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Protective Effects of Steroid Saponins from *Paris polyphylla* var. *yunnanensis* on Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats: Structural Requirement for Activity and Mode of Action

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Abstract—The methanolic extract from the rhizomes of *Paris polyphylla* Sm. var. *yunnanensis* (Fr.) H-M. was found to potently inhibit ethanol-induced gastric lesions in rats. Through bioassay-guided separation, four known spirostanol-type steroid saponins, pennogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**1**), pennogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (**2**), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**3**), and diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (**4**), and a new furostanol-type steroid saponin, parisaponin I (**5**), together with two known furostanol-type steroid saponins, trigofoenoside A (**6**) and protogracillin (**7**), were isolated from the active fraction. Compounds **1**–**4** (1.25–10 mg/kg, po) strongly inhibited gastric lesions induced by ethanol and indomethacin. With regard to structural requirement of steroid saponins, the 3-*O*-glycoside moiety and spirostanol structure were found to be essential for the activity and the 17-hydroxyl group in the aglycon part enhanced the protective effects against ethanol-induced gastric lesions. The protective effects of **1** and **3** against ethanol-induced gastric lesions were attenuated by pretreatment with indomethacin and *N*-ethylmaleimide. Compounds **1** and **3** weakly inhibited acid secretions in pylorus-ligated rats. These findings suggested that endogenous prostaglandins and sulfhydryl compounds were involved in the protective activity.

Introduction

Plants of the genus *Paris* (Liliaceae) are distributed in many regions of the world, such as India, China, Veitnam, and Germany. There are many reports as to the isolation of steroid saponins from the rhizomes of these plants.[1] Recently, steroid saponins have got in scientific attention because of their structural diversity and significant of the biological activities, such as hypocholesterolemic, antitumour, antidiabetic, antiinflammatory, inhibitory activities against platelet aggregation and cAMP phosphodiesterase, and antifungal.[2] However, the effects of steroid saponins on gastrointestinal function have not been reported to date.

In the course of our studies of bioactive saponin constituents from natural medicines and medicinal foodstuffs,[3] we previously reported the gastroprotective effect of some triterpenoid saponins on ethanol- or indomethacin-induced gastric mucosal lesions in rats.[4] In our continuing study, we found that the methanolic extract from the rhizomes of *Paris* (*P.*) *polyphylla* Sm. var. *yunnanensis* (Fr.) H-M., collected in Yunnan, China, showed protective effects against ethanol-induced gastric mucosal lesions in rats. The rhizomes of this plant are used in a traditional Chinese medicine for treatment of chronic bronchitis, mastitis, parotitis, etc.[1b,c] By bioassay-guided separation, four known spirostanol-type steroid saponins, pennogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**1**), pennogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl (1→2)]-β - D - glucopyranoside (**2**),

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diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**3**), and diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl (1→4) - [α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (**4**), and a new furostanol-type steroid saponin, parisaponin I (**5**), together with two known furostanol-type steroid saponins, trigofoenoside A (**6**) and protogracillin (**7**), were isolated from the active fraction.

In the present study, we examined the effects of steroid saponins isolated from the rhizomes of *P. polyphylla* var. *yunnanensis* on ethanol- or indomethacin-induced gastric mucosal lesions in rats. In addition, the structural requirements of steroid saponins for this activity, and mode of action of **1** and **3** including the involvement of prostaglandins (PGs), nitric oxide (NO) and sulfhydryl compounds (SHs), as well as effects of **1** and **3** on gastric secretion, were discussed.

