15**),[22] progracillin (**16**),[23] parisaponin I (**17**),[24] dichotomin (**18**),[17] Th (**20**),[18] diosgenin (**21**)[14] and pennogenin (**22**),[14] respectively, by comparison of their NMR data with literature.

Animal preparation

Virgin female Wistar rats, 240–280 g, were purchased from the Laboratory Animal Center, Chinese Academy of Medical Sciences, and housed with free access to food and water. The rats were pretreated intraperitoneally with estrostilben (0.1 mg/kg) at 48 h before the experiments.[25] Timed-pregnant Wistar rats were prepared as described before.[26] The study was approved by the Institute Animal Ethics Committee and all animal care and experimental protocols were in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (GB14925-2001).

Myometrial contraction studies in virgin rats

Bilateral uterine horns were excised from the rats, which had been killed by cervical dislocation. After cleaning of adherences, the myometrial tissue was cut into 10 × 2 × 2 mm strips along the longitudinal axis of uterus. Strips were suspended vertically in 5-ml organ baths containing modified Krebs' solution (composition in mM: NaCl 136, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.32 and glucose 5.04, pH 7.2), bubbled continuously with 95% O_2–5% CO_2 and warmed to 37.2°C. The muscle strips were loaded with weights of 1.0 g as initial tension and recorded isometrically with a tension transducer connected to a polygraph system (Pclab, Beijing Microsignalstar Techology Development Co. Ltd). Each strip was firstly challenged with 40 mM K^+ for 10 min for determining viability and maximum contraction force, and the recorded value was taken as the control (100%). Those strips that did not respond to KCl were discarded. A 30 min equilibration period was allowed to establish reproducible spontaneous contractions before recording 10-min spontaneous uterine contraction, which was taken as the basal value.

For structure–activity relationship studies, steroidal saponins were applied cumulatively in the bath at

10-min intervals, respectively. Mechanical responses of uterine strips were analysed as the area during a 10-min period after application of the samples. The change in contractile activity was expressed as a percentage of the 40 mM K^+-induced AUC during the 10-min period and the change in inhibitory activity was expressed as a percentage of the spontaneous contraction obtained before application of the saponin. The EC50/IC50 values (concentration producing 50% maximal and stimulation and inhibition, respectively) and the maximal stimulatory/inhibitory responses (Emax/Imax) were estimated from the concentration–response curves by least squares nonlinear regression analysis.

Myometrial contraction studies in pregnant rats

Virgin female rats, 200–250 g, were placed in separate cages with one male each and left overnight. Pregnancy was dated by defining the morning of sperm positivity as day 0 of gestation. Some rats were left untreated and were decapitated at days 7, 14 and 21 of pregnancy. After removing the fetus, the myometrial tissue was cut into 10 × 2 × 2 mm strips along the longitudinal axis of the uterus. The rest of the operation was as for the myometrium contraction studies in virgin rats.

Statistical analysis

Results are expressed as means ± SEM. One strip obtained from one rat was used for each experiment. Therefore, the number of experiments (*n* value) also indicates the number of rats. Student's *t*-test was applied for comparison of the means of two groups based on the number of replicates, and analysis of variance or the Kruskal–Wallis test were used for the means of multiple groups. $P < 0.05$ was considered significant. Statistical analyses were performed using the SAS software package (SAS Institute, Cary, USA).

Results

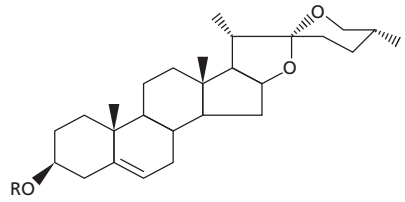
Structure–activity relationships of spirostanol glycosides on rat uterine contractility

Previous studies indicate that, when total spirostanol saponins isolated from *P. polyphylla* Smith var. *yunnanensis* expressed significant uterine contractile activity, their corresponding total furostanol saponins usually showed no activity.[7] Thus, from the perspective of structure–activity relationships (SAR), fifteen naturally occurring spirostanol glycosides isolated from *P. polyphylla* Smith var. *yunnanensis*, including eight diosgenin glycosides, five pennogenin glycosides and two other glycosides with structurally different aglycones, were selected for this study. In addition, for providing meaningful SAR information, five corresponding furostanol glycosides and two steroidal sapogenins were included.

