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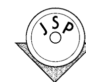

NOTE

New maltol glycosides from Flos Sophorae

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Abstract Three new maltol glycosides, designated soyamalosides A (**1**), B (**2**), and C (**3**), together with eight known compounds (**4–11**), were obtained from a 70 % EtOH extract of Flos Sophorae. Their structures were elucidated by chemical and spectroscopic methods. Of the known compounds, this is the first report of **4–6**, **9**, and **11** in the *Sophora* genus. Compounds **2**, **3**, and **10** showed significant protective effects against antimycin A-induced L6 cell injury.

Keywords Flos Sophorae · Maltol glycosides · Soyamaloside · L6 cell · Antioxidative activity

Introduction

Sophora japonica L. belongs to the Leguminosae family, and it is mainly distributed in Hebei, Henan, Jiangsu, Guangdong, and Guangxi provinces in China. As a Traditional Chinese Medicine (TCM), the flowers of *S. japonica* (Flos Sophorae) can be used for bloody diarrhea, bleeding, vomiting, epistaxis, headaches, dizziness, etc. [1]. Pharmacologic studies and clinical practice have demonstrated that it has anti-tumour, anti-fertility, and antioxidant activities [2, 3]. The main components in this plant were phenolic acids, flavonoids, and saponins [4]. During the course of our characterization studies on the constituents from Flos Sophorae, we have reported the isolation and structure elucidation of 15 constituents [5–7]. As a continuing study on this herbal medicine, three new maltol glycosides, designated soyamalosides A (**1**), B (**2**), and C (**3**), together with eight known ones were obtained from this TCM. In this paper, we describe the isolation and structure elucidation of the three new isolates. Furthermore, the protective effects of the isolated compounds against antimycin A-induced L6 cell injury were studied.

Results and discussion

The dried Flos Sophorae (8.0 kg) was extracted with 70 % ethanol–water 3 times to provide a 70 % ethanol–water extract (2.3 kg). The above-mentioned extract (670.0 g) was extracted with EtOAc–H_2O (1:1) to afford an EtOAc layer (78.0 g), EtOAc precipitation (197.0 g), and H_2O layer (385.0 g). The H_2O layer (348.3 g) was subjected to D101 column chromatography (CC) (H_2O → 95 % EtOH → acetone), and H_2O (213.1 g), 95 % EtOH (122.1 g), and acetone (8.2 g) eluted fractions were obtained.

The EtOAc layer and 95 % EtOH-eluted fraction was subjected to normal- and reversed-phase silica gel and Sephadex LH-20 column chromatography, and finally HPLC to give three new compounds, designated soyamalosides A (**1**), B (**2**), and C (**3**), along with eight known ones, 3-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl] maltol (**4**) [8], *N*,*N*'-dicoumaroylputrescine (**5**) [9], kakkasaponin II (**6**) [10], kaikasaponin I (**7**) [11], kaikasaponin III (**8**) [12], soyasaponin I (**9**) [13], dehydrosoyasaponin I (**10**) [12], and phaseoside IV (**11**)

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[13] (Fig. 1). Of the known isolates, this is the first report of **4–6**, **10** and **11** in the *Sophora* genus.

Soyamaloside A (**1**) was isolated as a white amorphous powder with negative optical rotation {$[\alpha]_D^{25}$ −20.5° (MeOH)}. Its molecular formula was determined to be $C_{21}H_{22}O_{10}$ by HRESI-TOF-MS (*m/z* 457.1109 $[M + Na]^+$, calcd for $C_{21}H_{22}O_{10}Na$, 457.1105). Its IR spectrum showed absorption bands ascribable to hydroxy (3446 cm^{-1}) and unsaturated ketone (1642 cm^{-1}) functions, aromatic ring (1618, 1514, 1446 cm^{-1}), and *O*-glycosidic linkage (1081 cm^{-1}). The ^{1}H-NMR (C_5D_5N), ^{13}C-NMR (Table 1) and various types of 2D-NMR experiments including ^{1}H–^{1}H COSY, HSQC, and HMBC spectra suggested the presence of a *trans*-*p*-coumaroyl [δ 6.57 (1H, d, $J = 16.0$ Hz, H-8″), 7.17 (2H, d, $J = 8.0$ Hz, H-3″,5″), 7.54 (2H, d, $J = 8.0$ Hz, H-2″,6″), 7.94 (1H, d, $J = 16.0$ Hz, H-7″)], a β-D-glucopyranosyl [δ 5.63 (1H, d, $J = 8.0$ Hz, H-1′)], and a maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one) moiety [8] [δ 2.38 (3H, s, 2-CH_3), 6.50 (1H, d, $J = 5.0$ Hz, H-5), 7.81 (1H, d, $J = 5.0$ Hz, H-6)]. The assignment of glycoside protons was made by proton–proton correlations observed from the ^{1}H–^{1}H COSY experiment (Fig. 2). Furthermore, the linkage positions of *trans*-*p*-coumaroyl and β-D-glucopyranosyl on the aglycone of **1** were determined by the HMBC experiment, which showed long-range correlations between δ_H 5.63 (H-1′) and δ_C 143.1 (C-3), δ_H 5.80 (H-4′) and δ_C 167.2 (C-9″) (Fig. 2). Finally, on acid hydrolysis and identification with HPLC analysis, the presence of D-glucose in **1** was clarified [14, 15]. Consequently, the structure of soyamaloside A was elucidated as maltol 3-*O*-(4′-*O*-*trans*-*p*-coumaroyl)-*β*-D-glucopyranoside (**1**).