Isolation of Steroid Saponins (1–7) and Structure of Parisaponin I (5)

The dried rhizomes of *P. polyphylla* var. *yunnanensis* (2.5 kg, cultivated in Yunnan, China) were extracted with methanol 3 times under reflux for 3 h. The methanolic extract (12.4% from this natural medicine) was subjected to Diaion HP-20 column chromatography (H_2O→MeOH) to give the water-eluted fraction (8.78%) and the methanol-eluted fraction (3.62%). The methanol-eluted fraction was then subjected to ordinary-phase silica-gel (SiO_2) [$CHCl_3$–MeOH→$CHCl_3$–MeOH–H_2O→MeOH] and reverse-phase silica-gel (ODS) column chromatography [MeOH–H_2O→MeOH], and finally HPLC [YMC Pack ODS-AL or Develosil C_{30}, 250×20 mm i.d., CH_3CN–H_2O] to give **1**[1b] (0.014%), **2**[1b,d] (0.019%), **3**[1b,c] (0.12%), **4**[1b,c] (0.16%), **5**[5] (0.009%), **6**[6] (0.010%), and **7**[7] (0.11%). Their aglycons, pennogenin (**8**)[8] and diosgenin (**9**),[8] were obtained by methanolysis of **1** and **3**.

Parisaponin I (**5**) was isolated as a white powder and was deduced to possess a furostanol structure based on TLC examination using the Ehrlich reagent.[9] The IR spectrum of **5** showed strong absorption bands at 3410, 1074, and 1055 cm^{-1} suggestive of the oligoglycosidic structure. In the negative- and positive-ion fast atom bombardment (FAB)-MS, quasimolecular ion peaks were observed at *m/z* 1033 $(M–H)^-$ and 1057 $(M+Na)^+$, respectively, and high-resolution MS analysis revealed the molecular formula of **5** to be $C_{50}H_{82}O_{22}$.[5] On acid hydrolysis with 1 M hydrochloric acid (HCl), **5** liberated diosgenin (**9**) as an aglycon, in addition to D-glucose, L-rhamnose, and L-arabinose, which were identified by HPLC analysis using an optical rotation detector.[10] The ^1H NMR (pyridine-d_5) and ^{13}C NMR[5] spectra of **5**, which were assigned by various NMR analytical methods,[5b] showed signals assignable to a furost-5-ene part {δ 0.89, 1.05 (3H each, both s, 18, 19-H_3), 0.98 (3H, d, J=6.7 Hz, 27-H_3), 1.30 (3H, d, J=6.7 Hz, 21-H_3), [δ 3.59 (1H, dd, J=5.8, 9.2 Hz), 3.92 (1H, m), 26-H_2], 3.82 (1H, m, 3-H), 4.91 (1H, m, 16-H), 5.31 (1H, br s, 6-H)}, two β-D-glucopyranosyl moieties [δ 4.74 (1H, d, J=7.3 Hz, 1‴-H), 4.88 (1H, d, J=7.3 Hz, 1′-H)], a α-L-rhamnopyranosyl moiety [δ 1.71 (3H, d, J=6.1 Hz, 6″-H_3), 6.15 (1H, br s, 1″-H], and a α-L-arabinofuranosyl moiety [δ 5.81 (1H, br s, 1⁗-H)]. The 3,26-bisdesmoside structure of **5** was characterized by a heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 1″-proton and the 2′-carbon, between the 1‴-proton and the 4′-carbon, and between the 1⁗-proton and the 26-carbon. Furthermore, the stereostructure at the 25-position of **5** was elucidated on the basis of the proton signals assignable to the 26-methylene group, which showed the characteristic 25*R* configuration.[6b] Consequently, the stereostructure of parisaponin I was formulated as 26-β-D-glusopyranosyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**5**) (Chart 1).

Gastroprotective Activities of Saponin Constituents (1–7) from *P. polyphylla* var. *yunnanensis*

