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New steroidal saponins from the roots of *Solanum melongena* L.

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ABSTRACT

Phytochemical investigation of the roots of *Solanum melongena* L. resulted in the isolation of six new steroidal saponins, including five new cholestane saponins (**1-5**) and one new steroidal alkaloid (**6**), along with one new natural product (**7**) and three know steroids (**8-10**). The structures of all isolated compounds were determined by 1D and 2D NMR experiments and by comparison of their spectroscopic and physical data with literature values. The inhibitory activities on nitric oxide (NO) production stimulated by lipopolysaccharide (LPS) in a RAW 264.7 cell line were assayed for all the isolated compounds. Compounds **1**, **2** and **4–9** exhibited moderate inhibition of NO production with IC_{50} values ranging from 12.6 to 59.5 μM.

1. Introduction

Solanum melongena L. (Solanaceae) is widely distributed in Southern Asia, the Middle East and Northern Africa [1], and its unripe fruit is primarily used as a vegetable. As a traditional Chinese medicine, the roots of *Solanum melongena* L. are used to treat chilblains, beriberi, pruritus, toothache [2], asthma, syphilis [3] and so on. A series of biological activities, such as anti-inflammatory, sedative, hypnotic, analgesic, neuroprotective and blood circulation-promoting effects [4,5] have been reported for pure compounds and crude extracts from the roots of *Solanum melongena* L. Previous phytochemical investigations have afforded the isolation of alkaloids [6], steroids [7], flavonoids [8], phenylpropanoid amides [9], coumarins [9] and lignans [10]. In this paper, the phytochemical composition of the roots of *Solanum melongena* L. were studied, resulting in six new steroidal saponins (**1-6**), one new natural product (**7**) and three know steroids (**8-10**) (Fig. 1). The isolation and structural determination of the new compounds are described here. The inhibitory activities of these compounds on nitric oxide (NO) production were stimulated with lipopolysaccharide (LPS) in the RAW 264.7 cell line.

2. Experimental

2.1. General experimental procedures

High-resolution electrospray ionization mass spectrometry (HRESIMS) was conducted using a Waters Xevo-TOF-MS™ instrument. Optical rotations were measured on a JASCO P-2000 instrument. The NMR spectra were recorded on a Bruker DPX 400 instrument (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Semi-preparative HPLC was performed with a Waters SunFireTM C_{18} (250 × 10 mm, 5 μm) column (Waters Corporation), and the HPLC system was equipped with a Shimadzu CBM-20A, RID (reflective index detector) detector and LC-6AD pump (Shimadzu Corporation, Japan). Column chromatography was performed using Sephadex LH-20 (Pharmacia) and silica gel (100–120 mesh and 200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China). The thin-layer chromatography used GF_{254}, and spots were detected by spraying the plates with 10% H_2SO_4-EtOH reagent followed by heating at 120 °C for 5 min.

2.2. Plant material

The roots of *Solanum melongena* L. were collected from Anguo of Hebei Province (China), and identified by Prof. Rui-Feng Fan of Heilongjiang University of Chinese Medicine. The voucher specimen (No. 20160918) was deposited at Heilongjiang University of Chinese Medicine.

2.3. Extraction and isolation

The air-dried roots of *Solanum melongena* L (12 kg) were cut into approximately 2 cm pieces and extracted under reflux with EtOH-H_2O (70:30 v/v) (3 × 120 L, 3 h each). The resulting extracts (940 g) were concentrated under vacuum (40 °C), suspended in H_2O (4.0 L), and partitioned successively with petroleum ether (PE) (3 × 4.0 L, 24 h each), EtOAc (3 × 4.0 L, 24 h each), and *n*-BuOH (3 × 4.0 L, 24 h each) to give EtOAc (88 g) and *n*-BuOH (135 g) fractions. The *n*-BuOH-soluble portion (135 g) was separated by silica gel column chromatography