Under the conditions of our experiments, it was shown that spirostanol glycosides **1**–**12** all exerted stimulating actions on rat myometrial strips, which were characterized by increasing amplitude and frequency of spontaneous contraction in a dose-dependent manner, while the remaining spirostanol glycosides (compounds **13**–**15**) dose-dependently induced uterine relaxation on spontaneous contractile activity. Consistent with previous study, five corresponding furostanol saponins and two steroidal sapogenins (diosgenin and pennogenin) were inactive even at the highest test concentration of 80 μM (Figure 1). These findings suggest that the spirostanol structure and the 3-*O*-glycoside moiety are both essential for rat uterine contractility.

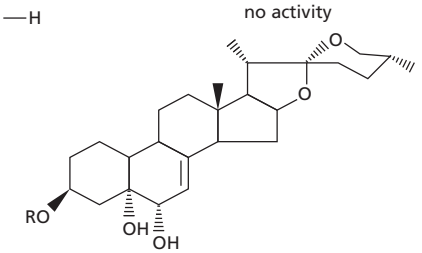
Among the spirostanol glycosides with uterine contractile activity, diosgenin-L-rhamnosyl-(1-2)-β-D-glucoside (compound **1**) showed the lowest contractile activity in myometrial strips, and the attachment of an α-L-rhamnosyl (compound **3**) or α-L-arabinofuranosyl (compound **4**) to C-4, or a β-D-glucosyl (compound **2**) to C-3 of the inner glucosyl moiety significantly increased the Emax values of myometrial strips to some different extents. Further addition of a β-D-glucosyl (compound **5**) or α-L-rhamnosyl (compound **6**) to C-5 of the arabinofuranosyl residue of compound **4** led to stronger contractile activity, both possessing the highest Emax values among the diosgenin glycosides, but further addition of an α-L-rhamnosyl (compound **7**) to C-4 of the rhamnosyl residue of compound **3** significantly decreased its activity, indicating that the structure but not the number of monosaccharide units in the diosgenin glycoside plays an important role in its uterine contractile activity (Figure 2a). In addition, the introduction of a C-17 α-hydroxyl group (compounds **8**–**11**) to the aglycones of compounds **1**, **2**, **4** and **7** led to a considerable increase in their activity, although the structures of the glycoside moieties of compounds **1**, **2**, **4** and **7** are identical to those of compounds **8**–**11**, respectively (Figure 2b). In contrast, compound **12**, a chiapagenin glycoside with the same sugar moiety as compounds **7** and **11**, exerted a stimulating action on rat myometrium until its concentration increased to 80 μM (data not shown). Therefore, the above results indicated that the uterine contractile activity of these spirostanol glycosides is associated with their individual aglycone moieties as well as the number and structure of monosaccharide units in their sugar chains.

An additional, unexpected finding was that selective hydrolysation of α-L-rhamnosyl at C-2 of the glucosyl moiety from compounds **4** and **10** into compounds **13** and **14** converted their contractile activity into a relaxant effect on rat myometrial contractions. In addition, the introduction of a C-17 α-hydroxyl group (compound **14**) to the aglycones of compound **13** led to a significant decrease in its relaxing effect, although the structures of the glycoside moieties of **13** are identical to those of **14**. Furthermore, compound **15**, which had an aglycone with two α-hydroxy groups at C-5 and C-6 and a double bond between C-7 and C-8, significantly decreased the amplitude and frequency of spontaneous myometrial contraction in a dose-dependent manner, although it had the same sugar chain as that in compounds **2** and **9**, which both exhibited uterine contractile activity (Figure 2c). In contrast, most sarsasapogenin glycosides with a spirostanol structure isolated from *Anemarrhena asphodeloides* Bge showed little contractile activity (data not shown). These data led us to assume that the composition of