Soyamaloside B (**2**) was obtained as a white amorphous powder with negative optical rotation {$[\alpha]_D^{25}$ –15.0° (MeOH)}. HRESI-TOF-MS analysis revealed its molecular formula to be $C_{27}H_{30}O_{14}$ (*m/z* 577.1541 $[M-H]^-$, calcd for $C_{27}H_{29}O_{14}$, 577.1563). Treatment of **2** with 1 M HCl yielded D-glucose [14, 15]. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1), together with ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **2** indicated that there were the same moieties as **1**, i.e. a maltol aglycone [δ 2.42 (3H, s, 2-CH_3), 6.46 (1H, d, $J = 4.5$ Hz, H-5), 8.01 (1H, d, $J = 4.5$ Hz, H-6)], a β-D-glucopyranosyl [δ 5.00 (1H, d, $J = 8.0$ Hz, H-1′)], and a *trans*-*p*-coumaroyl [δ 6.41 (1H, d, $J = 16.0$ Hz, H-8″), 6.81 (2H, d, $J = 8.0$ Hz, H-3″,5″), 7.48 (2H, d, $J = 8.0$ Hz, H-2″,6″), 7.68 (1H, d, $J = 16.0$ Hz, H-7″)]. Additionally, the above-mentioned spectra further suggested there was a 3-hydroxy-3-methylglutaric acid moiety [4] [δ_H: 1.34 (3H, s, 3‴-CH_3), 2.60 (2H, m, H_2-2‴), 2.63, 2.69 (1H each, both d, $J = 14.0$ Hz, H_2-4‴)]. The long-range correlations between δ_H 5.00 (H-1′) and δ_C 143.3 (C-3), δ_H 5.11 (H-3′) and δ_C 168.9 (C-9″), δ_H 4.24, 4.46 (H_2-6′) and δ_C 172.3 (C-1‴) elucidated the linkage positions of the maltol, *trans*-*p*-coumaroyl, β-D-glucopyranosyl, and 3-hydroxy-3-methylglutaric acid moieties (Fig. 2). This evidence led us to formulate the structure of soyamaloside B to be maltol 3-*O*-(3′-*O*-*trans*-*p*-coumaroyl-6′-*O*-(3-hydroxy-3-methylglutaroyl))-β-D-glucopyranoside (**2**).