The methanolic extract (25–200 mg/kg, po) and methanol-eluted fraction (10–50 mg/kg, p.o.) from the rhizomes of *P. polyphylla* var. *yunnanensis* inhibited ethanol-induced gastric mucosal lesions in a dose-dependent manner (Table 1). First, steroid saponin constituents (**1**–**7**) from the active fraction were examined. As shown in Table 2, spirostanol-type steroid saponins, pennogenin 3-*O*-glycosides (**1** and **2**, 1.25–5.0 mg/kg, po) and diosgenin 3-*O*-glycosides (**3** and **4**, 2.5–10 mg/kg, po) showed potent protective effects on ethanol- and indomethacin-induced gastric lesions in rats, and their effects on ethanol-induced gastric lesions were stronger than those of reference compounds, omeprazole and cetraxate hydrochloride. However, furostanol-type steroid saponins (**5**–**7**, 5.0 mg/kg, po) did not show such effects. Among spirostanol-type steroid saponins, **1** and **2** having the 17-hydroxyl group showed stronger effects than **3** and **4** lacking of this group against ethanol-induced gastric lesions, although the structures of the glycoside moieties of **1** and **2** are identical to those of **3** and **4**, respectively. Furthermore, two aglycons, pennogenin (**8**) and diosgenin (**9**), lacked the effect at a dose of 10 mg/kg. These findings suggest that the spirostanol structure and the 3-*O*-glycoside moiety are essential for the gastroprotective and the 17-hydroxyl group enhance the protective effects against ethanol-induced gastric lesions.

Effects of Spirostanol-Type Steroid Saponins (1, 3) on Gastric Secretions in Rats

Next, effects of the spirostanol-type steroid saponins (**1**, **3**) on gastric secretion in pylorus-ligated rats were examined. Compounds **1** (5.0 mg/kg) and **3** (10 mg/kg) showed the significant change in the acid output at their effective doses against the gastric lesions, but their inhibitions were weaker than that of omeprazole (Table 3). This finding suggested that the gastroprotective effects of **1** and **3** partly depended on their inhibitory effects on acid secretion.