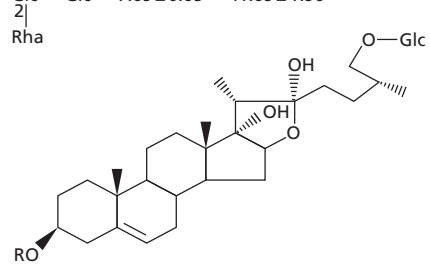


	R	EC50 (μM)	Emax (%)
1	Glc-(2)-Rha	4.15±0.32	11.45±0.81
2	Glc(3)-Glc-(2)-Rha	3.03±0.44	17.92±0.38
3	Glc(4)-Rha-(2)-Rha	3.84±0.20	16.40±0.28
4	Glc(4)-Ara-(2)-Rha	2.74±0.40	18.71±0.24
5	Glc(4)-Ara(5)-Glc-(2)-Rha	3.79±0.12	31.41±0.90
6	Glc(4)-Ara(5)-Rha-(2)-Rha	3.79±0.43	31.00±0.52
7	Glc(4)-Rha(4)-Rha-(2)-Rha	4.63±0.41	11.60±0.37

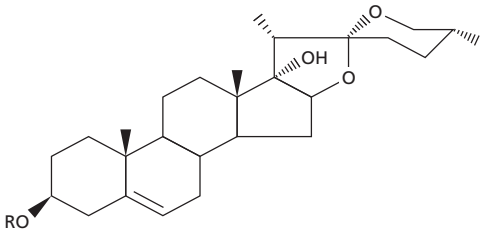
	R	IC50 (μM)	Imax (%)
13	Glc(4)-Ara	9.94±0.41	43.41±1.20
21	H	no activity	



	R	IC50 (μM)	Imax (%)
15	Glc(3)-Glc-(2)-Rha	7.69±0.65	41.69±1.96

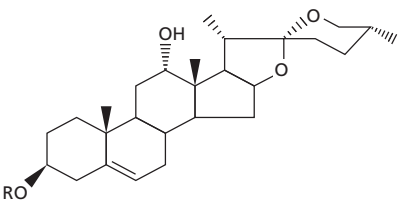


	R	EC50/IC50 (μM)
16	Glc(4)-Ara-(2)-Rha	no activity
17	Glc(4)-Rha(4)-Rha-(2)-Rha	no activity

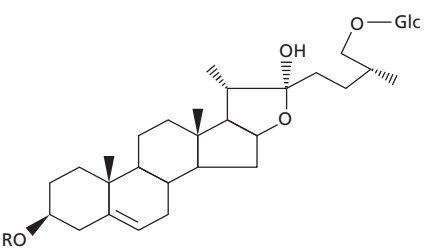


	R	EC50 (μM)	Emax (%)
8	Glc-(2)-Rha	12.51±0.49	24.82±0.77
9	Glc(3)-Glc-(2)-Rha	9.83±0.44	31.50±0.26
10	Glc(4)-Ara-(2)-Rha	3.16±0.15	27.62±0.59
11	Glc(4)-Rha(4)-Rha-(2)-Rha	3.86±0.08	39.64±0.71

	R	IC50 (μM)	Imax (%)
14	Glc(4)-Ara	127.40±17.72	63.60±2.08
22	H	no activity	



	R	EC50 (μM)	Emax (%)
12	Glc(4)-Rha(4)-Rha-(2)-Rha	>50	>25



	R	EC50/IC50 (μM)
18	Glc(3)-Glc-(2)-Rha	no activity
19	Glc(4)-Ara-(2)-Rha	no activity
20	Glc(4)-Rha(4)-Rha-(2)-Rha	no activity

Figure 1 Structure–activity relationships of spirostanol glycosides on rat uterine contractility. Fifteen spirostanol glycosides, five furostanol glycosides and two steroidal sapogenins were isolated from *Paris polyphylla* Smith var. *yunnanensis*, and their contractilities were evaluated in isolated uterine strips from estrogen-primed rats. The EC50/IC50 values and the maximal stimulatory/inhibitory responses (Emax/Imax) were estimated from concentration–response curves by least squares nonlinear regression analysis