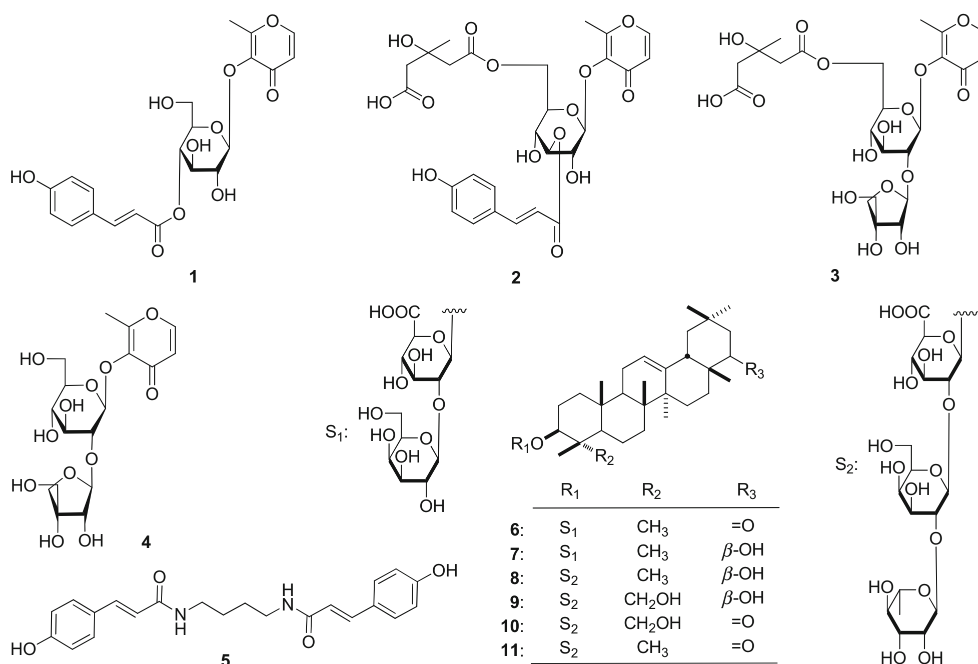

Fig. 1 Structures of compounds **1–11** obtained from Flos Sophorae

Table 1 ^{13}C-NMR data for **1–3**

No.	1[a]	1[b]	2[b]	3[b]	3[c]
2	161.7	164.5	164.6	164.1	160.2
3	143.1	143.6	143.3	142.5	140.6
4	175.2	177.0	177.0	176.3	172.9
5	117.0	117.4	117.5	117.8	116.5
6	155.2	157.1	157.3	156.6	154.8
1′	105.3	105.2	104.7	101.3	99.0
2′	75.8	75.7	73.8	78.6	76.3
3′	75.7	75.5	78.5	78.3	76.5
4′	72.1	71.9	69.7	71.6	70.1
5′	77.0	76.7	75.8	75.5	73.6
6′	62.1	62.1	64.0	64.3	62.8
1″	126.0	127.0	127.3	110.5	108.5
2″	130.8	131.2	131.2	78.0	76.0
3″	116.8	116.8	116.9	81.0	79.2
4″	161.6	161.4	161.3	75.6	74.0
5″	116.8	116.8	116.9	66.4	64.5
6″	130.8	131.2	131.2		
7″	145.8	147.3	146.8		
8″	114.9	114.6	115.4		
9″	167.2	168.5	168.9		
1‴			172.3	172.3	170.2
2‴			46.6	46.5	45.7
3‴			70.8	70.7	68.8
4‴			46.6	46.4	45.8
5‴			–	176.3	173.9
2-CH_3	15.4	15.8	15.8	15.8	14.8
3‴-CH_3			27.8	27.8	27.2

– not detected

[a] Measured in C_5D_5N

[b] Measured in CD_3OD

[c] Measured in DMSO-d_6

Soyamaloside C (**3**) was isolated as a white amorphous powder, which exhibited negative optical rotation {$[\alpha]_D^{25}$ −85.7° (MeOH)}. Its molecular formula was revealed to be $C_{23}H_{32}O_{16}$ by positive HRESI-TOF-MS analysis (*m/z* 587.1589 $[M + Na]^+$, calcd for $C_{23}H_{32}O_{16}$ Na, 587.1583). The IR spectrum absorption bands indicated the presence of an ester carbonyl (1725 cm^{-1}), an unsaturated ketone (1642 cm^{-1}), and an *O*-glycosidic linkage (1062 cm^{-1}). The ^{1}H-NMR (DMSO-d_6), ^{13}C-NMR (Table 1) and various 2D-NMR experiments, and references [8, 16], suggested that **3** possessed the same moiety as known compound **4**, maltol 3-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranosyl [δ 2.23 (3H, s, 2-CH_3), 5.05 (1H, d, $J = 7.0$ Hz, H-1′), 5.31 (1H, br. s, H-1″), 6.32 (1H, d, $J = 5.5$ Hz, H-5), 8.02 (1H, d, $J = 5.5$ Hz, H-6)]. In addition, the result of acid hydrolysis further validated the presence of D-glucose and D-apiose [14, 15]. Furthermore, there was a 3-hydroxy-3-methylglutaric acid functional group [δ_H: 1.15 (3H, s, 3‴-CH_3), 2.30, 2.35 (1H each, both d, $J = 16.0$ Hz, H_2-4‴), 2.41, 2.51 (1H each, both d, $J = 14.0$ Hz, H_2-2‴); δ_C: 27.2 (3‴-CH_3), 45.7 (C-2‴), 45.8 (C-4‴), 68.8 (C-3‴), 170.2 (C-1‴), 172.9 (C-5‴)] in **3**. In the HMBC experiment, a long-range correlation between δ_H [4.00 (1H, dd, $J = 6.0$, 11.5 Hz), 4.23 (1H, br. d, ca. $J = 12$ Hz), H_2-6′] and δ_C 170.2 (C-1‴) was observed. Consequently, the structure of soyamaloside C (**3**) was identified to be as shown in Fig. 1.