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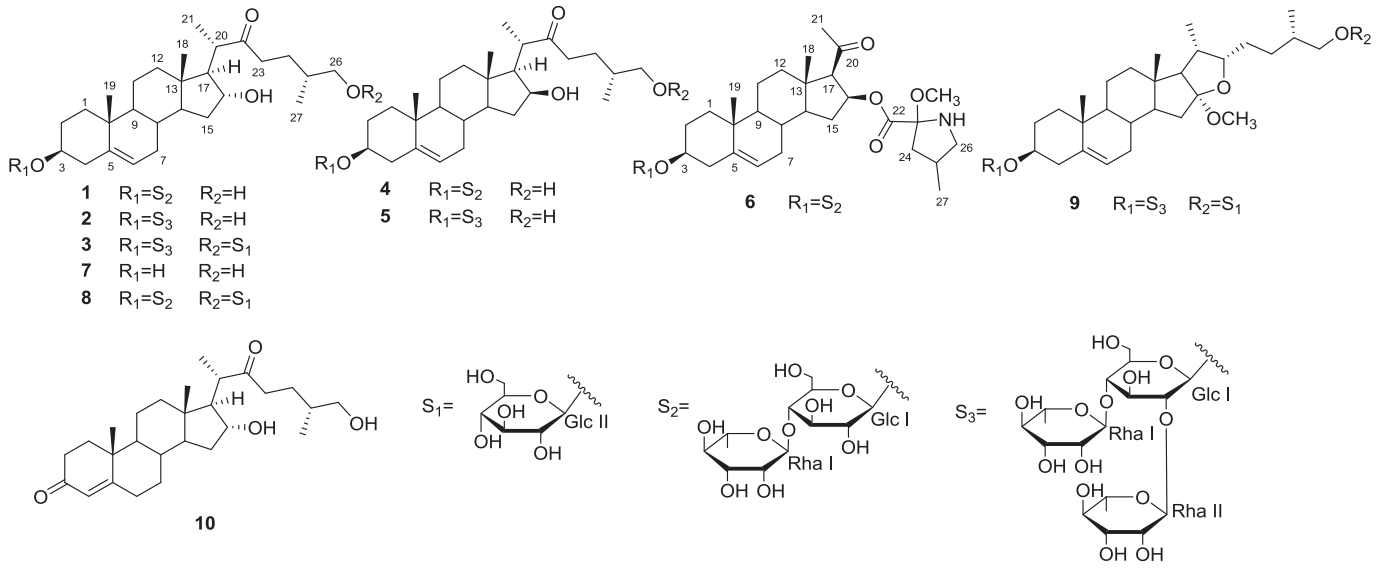

Fig. 1. Chemical structures of compounds **1-10.**

Table 1
^{1}H and ^{13}C NMR data for aglycons of compounds **1-5** (δ in ppm).