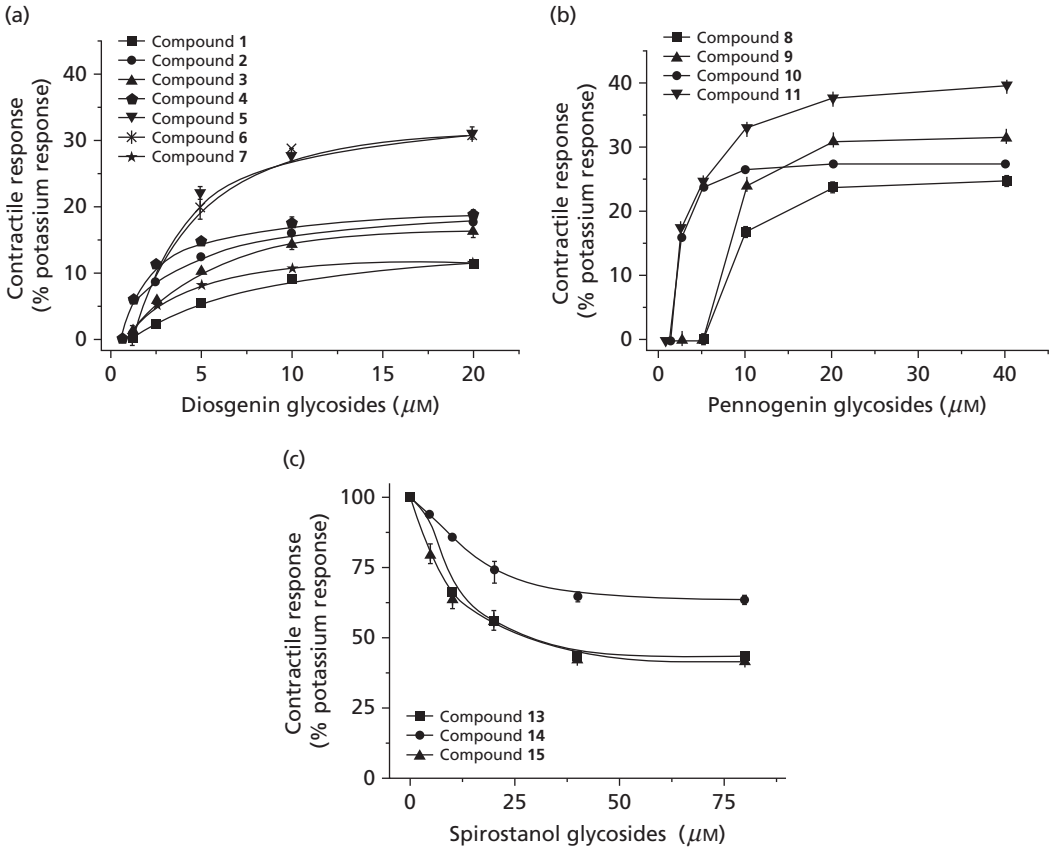


Figure 2 Concentration–response curves of spirostanol glycosides in stimulating or inhibiting rat myometrial contractions. (a, b) Dose–response curves for spirostanol-type diosgenin glycoside or pennogenin glycoside-induced rat myometrial contractions, respectively (n = 3). Contraction was measured as area under the curve (AUC) and expressed as a percentage of 40 mM potassium response. (c) Dose–response curves of spirostanol glycosides in inhibiting the spontaneous contractions of isolated uterine strips from estrogen-primed rats (n = 3). The relaxant activity was expressed as a percentage of the spontaneous contraction obtained before application of the saponin. The data are expressed as means ± SEM

the sugar moiety as well as the structure of the aglycone is essential for the uterine contractile or relaxant activity of these spirostanol glycosides.