Antimycin A is known to bind to cytochrome C reductase, thereby inhibiting the oxidation of ubiquinol in the electron transport chain of oxidative phosphorylation, causing the inhibition of mitochondrial electron transport [17].

Compared with the normal group, 100 μg/mL antimycin A significantly induced an L6 cell survival rate of 73.5 %, while 10 μM probucol produced increased cell survival rate effects compared with the antimycin-treated group. Of the compounds **1–11**, **2**, **3**, and **10** showed significant protective effects against antimycin A-induced L6 cell injury (Table 2). Further studies of the antioxidant mechanisms of compounds **2** and **3** are necessary.

Experimental

General

Optical rotations were recorded on a Rudolph Autopol® IV automatic polarimeter. IR spectra were measured on a Varian 640-IR FT-IR spectrophotometer. UV spectra were determined on a Varian Cary 50 UV–Vis spectrophotometer. NMR spectra were conducted on a Bruker 500-MHz NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR, with TMS as an internal standard. Positive- and negative-ion HRESI-TOF-MS were recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer. Column chromatographies (CC) were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), Silica gel (74-149 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and ODS (50 μm, YMC Co., Ltd., Tokyo, Japan). Pre-coated TLC plates with Silica gel GF_{254} (Tianjin Silida Technology Co., Ltd., Tianjin, China) were used to detect the purity of isolates achieved by spraying with 10 % aqueous H_2SO_4–EtOH, followed by heating. Preparative HPLC (Prep-HPLC) column, Cosmosil $5C_{18}$-MS-II (20 mm i.d. × 250 mm, Nakalai Tesque, Inc., Tokyo, Japan), was used to purify the constituents.

Plant material

The Flos Sophorae were collected from Tangshan city, Hebei province, China and identified by Dr. Li Tianxiang.

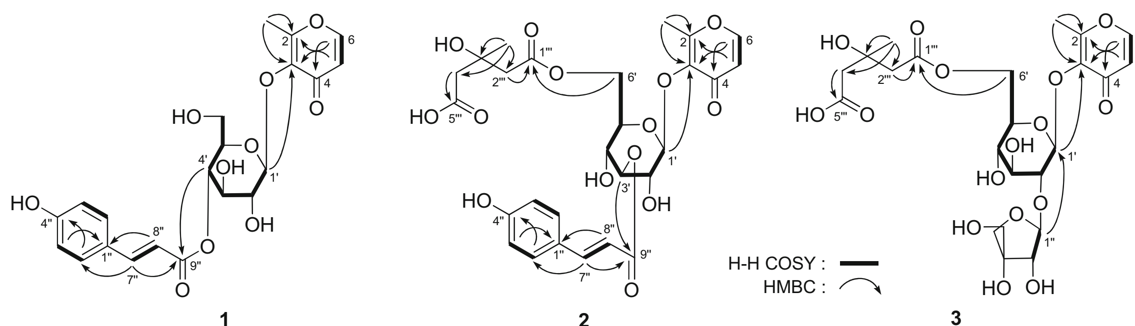

Fig. 2 The main ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**3**

Table 2 Effect of compounds **1**–**11** on cell survival rate of L6 cells treated with antimycin A

Sample	Cell survival rate (%)
Normal	100.0 ± 4.3**
Control	73.5 ± 0.8
Probucol	83.9 ± 0.9**
1	77.4 ± 1.4
2	80.4 ± 1.6*
3	88.6 ± 3.5**
4	67.4 ± 2.3
5	75.3 ± 1.9
6	67.7 ± 2.1
7	70.2 ± 2.4
8	79.5 ± 2.0
9	73.8 ± 3.0
10	82.0 ± 0.7*
11	73.1 ± 5.8

Values represent the mean ± SD of 8 determinations. The concentration of probucol and **1**–**11** administered was 10 μmol/L, $N = 6$

* $p < 0.05$ vs. control group

** $p < 0.01$ vs. control group

The voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM (No. 20120909).

Extraction and isolation

The dried Flos Sophorae (8.0 kg) was extracted with 70 % ethanol–water 3 times. Evaporation of the solvent under reduced pressure provided a 70 % ethanol–water extract (2.3 kg, 29.4 %). The 70 % ethanol–water extract (670.0 g) was partitioned with EtOAc–H_2O (1:1, v/v) to afford an EtOAc layer (SoE, 78.0 g, 3.4 %), EtOAc precipitation (197.0 g, 9.1 %), and H_2O layer (385.0 g, 16.9 %). The H_2O layer (348.3 g) was subjected to D101 CC (H_2O → 95 % EtOH → acetone), and H_2O (213.1 g, 10.9 %), 95 % EtOH (SoH, 122.1 g, 6.2 %), and acetone (8.2 g, 0.4 %) eluted fractions were obtained.