	1[a]		2[b]		3[a]		4[a]		5[a]	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	37.7	1.66 (m) 0.91 (m)	38.5	1.88 (m) 1.08 (m)	37.8	1.69 (m) 0.90 (m)	37.3	1.63 (m) 0.89 (m)	37.4	1.69 (m) 0.90 (m)
2	30.6	2.03 (m) 1.68 (m)	30.7	1.92 (m) 1.61 (m)	30.6	2.03 (m) 1.82 (m)	30.2	2.02 (m) 1.66 (m)	30.1	2.03 (m) 1.80 (m)
3	78.6	3.85 (m)	79.2	3.61 (m)	78.5	3.84 (m)	78.2	3.82 (m)	78.0	3.82 (m)
4	39.7	2.67 (dd, 13.2, 2.3) 2.42 (dd, 13.2, 2.5)	39.5	2.45 (dd, 13.2, 2.4) 2.29 (dd, 13.2, 2.8)	39.4	2.86 (overlap) 2.70 (overlap)	39.3	2.68 (dd, 11.8, 2.2) 2.42 (dd, 11.8, 2.4)	38.9	2.85 (overlap) 2.70 (overlap)
5	141.3	-	141.9	-	141.2	-	140.9	-	140.8	-
6	122.2	5.28 (br.d, 4.9)	122.6	5.37 (br.d, 4.9)	122.3	5.28 (br.d, 4.8)	121.8	5.29 (br.d, 4.7)	121.9	5.28 (br.d, 4.4)
7	32.6	1.84 (m) 1.50 (m)	32.9	1.95 (m) 1.55 (m)	32.6	1.83 (m) 1.48 (m)	32.2	1.88 (m) 1.50 (m)	32.2	1.88 (m) 1.53 (m)
8	32.0	1.34 (m)	32.6	1.45 (m)	32.1	1.33 (m)	31.6	1.36 (m)	31.6	1.37 (m)
9	50.7	0.90 (m)	51.6	0.99 (m)	50.8	0.87 (m)	50.3	0.87 (m)	50.3	0.87 (m)
10	37.3	-	37.9	-	37.4	-	36.9	-	37.0	-
11	21.3	1.37 (m)	21.7	1.55 (m) 1.48 (m)	21.3	1.36 (m)	20.8	1.31 (m) 1.23 (m)	20.8	1.32 (m) 1.22 (m)
12	40.3	1.85 (m) 1.22 (m)	41.0	1.98 (m) 1.37 (m)	40.3	1.84 (m) 1.22 (m)	38.9	1.58 (m) 1.22 (m)	39.1	1.58 (m) 1.20 (m)
13	44.5	-	45.1	-	44.5	-	43.6	-	43.5	-
14	54.4	1.42 (m)	54.9	1.40 (m)	54.4	1.44 (m)	53.6	1.51 (m)	53.6	1.51 (m)
15	37.5	1.78 (m) 1.65 (m)	37.1	1.64 (m) 1.51 (m)	37.5	1.78 (m) 1.65 (m)	37.6	1.86 (m) 1.74 (m)	37.6	1.88 (m) 1.75 (m)
16	76.4	4.26 (m)	77.1	3.91 (m)	76.4	4.27 (m)	75.0	4.30 (m)	75.0	4.31 (m)
17	63.7	1.85 (m)	63.8	1.51 (m)	63.7	1.84 (m)	62.1	2.15 (m)	62.1	2.14 (m)
18	13.8	0.67 (s)	13.7	0.76 (s)	13.8	0.67 (s)	14.5	0.72 (s)	14.5	0.72 (s)
19	19.8	0.89 (s)	19.8	1.02 (s)	19.8	1.03 (s)	19.4	0.87 (s)	19.4	1.01 (s)
20	49.9	2.79 (m)	50.5	2.68 (m)	49.8	2.76 (m)	47.3	2.81 (m)	47.3	2.80 (m)
21	17.2	1.17 (d, 6.8)	16.9	1.14 (d, 6.8)	17.2	1.18 (d, 6.8)	17.5	1.47 (d, 6.9)	17.4	1.46 (d, 6.9)
22	215.4	-	218.1	-	215.2	-	214.7	-	214.8	-
23	39.6	2.85 (m)	39.7	2.59 (m)	39.4	2.85 (m)	39.1	2.83 (m) 2.73 (m)	39.1	2.82 (m) 2.74 (m)
24	28.3	2.15 (m) 1.76 (m)	28.0	1.62 (m) 1.36 (m)	28.5	1.98 (m) 1.71 (m)	27.9	2.14 (m) 1.68 (m)	27.9	2.12 (m) 1.69 (m)
25	36.6	1.90 (m)	36.4	1.58 (m)	33.9	1.97 (m)	36.1	1.88 (m)	36.1	1.88 (m)
26	67.9	3.75 (dd, 10.4, 5.8) 3.68 (dd, 10.4, 6.1)	68.1	3.39 (overlap) 3.33 (overlap)	75.6	3.92 (dd, 9.4, 5.1) 3.58 (dd, 9.4, 5.8)	67.4	3.76 (overlap)	67.4	3.75 (overlap)
27	17.8	1.06 (d, 6.6)	17.0	0.90 (d, 6.6)	18.0	0.96 (d, 6.4)	17.2	1.08 (d, 6.7)	17.2	1.08 (d, 6.7)