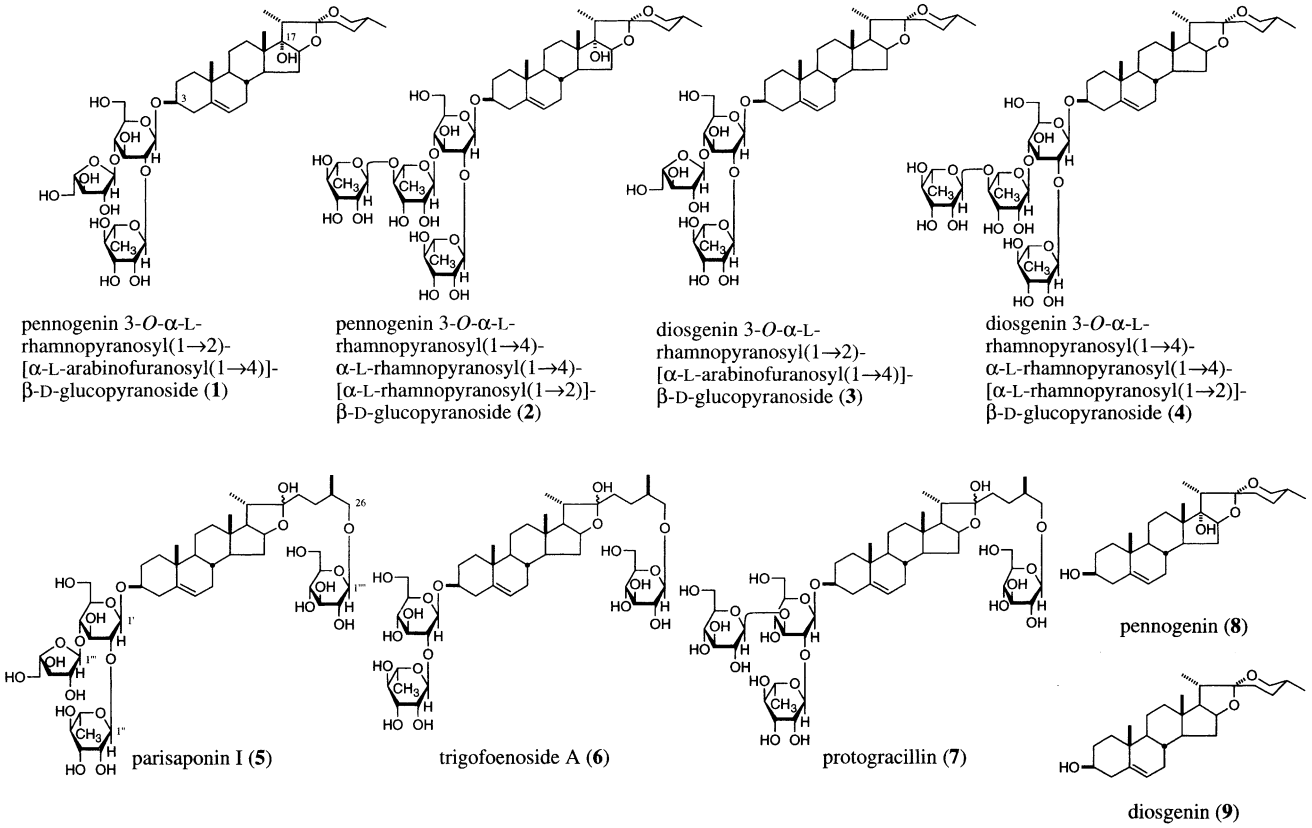

Chart 1. Chemical structures of steroid saponins from *P. polyphylla* var. *yunnanensis*.

Table 1. Effects of the methanolic extract and the methanol- and water-eluted fractions from *P. polyphylla* var. *yunnanensis* on gastric lesions induced by ethanol in rats

Treatment	Dose (mg/kg, po)	*N*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	6	136.5±3.6	—
MeOH extract	25	6	91.0±10.7**	33.3
	50	6	52.9±13.7**	61.2
	100	6	20.6±6.0**	84.9
	200	6	2.6±1.5**	98.1
Control	—	8	140.4±5.5	—
MeOH-eluted fraction	10	8	82.7±9.7**	41.1
	25	8	65.8±7.2**	53.1
	50	8	24.2±7.7**	82.8
H_2O-eluted fraction	10	6	117.4±9.0	16.4
	25	6	110.2±7.7*	21.5
	50	6	113.7±4.9*	19.0

The acute gastric lesions were induced by oral administration of ethanol, as previously reported.[4] Briefly, 99.5% ethanol was administered to male Sprague–Dawley rats (230–250 g, b.w.). One hour later, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed, inflated by injection of 10 mL 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of gastric lesions were measured. Test substances were administered orally 1 h prior to the application of ethanol.[11] Values represent the means±SEM. Significantly different from the control group, $^*P<0.05$, $^{**}P<0.01$.

Mode of Action of Spirostanol-Type Steroid Saponins (1, 3) Against Ethanol-Induced Gastric Lesions

Finally, we investigated the involvement of endogenous PGs, NO, and SHs in the protective effects of spirostanol-type steroid saponins (**1**, **3**). PGs are known to play an important role in cytoprotection. It was reported that exogenous PGs protect gastric mucosa against necrotizing agents, and mild irritants protect the gastric mucosa against damage via induction of endogenous PGs as well.[15] In the present study, pretreatment with indomethacin (10 mg/kg, sc) markedly attenuated the protection afforded by **1** and **3** (Table 4). This finding suggests that endogenous PGs play an important role in the protective effect of **1** and **3**.

Since vascular changes in gastric mucosa appear to be the most pronounced feature of absolute ethanol-induced injury, maintenance of mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection. It has been demonstrated that the gastric mucosa produces endogenous NO derived from L-arginine, and that NO participates in gastric defence mechanisms by regulating the gastric mucosal blood flow and gastric mucus secretion.[16] As shown in Table 4, an NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg, ip) slightly attenuated the gastroprotection of **1** and **3**, but the effects were not significant, except for 2.5 mg/kg of **1**. This finding suggests that endogenous NO slightly participate in the protective effect of **1**.