SoE (50.0 g) was isolated by silica gel CC [$CHCl_3$ → $CHCl_3$–MeOH (100:2 → 100:3 → 100:5, v/v) → $CHCl_3$–MeOH–H_2O (10:3:1, v/v/v, the lower layer) → MeOH], and seven fractions (SoE 1–7) were obtained. SoE 5 (7.0 g) was separated by ODS CC [MeOH–H_2O (30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, v/v) to give 12 fractions (SoE 5-1–5-12). SoE 5-4 (136.1 mg) was purified by Prep-HPLC [CH_3CN–(H_2O + 1 % HAc) (20:80, v/v)] to afford soyamaloside A (**1**, 15.2 mg, 0.00066 %). SoE 6 (7.5 g) was subjected to ODS CC [MeOH–H_2O (10:90 → 45:55 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, v/v)] to yield 12 fractions (SoE 6-1–6-12). SoE 6-3 (1462.0 mg) was isolated by Prep-HPLC [MeOH–H_2O (45:55, v/v)], and 11 fractions (SoE 6-3-1–6-3-11) were obtained. SoE 6-3-9 (828.3 mg) was separated by Prep-HPLC [CH_3CN–H_2O (23:77, v/v)], yielding soyamaloside B (**2**, 132.0 mg, 0.0082 %). SoE 6-7 (200.3 mg) was isolated by Prep-HPLC [CH_3CN–H_2O (45:55, v/v)] to afford *N*,*N*'-dicoumaroylputrescine (**5**, 12.1 mg, 0.00098 %).

SoH (72 g) was subjected to silica gel CC [$CHCl_3$ → $CHCl_3$–MeOH (100:2 → 100:5 → 100:7, v/v) → $CHCl_3$–MeOH–H_2O (10:3:1 → 7:3:1 → 6:4:1, v/v/v, lower layer) → MeOH], and 19 fractions (SoH 1–19) were obtained. SoH 12 (3.2 g) was isolated by Prep-HPLC [MeOH–H_2O (20:80 → 40:60 → 50:50 → 60:40 → 100:0, v/v)] to yield 21 fractions (SoH 12-1–12-21). SoH 12-5 (64.3 mg) was purified by Prep-HPLC [MeOH–(H_2O + 1 % HAc) (10:90, v/v)] to give 3-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl] maltol (**4**, 6.8 mg, 0.00055 %). SoH 12-15 (238.7 mg) was further separated by Prep-HPLC [CH_3CN–(H_2O + 1 % HAc) (23:77, v/v)], and soyamaloside B (**2**, 31.3 mg, 0.0083 %) was obtained. SoH 13 (5.0 g) was subjected by ODS CC [MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 100:0, v/v)] to give 15 fractions (SoH 13-1 → 13-15). SoH 13-5 (50.4 mg) was purified by Prep-HPLC [MeOH-(H_2O + 1 % HAc) (12:88, v/v)] to yield 3-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucopyranosyl] maltol (**4**, 4.0 mg, 0.00055 %). SoH 13-8 (188.8 mg) was separated by MeOH–(H_2O + 1 % HAc) (12:88, v/v) to obtain soyamaloside C (**3**, 16.1 mg, 0.00082 %). SoH 15 (6.0 g) was

subjected to ODS CC [MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20 → 100:0, v/v)] to give 20 fractions (SoH 15-1–15-20). SoH 15-19 (272.8 mg) was centrifuged repeatedly to give 2 fractions (SoH 15-19-1 and 15-19-2). SoH 15-19-2 (103.2 mg) was subjected to silica gel CC [$CHCl_3$–MeOH–H_2O (15:3:1, v/v/v, lower layer)] to produce kaikasaponin I (**7**, 28.9 mg, 0.0010 %). SoH 16 (8.5 g) was subjected to Sephadex LH-20 CC [MeOH–H_2O (1:1, v/v)] to give 4 fractions (SoH 16-1–16-4). SoH16-2 (1.54 g) was isolated by Prep-HPLC [MeOH–H_2O (70:30 → 90:10 → 100:0)] to give 21 fractions (SoH 16-2-1–16-2-21). SoH 16-2-18 (300.5 mg) was isolated by Prep-HPLC [MeOH–H_2O (72:28), v/v] and 10 fractions (SoH 16-2-18-1–16-2-18-10) were obtained. SoH 16-2-18-3 (123.4 mg) was further purified by silica gel CC [$CHCl_3$–MeOH–H_2O (7:3:1, v/v/v, lower layer)] to produce soyasaponin I (**9**, 70.1 mg, 0.0036 %). SoH 16-2-18-6 (89.1 mg) was pure and was identified as kaikasaponin III (**8**, 89.1 mg, 0.0046 %). SoH 16-2-19 (74.2 mg) was separated by silica gel CC [$CHCl_3$–MeOH–H_2O (10:3:1 → 7:3:1, v/v/v, lower layer)] to produce dehydrosoyasaponin I (**10**, 15.2 mg, 0.00078 %). SoH 16-2-20 (103.2 mg) was isolated by silica gel CC [$CHCl_3$-MeOH-H_2O (20:3:1 → 10:3:1, v/v/v, lower layer)] to produce kakkasaponin II (**6**, 6.8 mg, 0.00035 %) and phaseoside IV (**9**, 42.3 mg, 0.0022 %).