[a] Measured in C_5D_5N(^{1}H: 400 MHz;^{13}C: 100 MHz).
[b] Measured in CD_3OD(^{1}H: 400 MHz;^{13}C: 100 MHz).

with CH_2Cl_2-MeOH mixtures of increasing polarity to give ten fractions (A1-A10). Fraction A2 (9 g) was subjected to ODS column chromatography with MeOH-H_2O (1:9 to 1:0) to afford sub-fractions A2A-A2I. Sub-fraction A2G (890 mg) was subjected to a Sephadex LH-20 column eluted with MeOH (flow rate: 0.8 mL/min) to yield subfraction A2G4 and then purified by semi-preparative HPLC using Waters SunFire™

Table 2
^{1}H and ^{13}C NMR data for sugar moieties of compounds **1-5** (δ in ppm).

	1[a]		2[b]		3[a]		4[a]		5[a]	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
Glc I										
1	102.9	4.94 (d, 7.7)	100.4	4.49 (d, 7.8)	100.7	4.93 (d, 6.2)	102.4	4.93 (d, 7.7)	100.3	4.90 (d, 7.1)
2	76.0	3.98 (dd, 9.3, 7.7)	79.3	3.38 (m)	79.0	4.40 (m)	75.5	3.98 (dd, 8.8, 7.7)	78.5	4.39 (m)
3	77.1	4.23 (m)	78.0	3.59 (m)	77.4	3.64 (m)	76.7	4.22 (m)	77.0	3.62 (m)
4	78.7	4.47 (m)	79.9	3.51 (m)	78.4	4.21 (m)	78.2	4.48 (m)	78.0	4.20 (m)
5	77.6	3.73 (m)	76.6	3.32 (m)	78.2	4.21 (m)	77.1	3.72 (m)	77.7	4.20 (m)
6'	61.9	4.26 (dd, 12.0, 5.1)	61.9	3.79 (dd, 12.2, 3.2)	61.7	4.18 (overlap)	61.5	4.26 (dd, 12.3, 4.9)	61.2	4.19 (overlap)
		4.13 (dd, 12.0, 3.4)		3.65 (dd, 12.2, 5.1)		4.02 (overlap)		4.13 (dd, 12.3, 3.5)		4.07 (overlap)
Rha I										
1	103.1	5.91 (br.s)	103.0	4.83 (d, 1.5)	103.4	5.86 (br.s)	102.7	5.91 (br.s)	102.9	5.85 (br.s)
2	73.1	4.71 (m)	72.3	3.91 (m)	73.0	4.83 (m)	72.6	4.71 (m)	72.5	4.82 (m)
3	73.2	4.58 (dd, 9.2, 3.3)	72.1	3.62 (m)	73.3	4.56 (m)	72.8	4.59 (dd, 9.2, 3.3)	72.8	4.53 (m)
4	74.4	4.35 (dd, 9.3, 9.2)	73.9	3.38 (m)	74.6	4.33 (m)	74.0	4.35 (dd, 9.3, 9.2)	74.1	4.32 (m)
5	70.8	5.05 (m)	70.6	3.92 (m)	70.9	4.92 (m)	70.3	5.04 (m)	70.4	4.93 (m)
6	19.0	1.72 (d, 6.2)	17.8	1.25 (d, 6.2)	19.0	1.62 (d, 6.2)	18.5	1.72 (d, 6.2)	18.5	1.62 (d, 6.2)
Rha II										
1			102.3	5.20 (d, 1.4)	102.5	6.40 (br.s)			102.0	6.39 (br.s)
2			72.1	3.92 (m)	73.0	4.83 (m)			72.6	4.82 (m)
3			72.4	3.65 (m)	73.2	4.62 (m)			72.8	4.61 (m)
4			73.7	3.41 (m)	74.4	4.31 (m)			74.0	4.32 (m)
5			69.7	4.12 (m)	70.0	4.94 (m)			69.5	4.94 (m)
6			17.9	1.23 (d, 6.3)	19.1	1.76 (d, 6.2)			18.7	1.74 (d, 6.2)
Glc II										
1					105.4	4.81 (d, 7.8)				
2					75.7	4.02 (m)				
3					79.0	4.38 (m)				
4					72.1	4.22 (m)				
5					79.1	4.24 (m)				
6					63.3	4.53 (overlap)				
						4.38 (overlap)				

[a] Measured in C_5D_5N(^{1}H: 400 MHz;^{13}C: 100 MHz).
[b] Measured in CD_3OD(^{1}H: 400 MHz;^{13}C: 100 MHz).