Oxygen-derived free radicals and lipid peroxidation are associated with gastrointestinal lesions, and antioxidants prevent the lesions by various ulcerogens. Gastric mucosal SHs act as antioxidant and are important for maintenance of mucosal integrity in the stomach. Ethanol-induced

Table 2. Effects of steroid saponins (**1**–**7**) from *P. polyphylla* var. *yunnanensis*, pennogenin (**8**), and diosgenin (**9**) on gastric lesions induced by ethanol and indomethacin in rats

Treatment	Dose (mg/kg, po)	Ethanol			Indomethacin		
		N	Length (mm)	Inhibition (%)	*N*	Length (mm)	Inhibition (%)
Control	—	9	141.0±14.3	—	8	70.4±2.3	—
1	1.25	6	72.6±12.0**	48.5	6	34.9±9.1**	50.4
	2.5	9	24.1±7.4**	82.9	5	30.3±11.6**	57.0
	5.0	9	8.9±4.4**	93.7	8	21.0±3.6**	70.2
2	1.25	6	66.0±7.2**	53.2	5	58.4±16.4	17.0
	2.5	9	44.0±10.4**	68.8	5	29.6±2.6**	58.0
	5.0	9	20.0±3.6**	85.8	8	38.0±9.2**	46.0
Control	—	6	126.1±5.7	—	6	70.8±5.3	—
3	2.5	6	76.1±17.9**	39.7	6	27.9±6.6**	60.6
	5.0	9	46.5±11.3**	63.1	6	23.2±5.8**	67.2
	10	6	11.4±4.2**	91.0	6	10.9±5.1**	84.6
4	2.5	6	120.4±9.7	4.5	6	57.3±8.0**	19.1
	5.0	9	96.4±17.8*	23.6	6	31.3±8.3**	55.8
	10	9	60.6±11.2**	51.9	6	19.2±4.1**	72.9
Control	—	6	126.0±9.1	—	5	88.9±5.5	—
Parisaponin I (**5**)	2.5	6	114.4±10.5	9.2	—	—	—
	5.0	6	106.3±10.7	15.6	5	82.5±14.7	7.2
Trigofoenoside A (**6**)	2.5	6	93.0±8.9	26.2	—	—	—
	5.0	6	111.2±14.1	11.7	5	74.7±26.6	16.0
Protogracillin (**7**)	2.5	5	115.3±7.6	8.5	—	—	—
	5.0	6	108.2±9.5	14.1	—	—	—
Control	—	5	138.0±12.1	—	6	62.8±12.4	—
Pennogenin (**8**)	10	5	134.0±10.6	2.9	6	67.1±10.8	−6.8
Control	—	5	107.0±14.5	—	5	54.6±16.5	—
Diosgenin (**9**)	10	5	76.9±9.4	28.1	5	49.9±16.9	8.6
Control	—	6	159.2±21.0	—	6	59.8±6.0	—
Omeprazole	2.5		—	—	6	34.4±4.1**	42.5
	5.0		—	—	6	24.6±3.7**	58.9
	7.5		—	—	6	8.0±3.3**	86.6
	10	6	90.6±21.2**	43.1	6	1.1±1.1**	98.2
	15	6	28.6±13.4**	82.0	—	—	—
	20	6	16.9±6.1**	89.4	—	—	—
Control	—	6	148.4±9.8	—	6	81.3±6.7	—
Cetraxate hydrochloride	75	6	87.2±7.4**	41.2	6	58.7±7.5*	27.8
	150	6	51.0±4.0**	65.6	6	13.4±3.2**	83.5
	300	6	30.5±8.3**	79.4	6	1.4±0.5**	98.3

Ethanol-induced gastric lesions in rats: See footnote of Table 1. *Indomethacin-induced gastric lesions in rats*: The acute gastric mucosal lesion in rats by indomethacin was induced by the methods described by Wallace et al.[12] and Morise et al.[13] with slight modifications. Briefly, indomethacin (20 mg/kg, dissolved in 5% $NaHCO_3$, and then diluted in water and neutralized with 0.2 M HCl) was administered orally to rats (1.5 mL/rat). Four h later, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed, inflated by injection of 1.5% formalin (10 mL) to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of gastric lesions were measured. Reference compounds, omeprazole and cetraxate hydrochloride, were administered orally 1 h prior to the application of indomethacin.Values represent the means±SEM. Significantly different from the control group, $^*P<0.05$, $^{**}P<0.01$.