Soyamaloside A (**1**): white powder. $[\alpha]_D^{25}$ –20.5° (c = 0.42, MeOH); IR ν_{max} (KBr) cm^{-1}: 3446, 2955, 2694, 2286, 1642, 1618, 1514, 1446, 1293, 1255, 1167, 1081, 994, 922, 834; UV λ_{max} (MeOH) nm (log ε): 201 (4.50), 295 (3.99). ^{1}H-NMR (500 MHz, C_5D_5N): δ 6.50 (1H, d, J = 5.0 Hz, H-5), 7.81 (1H, d, J = 5.0 Hz, H-6), 5.63 (1H, d, J = 8.0 Hz, H-1′), 4.27 (1H, dd, J = 8.0, 9.0 Hz, H-2′), 4.45 (1H, dd, J = 9.0, 9.5 Hz, H-3′), 5.80 (1H, dd, J = 9.5, 9.5 Hz, H-4′), 4.08 (1H, m, H-5′), [4.12 (1H, dd, J = 6.0, 11.0 Hz), 4.21 (1H, br. d, ca. J = 11 Hz), H_2-6′], 7.54 (2H, d, J = 8.0 Hz, H-2″,6″), 7.17 (2H, d, J = 8.0 Hz, H-3″,5″), 7.94 (1H, d, J = 16.0 Hz, H-7″), 6.57 (1H, d, J = 16.0 Hz, H-8″), 2.38 (3H, s, 2-CH_3); ^{1}H NMR (500 MHz, CD_3OD): δ 6.46 (1H, d, J = 5.5 Hz, H-5), 8.01 (1H, d, J = 5.5 Hz, H-6), 4.90 (1H, d, J = 8.0 Hz, H-1′), 3.53 (1H, dd, J = 8.0, 9.0 Hz, H-2′), 3.72 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 4.93 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.53 (1H, m, H-5′), [3.54 (1H, dd, J = 5.0, 11.5 Hz), 3.85 (1H, br. d, ca. J = 12 Hz), H_2-6′], 7.46 (2H, d, J = 8.5 Hz, H-2″,6″), 6.81 (2H, d, J = 8.5 Hz, H-3″,5″), 7.67 (1H, d, J = 16.0 Hz, H-7″), 6.35 (1H, d, J = 16.0 Hz, H-8″), 2.48 (3H, s, 2-CH_3); ^{13}C-NMR (125 MHz, C_5D_5N) and (125 MHz, CD_3OD) spectroscopic data, see Table 1. HRESI-TOF-MS: positive-ion mode m/z 457.1109 $[M + Na]^+$ (calcd for $C_{21}H_{22}O_{10}Na$, 457.1105).

Soyamaloside B (**2**): white powder. $[\alpha]_D^{25}$ –15.0° (c = 0.56, MeOH); IR ν_{max} (KBr) cm^{-1}: 3312, 2941, 1720, 1621, 1604, 1514, 1441, 1386, 1329, 1243, 1165, 1062, 1026, 926, 835; UV λ_{max} (MeOH) nm (log ε): 208 (4.14), 259 (3.90), 309 (4.26). ^{1}H-NMR (500 MHz, CD_3OD): δ 6.46 (1H, d, J = 4.5 Hz, H-5), 8.01 (1H, d, J = 4.5 Hz, H-6), 2.42 (3H, s, 2-CH_3), 5.00 (1H, d, J = 8.0 Hz, H-1′), 3.62 (1H, dd, J = 8.0, 9.0 Hz, H-2′), 5.11 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 3.60 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.55 (1H, m, H-5′), [4.24 (1H, dd, J = 5.0, 12.0 Hz), 4.46 (1H, br. d, ca. J = 12 Hz), H_2-6′], 7.48 (2H, d, J = 8.0 Hz, H-2″,6″), 6.81 (2H, d, J = 8.0 Hz, H-3″,5″), 7.68 (1H, d, J = 16.0 Hz, H-7″), 6.41 (1H, d, J = 16.0 Hz, H-8″), 2.60 (2H, m, H_2-2‴), 2.63, 2.69 (1H each, both d, J = 14.0 Hz, H_2-4‴), 1.34 (3H, s, 3‴-CH_3); ^{13}C-NMR (125 MHz, CD_3OD) spectroscopic data, see Table 1. HR-ESI-TOF-MS: negative-ion mode m/z 577.1541 $[M-H]^-$ (calcd for $C_{27}H_{29}O_{14}$, 577.1563).