C_{18} column (MeOH-H_2O, 73:27; flow rate: 3 mL min^{-1}) to yield compound **10** (24 mg, t_R 20.5 min). Sub-fraction A2H (782 mg) was also separated by semi-preparative HPLC (MeOH-H_2O, 75:25; flow rate: 3 mL min^{-1}) to give compound **7** (18 mg, t_R 22.5 min). Fraction A7 (8.7 g) was subjected to ODS column chromatography with MeOH-H_2O (1:9 to 1:0) to afford sub-fractions A7A-A7J. Sub-fraction A7E was isolated by a Sephadex LH-20 column eluted with MeOH (flow rate: 0.8 mL/min) to afford subfraction A7E4, which was further purified by semi-preparative HPLC (MeOH-H_2O, 63:37; flow rate: 3 mL min^{-1}) to yield compound **6** (9.0 mg, t_R 21.4 min). Subfraction A7F (2.8 g) was chromatographed by semi-preparative HPLC (MeOH-H_2O, 59:41; flow rate: 3 mL min^{-1}) to give compounds **4** (12.3 mg, t_R 18.5 min), **5** (6.8 mg, t_R 23.0 min) and **8** (18.3 mg t_R 32.5 min). Subfraction A7G (1.3 g) was also isolated by semi-preparative HPLC (MeOH-H_2O, 66:34; flow rate: 3 mL min^{-1}) to afford compounds **2** (10.1 mg, t_R 23.4 min) and **1** (15.6 mg, t_R 28.5 min). Fraction A8 (7.8 g) was subjected to ODS column chromatography with MeOH-H_2O (1:9 to 1:0) to give sub-fractions A8A-A8H. Sub-fraction A8F (560 mg) was separated by semi-preparative HPLC (MeOH-H_2O, 61:39; flow rate: 3 mL min^{-1}) to yield compounds **3** (7.8 mg, t_R 27.8 min) and **9** (9.8 mg, t_R 37.5 min).

2.3.1. Abutiloside P (1)

White amorphous powder; $[\alpha]_D^{22} = -33.0$, (c = 0.1, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see Table 1; ^{1}H and ^{13}C NMR data for the sugar moiety, see Table 2; HRESIMS: m/z 763.4243 [M + Na]$^+$, (calcd. for $C_{39}H_{64}NaO_{13}$, m/z 763.4245)

2.3.2. Abutiloside Q (2)

White amorphous powder; $[\alpha]_D^{22} = -22.0$, (c = 0.1, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see Table 1; ^{1}H and ^{13}C NMR data for the sugar moiety, see Table 2; HRESIMS: m/z 909.4825 [M + Na]$^+$, (calcd. for $C_{45}H_{74}NaO_{17}$, m/z 909.4824)

2.3.3. Abutiloside R (3)

White amorphous powder; $[\alpha]_D^{22} = -31.0$, (c = 0.1, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see Table 1; ^{1}H and ^{13}C NMR data for the sugar moiety, see Table 2; HRESIMS: m/z 1071.5344 [M + Na]$^+$, (calcd. for $C_{51}H_{84}NaO_{22}$, m/z 1071.5352)

2.3.4. Abutiloside S (4)

White amorphous powder; $[\alpha]_D^{24}$ = -23.0, (c = 0.1, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see Table 1; ^{1}H and ^{13}C NMR data for the sugar moiety, see Table 2; HRESIMS: m/z 763.4255 [M + Na]$^+$, (calcd. for $C_{39}H_{64}NaO_{13}$, m/z 763.4245)