Table 3. Effects of spirostanol-type steroid saponins (**1**, **3**) from *P. polyphylla* var. *yunnanensis* on gastric secretion in pylorus-ligated rats

Treatment	Dose (mg/kg, po)	*N*	Volume (mL/4 h)	pH	Acid output (μ equiv/h)
Control	—	13	4.7±0.3	1.39±0.02	120.3±11.1
1	2.5	8	4.1±0.2	1.41±0.01	98.5±10.9
	5.0	8	3.3±0.1*	1.41±0.02	71.8±8.7*
Control	—	11	4.9±0.3	1.44±0.01	119.7±11.7
3	5.0	10	4.4±0.4	1.47±0.02	100.5±10.4
	10	11	3.6±0.3	1.48±0.02	82.1±7.6*
Control	—	6	4.7±0.4	1.42±0.02	120.3±19.7
Omeprazole	5.0	6	3.3±0.4	1.63±0.03*	62.6±10.5*
	10	6	2.5±0.4**	1.80±0.04**	46.8±6.6**
	20	6	2.2±0.2**	5.54±0.26**	14.6±3.6**

The pyloric ligation was carried out according to the methods of Shay et al.[14] Rats weighing about 240 g were anesthetized with ether, then the abdomen was incised and the pylorus was ligated. Four hours later, the rats were killed and the cardia was ligated. The stomach was then removed and the gastric contents were collected in a graduated centrifuged tube. After centrifugation, the volume and pH of the gastric juice were measured. Total acidity was determined by titration with 0.1 M NaOH and acid output (μ equiv/h) was calculated. Test samples were administered orally 1 h before the pyloric ligation.[11] Values represent the means±SEM. Significantly different from the control group, $^*P<0.05$, $^{**}P<0.01$.

Table 4. Effects of spirostanol-type steroid saponins (**1**, **3**) from *P. polyphylla* var. *yunnanensis* on gastric lesions induced by ethanol in indomethacin-, L-NAME- and NEM-pretreated rats

Treatment	Dose (mg/kg, po)	*N*	Gastric lesions	
			Length (mm)	Inhibition (%)
Normal rats				
Control	—	5	135.9±10.3	—
1	2.5	5	34.3±5.5**	74.8
	5.0	5	24.9±3.6**	81.7
Control	—	6	116.4±9.3	—
3	5.0	6	35.7±2.3**	69.3
	10	6	15.3±2.9**	86.9
Indomethacin-pretreated rats				
Control	—	6	152.9±9.7	—
1	2.5	6	139.3±11.2††	8.9
	5.0	6	126.6±16.9††	17.2
Control	—	6	124.3±7.8	—
3	5.0	6	102.2±8.1††	17.8
	10	6	96.1±13.7††	22.7
L-*NAME-pretreated rats*				
Control	—	6	190.1±9.3	—
1	2.5	6	92.8±8.0**††	51.2
	5.0	6	50.0±9.9**	73.7
Control	—	6	188.7±10.5	—
3	5.0	6	60.8±11.6**	67.8
	10	6	31.4±3.8**	83.4
NEM-pretreated rats				
Control	—	6	206.7±6.3	—
1	2.5	6	110.0±8.4**††	46.8
	5.0	6	63.0±8.7**	69.5
Control	—	6	169.6±1.9	—
3	5.0	6	92.3±9.0**††	45.6
	10	6	61.5±4.4**††	63.7

Indomethacin (10 mg/kg, dissolved in 5% $NaHCO_3$ solution, and diluted in distilled water, sc), L-NAME (70 mg/kg, dissolved in saline, ip), or NEM (10 mg/kg, dissolved in saline, sc), was injected 30 min before the administration of the sample. Ethanol was administered to rats 1 h after administration of test sample.[4b,19] Values represent the means±SEM. Significantly different from each control group, $^{**}P<0.01$, and from compound **1** or **3**-treated group in normal rats, $^{††}P<0.01$.

gastric damage is also associated with a depletion of endogenous SHs such as glutathione, and pretreatment with SH-blockers prevented the gastroprotection of SH-containing substances.[17,18] In the present study, pretreatment with *N*-ethylmaleimide (NEM, 10 mg/kg, s.c.), an SH-blocker, also reduced the protection afforded by **1** and **3**. This finding suggests that endogenous SHs is involved in the protection of **1** and **3**.