Soyamaloside C (**3**): white powder. $[\alpha]_D^{25}$ –85.7° (c = 0.85, MeOH); IR ν_{max} (KBr) cm^{-1}: 3377, 2926, 1725, 1642, 1616, 1568, 1434, 1252, 1194, 1062, 1010, 927, 843; UV λ_{max} (MeOH) nm (log ε): 207 (3.90), 255 (3.85). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 6.32 (1H, d, J = 5.5 Hz, H-5), 8.02 (1H, d, J = 5.5 Hz, H-6), 2.23 (3H, s, 2-CH_3), 5.05 (1H, d, J = 7.0 Hz, H-1′), 3.37 (1H, m, overlapped, H-2′), 3.39 (1H, m, overlapped, H-3′), 3.17 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.28 (1H, m, H-5′), [4.00 (1H, dd, J = 6.0, 11.5 Hz), 4.23 (1H, br. d, ca. J = 12 Hz), H_2-6′], 5.31 (1H, br. s, H-1″), 3.79 (1H, br. s, H-2″), [3.57 (1H, d, J = 9.0 Hz), 3.84 (1H, d, J = 9.0 Hz), H_2-4″], 3.37, 3.50 (1H each, both d, J = 9.5 Hz, H_2-5″), 2.41, 2.51 (1H each, both d, J = 14.0 Hz, H_2-2‴), 2.30, 2.35 (1H each, both d, J = 14.0 Hz, H_2-4‴), 1.15 (3H, s, 3‴-CH_3); ^{1}H-NMR (500 MHz, CD_3OD): δ 6.39 (1H, d, J = 5.5 Hz, H-5), 7.95 (1H, d, J = 5.5 Hz, H-6), 2.36 (3H, s, 2-CH_3), 5.11 (1H, d, J = 7.5 Hz, H-1′), 3.57 (1H, dd, J = 7.5, 9.0 Hz, H-2′), 3.55 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 3.36 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.38 (1H, m, H-5′), [4.20 (1H, dd, J = 5.5, 11.5 Hz), 4.37 (1H, dd, J = 1.5, 11.5 Hz), H_2-6′], 5.43 (1H, br. s, H-1″), 4.00 (1H, br. s, H-2″), 3.70, 4.05 (1H each, both d, J = 9.5 Hz, H_2-4″], 3.64, 3.72 (1H each, both d, J = 11.5 Hz, H_2-5″), 2.59, 2.64 (1H each, both d, J = 14.0 Hz, H_2-2‴), 2.53, 2.59 (1H each, both d, J = 15.5 Hz, H_2-4‴), 1.30 (3H, s, 3‴-CH_3); ^{13}C-NMR (125 MHz,DMSO-d_6) and (125 MHz, CD_3OD) spectroscopic data, see Table 1. HR-ESI-TOF-MS: positive-ion mode m/z 587.1589 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{16}Na$, 587.1583).

Acid hydrolysis of 1–3

Solutions of soyamalosides A–C (**1**–**3**, each 1.5 mg) were dissolved in 1 M HCl (1.0 mL) and heated under reflux for

1 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and removed by filtration. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co. Ltd., Tokyo, Japan); detection, optical rotation [Chiralyser (IBZ Messtechnik GMBH, Mozartstrasse 14–16 D-30173 Hannover, Germany)]; mobile phase, CH_3CN–H_2O (75:25, v/v); flow rate 1.0 mL/min. Identification of D-apiose (i) from **3** and D-glucose (ii) from **1–3** present in the aqueous layer was carried out by comparison of their retention times and optical rotations with those of authentic samples: (i) t_R 6.3 min (D-apiose, positive optical rotation), (ii) t_R 13.5 min (D-glucose, positive optical rotation).