Spirostanol glycosides potentiate each other as well as PGF-2α-induced uterine contractions

In our previous studies, total steroid saponins extracted from the rhizomes of *P. polyphylla* Sm. var. *yunnanensis* (TSSP) exhibit stronger contractile activity in rat myometrium than some of the purified steroid saponins, indicating potentiating or synergistic actions exist among the spirostanol glycosides. To test this hypothesis, rat myometrial strips were treated with compound **10**, a spirostanol-type pennogenin glycoside with stronger uterine contractile activity, alone or in pairs with pennogenin or disogenin glycosides. As shown in Figure 3a, while no significant contractile activity was seen when the threshold concentrations of pennogenin glycosides, including compounds **8**, **9** and **11**, were used alone, uterine contractility was significantly enhanced when used together with increasing doses of compound **10** under or near its threshold concentration, with a significant synergism in the pairs of compounds **10** and **11**, then compounds **10** and **8**, and no significant potentiation in the pairs of compounds **10** and **9**. The contractile response to combinations of compound **10** with diosgenin glycosides was also investigated. It was observed that there was strong synergism when compound **10** was used with compounds **4** and **7**, then compounds **3**, **1** and **2** on rat uterine contractility (Figure 3b).

It is accepted that PGF-2α can act pharmacologically as a uterine contractile agonist during the gestation period.[27] Thus, we wondered whether a synergistic effect would be observed between PGF-2α and spirostanol glycoside. As for spirostanol glycosides, the contractile response to a threshold concentration of compound **10** plus different concentrations of PGF-2α was shown (Figure 3c). An enhanced contractile effect of compound **10** was observed with increasing concentrations of PGF-2α and the optimal contractions were recorded when myometrial strips were challenged with the threshold concentrations of compound **10** and PGF-2α together. In contrast, a similar phenomenon was not observed for the combination of compound **10** with oxytocin or acetylcholine (data not shown). These results suggested that spirostanol glycosides may share, at least in part, the