2.3.5. Abutiloside T (5)

White amorphous powder; $[\alpha]_D^{21}$ = -55.0, (c = 0.2, MeOH); ^{1}H and ^{13}C NMR data for the aglycon moiety, see Table 1; ^{1}H and ^{13}C NMR data for the sugar moiety, see Table 2; HRESIMS: m/z 909.4846 [M + Na]$^+$, (calcd. for $C_{45}H_{74}NaO_{17}$, m/z 909.4824)

2.3.6. Abutiloside U (6)

White amorphous powder; $[\alpha]_D^{22} = -12.0$, (c = 0.1, MeOH); ^{1}H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS: m/z 804.4138 [M + Na]$^+$, (calcd. for $C_{40}H_{63}NaNO_{14}$, m/z 804.4146)

2.4. Acid hydrolysis of compounds 1-6

The compounds (**1-6**) (1 mg each) were hydrolyzed by refluxing with 1 M HCl (1 mL) for 4 h. After cooling, the dried residues obtained

Table 3
^{1}H and ^{13}C NMR data for compounds **6** and **7** (δ in ppm).

	6[a]		7[b]	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	37.4	1.67 (m)	38.4	1.87 (m)
		0.94 (m)		1.10 (m)
2	30.2	2.06 (m)	32.3	1.79 (m)
		1.24 (m)		1.49 (m)
3	78.1	3.86 (m)	72.4	3.40 (m)
4	39.3	2.68 (dd, 10.5, 2.7)	43.0	2.22 (m)
		2.40 (dd, 10.5, 7.1)		
5	140.9	-	142.3	-
6	121.8	5.30 (br.d, 5.1)	122.2	5.34 (br.d, 5.1)
7	32.1	1.86 (m)	32.9	1.97 (m)
		1.50 (m)		1.62 (m)
8	31.0	1.52 (m)	32.7	1.51 (m)
9	50.5	0.83 (m)	51.6	1.02 (m)
10	37.0	-	37.7	-
11	20.7	1.41 (m)	21.8	1.58 (m)
12	38.3	2.03 (m)	41.0	1.96 (m)
		1.00 (m)		1.40 (m)
13	42.4	-	45.1	-
14	54.3	0.69 (overlap)	54.9	1.44 (m)
15	37.6	2.39 (m)	37.2	1.66 (m)
		0.99 (m)		1.51 (m)
16	74.5	5.67 (ddd, 4.8, 8.2, 8.2)	77.1	3.91 (m)
17	67.8	2.43 (d, 8.2)	63.8	1.62 (m)
18	14.8	1.38 (s)	13.7	0.77 (s)
19	19.4	0.89 (s)	19.8	1.02 (s)
20	206.5	-	50.0	2.68 (m)
21	31.3	2.27 (s)	17.0	1.15 (d, 6.9)
22	167.4	-	218.1	-
23	97.2	-	39.7	2.59 (m)
24	40.7	2.08 (m)	28.0	1.65 (m)
		1.59 (m)		1.36 (m)
25	26.0	2.37 (m)	36.4	1.59 (m)
26	49.0	3.22 (overlap)	68.1	3.40 (dd, 10.8, 5.0)
		2.76 (overlap)		3.35 (dd, 10.8, 4.5)
27	18.6	0.73 (d, 6.6)	17.1	0.91 (d, 6.7)
Glc I				
1	102.4	4.95 (d, 7.8)		
2	75.6	3.98 (dd, 8.7, 7.8)		
3	76.7	4.22 (dd, 9.3, 8.7)		
4	78.2	4.47 (dd, 9.4, 9.3)		
5	77.2	3.73 (m)		
6	61.5	4.26 (dd, 12.2, 5.1)		
		4.13 (dd, 12.2, 3.4)		
Rha I				
1	102.7	5.90 (br.s)		
2	72.7	4.70 (m)		
3	72.8	4.58 (dd, 9.2, 3.3)		
4	74.0	4.34 (dd, 9.4, 9.2)		
5	70.4	5.03 (m)		
6	18.5	1.72 (d, 6.2)		
MeO	49.9	3.39 (s)		