Mitochondrial oxidative stress protection assay

Antimycin A was used to induce mitochondrial oxidative stress [18]. Briefly, L6 cells (Cell Resource Center, IBMS, CAMS/PUMC, Beijing, China) were plated at a density of 5×10^4 cells/well in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific, UT, USA) supplemented with 10 % calf serum (Thermo Scientific) in a 96-well plate and were incubated at 37 °C for 24 h. Cells were treated with or without 10 μmol/L sample DMSO solution (final DMSO concentration was 0.5 %). One hour later, medium was removed and 100 μg/mL antimycin A (Sigma Co. Ltd, MO, USA) in 100 μL DMEM was added to each well, The MTT assay was performed 4 h later to detect the cell survival rate. Probucol (10 μmol/L) was used as positive control.

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References

1. China Pharmacopoeia Committee (2009) Pharmacopoeia of the People's Republic of China, the First Division of 2010 Edition, China Chemical Industry Press, Beijing, p 83
2. de Lemos ML (2001) Effects of soy phytoestrogens genistein and daidzein on breast cancer growth. Ann Pharmacother 35:1118–1121
3. Han RM, Tian YX, Liu Y, Chen CH, Ai XC, Zhang JP, Skibsted LH (2009) Comparison of flavonoids and isoflavonoids as antioxidants. J Agric Food Chem 57:3780–3785
4. Kite GC, Stoneham CA, Veitch NC (2007) Flavonol tetraglycosides and other constituents from leaves of *Styphnolobium japonicum* (Leguminosae) and related taxa. Phytochemistry 68:1407–1416
5. Liu L, Li X, Chen Y, Sun J, Liu E, Zhang Y (2014) Study on phenolic acids from Flos Sophorae. Tianjin Zhongyiyao Daxue Xuebao 33:39–41
6. Liu L, Wang T, Li X, Shi P, Liu E, Zhang Y (2014) Isolation and identification of chemical constituents from Flos Sophorae II. Liaoning Zhongyiyao Daxue Xuebao 16:51–1653
7. Liu L, Li X, Chen Y, Shi P, Qu L, Zhang Y (2014) Isolation and identification of chemical constituents from Flos Sophorae I. Tianjin Zhongyiyao Daxue Xuebao 33:230–233
8. Djimtombaye BJ, Alankuş-Çalışkan Ö, Gülcemal D, Khan IA, Anıl H, Bedir E (2013) Unusual secondary metabolites from *Astragalus halicacabus* Lam. Chem Biodivers 10:1328–1334
9. Niwa T, Doi U, Osawa T (2003) Inhibitory activity of corn-derived bisamide compounds against α-glucosidase. J Agric Food Chem 51:90–94
10. Lu J, Sun JH, Tan Y, Kano Y, Yuan D (2013) New triterpenoid saponins from the flowers of *Pueraria thomsonii*. J Asian Nat Prod Res 15:1065–1072
11. Kitagawa I, Taniyama T, Wang H, Hori K, Yoshikawa M (1988) Saponin and sapogenol. XLV. Structures of kaikasaponins I, II, and III from sophorae flos, the buds of *Sophora japonica* L. Yakugaku Zasshi 108:538–546
12. Wen J, Shi H, Tu P (2006) Chemical constituents of *Abrus mollis*. Chin Tradit Herb Drug 37:658–660
13. Miyao H, Sakai Y, Takeshita T, Kinjo J, Nohara T (1996) Triterpene saponins from *Abrus cantoniensis* (Leguminosae). I. Isolation and characterization of four new saponins and a new sapogenol. Chem Pharm Bull 46:1222–1227
14. Morikawa T, Zhang Y, Nakamura S, Matsuda H, Muraoka O, Yoshikawa M (2007) Bioactive constituents from Chinese natural medicines. XXII. Absolute structures of new megastigmane glycosides, sedumosides E1, E2, E3, F1, F2, and G, from *Sedum sarmentosum* (Crassulaceae). Chem Pharm Bull 55:435–441
15. Yoshikawa M, Morikawa T, Zhang Y, Nakamura S, Muraoka O, Matsuda H (2007) Megastigmanes and their glucosides from the whole plant of *Sedum sarmentosum*. J Nat Prod 70:575–583
16. Liu Q, Liu YL (1989) Studies on chemical constituents *Glycyrrhiza eurycarpa* P. C. LI. Acta Pharm Sin 24:525–531
17. Piskernik C, Haindl S, Behling T, Gerald Z, Kehrer I, Redl H, Kozlov AV (2008) Antimycin A and lipopolysaccharide cause the leakage of superoxide radicals from rat liver mitochondria. Biochim Biophys Acta 1782:280–285
18. Zhang Y, Han LF, Sakah KJ, Wu ZZ, Liu LL, Agyemang K, Gao XM, Wang T (2013) Bioactive protopanaxatriol type saponins isolated from the roots of *Panax notoginseng* (Burk.) F. H Chen Mol 18:10352–10366