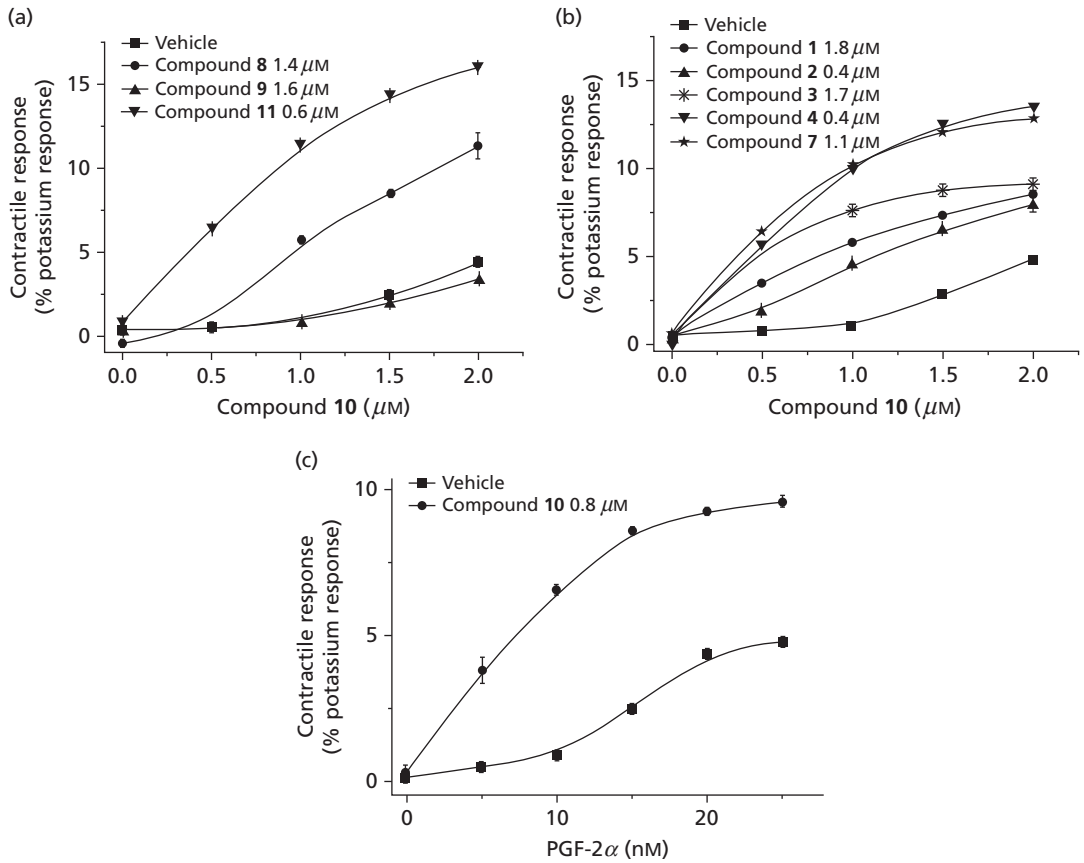


Figure 3 Spirostanol glycosides potentiate each other and prostaglandin $F_{2\alpha}$-induced uterine contractions. (a, b) Synergistic actions of compound **10** with spirostanol-type pennogenin glycosides or diosgenin glycosides, respectively, were examined on rat myometrium contraction (n = 3). (c) Synergistic action of compound **10** with prostaglandin $F_{2\alpha}$ (PGF-2α) was examined on rat myometrium contraction (n = 3). Data are expressed as means ± SEM

same signal pathways as PGF-2α in stimulating myometrial contractions.

Variation of spirostanol glycoside-induced rat myometrial contraction during pregnancy

Rhythmic uterine contractions play an important role in parturition.[28] To uncover the role of spirostanol glycosides in a more physiological setting, we examined the contractile response of isolated pregnant uterine strips to compound **11**, a pennogenin glycoside, and compound **4**, a diosgenin glycoside, and also compared them with PGF-2α in uterine contractility. As shown in Figure 4a, b, stimulation of isolated pregnant uterine strips with compounds **11** and **4** revealed dose–response curves, with maximal contractions reached at mid gestation with compound **11**, and at late gestation with compound **4**. Changes in sensitivity and maximum developed forces of isolated pregnant uterine strips to compounds **11** and **4** as well as PGF-2α are represented in Table 1. The EC50 values for these stimulators increased to some different extents with advancing pregnancy, reaching the highest value for compound **4** and PGF-2α at day 14 and for compounds **11** at day 21. Along with EC50 changes, the Emax values of the pregnant uterine strips induced by these stimulators increased significantly at a later stage of pregnancy, reaching the highest value before parturition for compound **4** and PGF-2α, while for compound **11**, the highest Emax value was obtained at day 14 of gestation. The enhancement in the contractile response to spirostanol glycosides with advancing pregnancy supported the possibility that spirostanol glycosides may represent a new type of contractile agonist for the uterus.

Discussion

Steroid saponins are present in plants and some marine animals, and possess a broad range of biological and pharmacological properties, such as hypocholesterolaemic, anti-tumour, antidiabetic, anti-inflammatory, antifungal and platelet agonistic and inhibitory activity.[8–10] In this study, we firstly analysed the structure–activity relationship of a series of spirostanol glycosides isolated from *P. polyphylla* Sm. var. *yunnanensis* in uterine contraction. It was shown that spirostanol glycosides exhibited inducible or inhibitory activity in rat uterine contraction based on the difference in their structures, which was not only attributed in part to the number, the length and the position of sugar side chains attached by a glycoside, but also related to the structure of the aglycone. Furthermore, the synergistic actions observed among pennogenin or disogenin glycosides as well as with the known inherent agonist PGF-2α further suggested that spirostanol glycosides and PGF-2α may share, at least in