In conclusion, four known spirostanol-type steroid saponins, pennogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**1**), pennogenin 3-*O*-α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside (**2**), diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-arabinofuranosyl(1→4)]-β-D-glucopyranoside (**3**), and diosgenin 3-*O*-α-L-rhamnopyranosyl(1→4) - α - L - rhamnopyranosyl(1→4) - [α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (**4**), strongly inhibited gastric lesions induced by ethanol and indomethacin. With regard to structural requirement of steroid saponins, the 3-*O*-glycoside moiety and spirostanol structure were found to be essential for the activity and the 17-hydroxyl group in the aglycon part enhanced the effects against ethanol-induced gastric lesions. The gastroprotective effects of **1** and **3** were attenuated by pretreatment with indomethacin and *N*-ethylmaleimide. These findings suggested that endogenous PGs and SHs were involved in the protective activity. To best our knowledge, this is the first report of steroid saponins with the strong gastroprotective effects, although various triterpenoid saponins with gastroprotective effects were reported.[4,20]

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5. (a) **5**: $[\alpha]_D^{26}$ −41.3° (*c* = 1.10, MeOH). High-resolution positive-ion FAB-MS: calcd for $C_{50}H_{82}O_{22}Na$ $(M+Na)^+$: 1057.5195. Found: 1057.5179. IR (KBr): 3410, 2936, 1074, 1055, 756 cm^{-1}. ^{13}C NMR (C_5D_5N) δc : 37.4 (C-1), 30.1 (C-2), 78.3 (C-3), 38.9 (C-4), 140.6 (C-5), 121.5 (C-6), 32.3 (C-7), 31.6 (C-8), 50.3 (C-9), 37.0 (C-10), 21.0 (C-11), 39.9 (C-12), 40.7 (C-13), 56.5 (C-14), 32.4 (C-15), 80.9 (C-16), 63.7 (C-17), 16.4 (C-18), 19.3 (C-19), 40.5 (C-20), 16.3 (C-21), 110.4 (C-22), 37.0 (C-23), 28.2 (C-24), 34.1 (C-25), 75.0 (C-26), 17.3 (C-27), 100.0 (C-1′), 78.3 (C-2′), 77.1 (C-3′), 76.4 (C-4′), 77.7 (C-5′), 62.4 (C-6′), 101.5 (C-1″), 72.5 (C-2″), 72.1 (C-3″), 73.9 (C-4″), 69.2 (C-5″), 18.5 (C-6″), 109.4 (C-1‴), 82.4 (C-2‴), 77.3 (C-3‴), 86.5 (C-4‴), 61.3 (C-5‴), 104.5 (C-1⁗), 74.9 (C-2⁗), 78.0 (C-3⁗), 71.5 (C-4⁗), 78.0 (C-5⁗), 62.7 (C-6⁗). Positive-ion FAB-MS *m/z*: 1057 $(M+Na)^+$. Negative-ion FAB-MS *m/z*: 1033 $(M-H)^-$. (b) The ^1H and ^{13}C NMR spectra of **5** were assigned with the aid of homo- and hetero-correlation spectroscopy (^1H–^1H, ^{13}C–^1H COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple bond connectivity (HMBC), and homo- and heteronuclear Hartmann-Hahn spectroscopy (^1H–^1H, ^{13}C–^1H HOHAHA) experiments. The detailed elucidation of chemical structure of **5** will be present in a full paper.
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