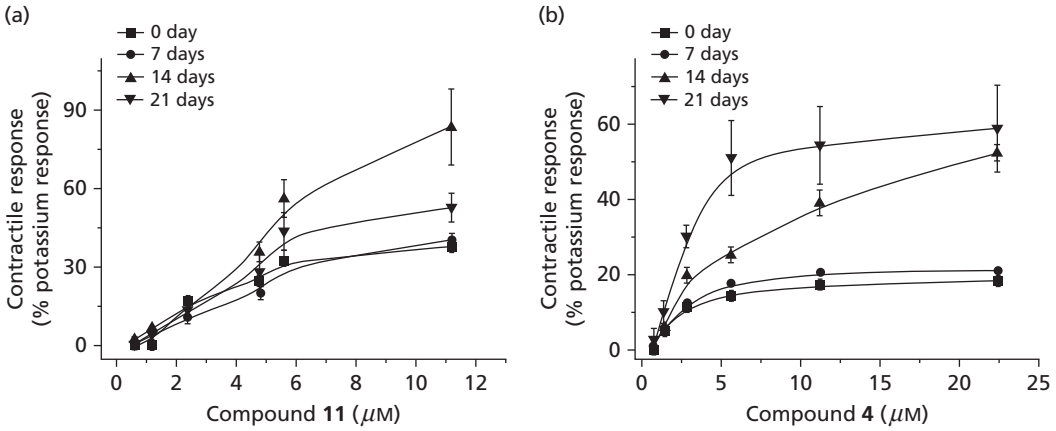


Figure 4 Variation of the spirostanol glycoside-stimulated rat myometrial contraction during pregnancy. (a, b) Representative recordings of cumulative dose–responses developed by compound **11** and compound **4**, respectively, in isolated pregnant rat uterine strips (n = 3~6). Strips were isolated from pregnant rats at different gestational ages. The change in contractile activity was expressed as a percentage of the 40 mM K^+-induced atrea during a 10-min period and the data are expressed as means ± SEM

Table 1 The EC50 and Emax values of pregnant rat uterine strips challenged with compound **11**, **4** and PGF-2α

Drug	Gestational age	EC50	Emax (% of KCl)
Compound **11**	Day 0	3.15 ± 0.03 μM	37.77 ± 0.53
	Day 7	4.39 ± 0.07 μM**	40.15 ± 0.75
	Day 14	4.13 ± 0.56 μM	83.44 ± 14.48**
	Day 21	4.82 ± 0.72 μM*	52.55 ± 5.75
Compound **4**	Day 0	2.54 ± 0.15 μM	18.76 ± 0.27
	Day 7	4.77 ± 0.26 μM**	20.81 ± 0.41*
	Day 14	4.56 ± 0.17 μM*	52.58 ± 2.16**
	Day 21	2.69 ± 0.32 μM	59.17 ± 11.65**
PGF-2α	Day 0	85.52 ± 3.77 nM	44.44 ± 0.82
	Day 7	259.38 ± 19.43 nM**	69.53 ± 2.81*
	Day 14	270.82 ± 37.12 nM**	69.88 ± 3.85*
	Day 21	211.17 ± 4.37 nM*	149.18 ± 1.46**

The EC50 values and the maximal stimulatory responses (Emax) were estimated from concentration–response curves by least squares nonlinear regression analysis. The data are expressed as means ± SEM, n = 3~6. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with control.

part, similar signal pathways in stimulating myometrial contractions. Finally, and an important observation, the enhancement in the contractile response to spirostanol glycosides with advancing pregnancy further supported the possibility that some spirostanol glycosides may represent a new type of contractile agonist for the uterus, as one of the most consistent findings is the increase in uterine contractility to some inherent contractile agonists, such as PGF-2α, oxytocin, etc, before the onset of labour in several species.[29,30]

Steroidal saponins are composed of a C-27 aglycone moiety and sugar chains with one or more monosaccharides. These compounds are classified as spirostanol glycosides, with a sugar chain at C_3 position, and furostanol glycosides with two sugar chains at both C_3 and C_{26} positions.[8,9] Structure–activity assay revealed that spirostanol glycosides from *P. polyphylla* var. *yunnanensis* elicited contractile responses, with a rank order of potency of tetraglycoside (with the exception of compound **7**) > triglycoside > diglycoside while they possess the same aglycone moieties, and the introduction of a C-17 α-hydroxyl group to the aglycone of diosgenin glycoside led to considerable increase in its contractile activity. In contrast, selective hydrolysation of α-L-rhamnosyl at C-2 of the inner glucosyl moiety or replacement of diosgenin or pennogenin with 3β,5α, 6α-trihydroxy-7(8)-en-isospirostanol converted the contractile activity of some spirostanol glycosides into relaxant activity. This indicated that both the sugar chain and the structure of sapogenin in each spirostanol glycoside are necessary and play a key role for its uterine contractility. Similar to these findings, Matsuda *et al.* reported the protective effect of spirostanol glycosides from *P. polyphylla* var. *yunnanensis* on ethanol- or indometacin-induced gastric mucosal lesions in rats.[24] It was shown that the 3-*O*-glycoside moiety and spirostanol structure were essential for the activity and that the 17-hydroxyl group in the aglycone part of saponins with pennogenin enhanced the protective effect against ethanol-induced gastric lesions.[24]

Saponins are generally considered to possess detergent-like surfactant properties. To determine the selectivity of spirostanol glycosides on uterine contractility, the in-vitro cytotoxicity was evaluated against human promyelocytic leukaemia HL-60 cells, and most spirostanol glycosides showed considerable cytotoxic activity,[11] which was found to be sensitive to the monosaccharides constituting the sugar moieties and their sequences, as well as to the structures of the aglycones, but their IC50s for cytotoxicity were not comparable to the EC50s in stimulating rat uterine contractions. For example, the increase in the number of the monosaccharides of a sugar chain at its C_3 position or introduction of a C-17 α-hydroxyl group to the aglycone of diosgenin glycoside, which has been found to significantly enhance the uterine contractile activity of spirostanol glycoside, sometimes, however, led to considerable decrease in its cytotoxic activity. In contrast, it was reported that spirostanol-type pennogenin glycosides from *P. polyphylla* Smith

var. *yunnanensis* are strong platelet agonists that cause platelet aggregation in a concentration-dependent manner, and the platelet activity of these glycosides is comparable to their uterine contractility.[31] Therefore, these studies provided an idea that many actions of the spirostanol glycosides, such as uterine contractility, platelet agonist and protective effects against ethanol-induced gastric lesions, were associated with their aglycone moieties as well as the constitution of monosaccharide units in their sugar chains, which may be mediated by similar targets or receptors.

Chinese herbal medicines are commonly used in combination to enhance their curative effects or decrease their toxicity. Synergistic or antagonistic actions among the ingredients in the formulas are proposed to be the basic rules for the combination, but little evidence from modern medicine is provided.[32] As presented in this paper and reported in our previous study,[7,31] total spirostanol saponins extracted from *P. polyphylla* Smith var. *yunnanensis* show stronger activity than some single compounds of spirostanol glycosides in uterine contraction or platelet aggregation, and the synergistic actions of spirostanol glycosides were observed in both activities, which in the body is of great clinical significance as it can lead to marked potentiation of the uterine contraction and platelet activation *in vivo*.[33] This may be the reason that the total steroidal saponin, but not its single compounds, are developed as medicines in traditional Chinese medicine. As the main ingredients of GongXueNing (GXN),[6] it is proposed that the strengthening of uterine contractions or promotion of haemostasis *in vivo* by steroidal saponins may be responsible for its therapeutic effect in abnormal uterine bleeding.

Conclusions

This study has shown that spirostanol glycosides purified from TSSP exhibited inducible or inhibitory activity in rat uterine contraction based on the difference of their structures, which was not only attributed in part to the number, the length and the position of sugar side chains attached by a glycoside, but also related to the structure of the aglycone. Furthermore, the synergistic actions were observed among pennogenin or disogenin glycosides as well as with the known inherent agonist PGF-2α. Therefore, ascertaining the chemical foundation of the spirostanol glycosides in inducing myometrial contractions and developing the combinations of these glycosides are worthy of exploration as leads for the drug discovery in the treatment of abnormal uterine bleeding.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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