[a] Measured in C_5D_5N(^{1}H: 400 MHz;^{13}C: 100 MHz).
[b] Measured in CD_3OD(^{1}H: 400 MHz;^{13}C: 100 MHz).

were partitioned between ethyl acetate and water. The residue from the water part was dissolved in 1-(trimethylsilyl)imidazole and pyridine (1 mL), and was maintained at 60 °C for 5 min. After drying the solution, the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC [detector, flame ionization detector (FID); detector temperature, 280 °C; injection temperature, 250 °C; DB-5 capillary column, 30 m × 0.25 mm × 0.25 μm; column temperature, 100 °C for 2 min and then increased to 280 °C at a rate of 10 °C/min; final temperature, 280 °C for 5 min; and carrier gas, N_2]. By comparison with the retention time of authentic sugars, the absolute configurations of sugar components were determined (D-glucose, 19.50 min; L-rhamnopyranose, 18.32 min) [11,12].

2.5. Cell culture and cell viability assay

RAW 264.7 cells (mouse macrophages) (Shanghai Institutes for Biological Sciences) were maintained in DMEM supplemented with 10% FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a humidified atmosphere containing 5% CO_2 at 37 °C. An MTT assay was used to determine cell viability [13]. In brief, RAW 264.7 macrophages were seeded in 96-well plastic plates and treated with 1–80 μM steroids from the roots of *Solanum melongena* L. for 24 h, respectively. MTT (5 mg/mL) reagent was then added to the wells and incubated for 4 h. The formazan formed in the cell pellets was dissolved by adding 100 μL of DMSO, and the absorbance at 570 nm was measured with a microplate reader.

2.6. Inhibitory assay of NO production

An inhibitory assay of NO production was examined in accordance with a method described previously [14] with minor modifications. Briefly, RAW 264.7 cells (1×10^5 cells in 100 μL) were seeded into a 96-well microplate, grown for 24 h, then stimulated with 1 μg/mL LPS in a serum-free medium containing various concentrations of the compounds (1, 5, 10, 20, 40 and 80 μM) for 24 h. Then the supernatant of culture medium was transferred to a new 96-well microplate and 50 μL of 0.15% *N*-(1-naphtyl)ethylenediamine in H_2O and 1.5% sulfanilamide in 7.5% phosphoric acid were added. The absorbance at 570 nm was measured with a microplate reader.

3. Results and discussion

Compound **1** was obtained as a white amorphous solid. High resolution-election impact (HRESIMS) showed a molecular ion at *m/z* 763.4243 ($[M+Na]^+$, calcd. for 763.4245), which agreed with the molecular formula $C_{39}H_{64}O_{13}$.

The ^{1}H-NMR spectrum of **1** (Tables 1 and 2) showed four methyl proton signals of a typical steroidal skeleton at δ 1.06 (3H, d, J = 6.6 Hz, H_3 -27), δ 1.17 (3H, d, J = 6.8 Hz, H_3-21), δ 0.67, δ 0.89 (3H each, both s, H_3-18, 19), an olefinic hydrogen δ 5.28 (1H br.d, J = 4.9 Hz, H-6), together with glucopyranosyl δ 4.94 (1H, d, J = 7.7 Hz, $H_{Glc\ I}$-1) and one rhamnopyranosyl δ 5.91 (1H, br. s, $H_{Rha\ I}$-1). The above ^{1}H NMR data, together with olefinic carbons signals at (δ 141.3, C-5) and (122.2, C-6) and a carbonyl carbon signal at (δ 215.4, C-22) in the ^{13}C-NMR spectrum, suggested **1** to be a $^{\Delta}$5,6-cholestane skeleton in the aglycone with two sugar units. The ^{13}C-NMR data of **1** were similar to abutiloside G obtained previously from the *Solanum abutiloides* [7], and the major difference between their ^{13}C-NMR spectra was the absence of a group of glucosyl carbon signals in compound **1**. In addition, further comparative study of ^{13}C-NMR spectrum between **1** and abutilosides G found that the chemical shifts of C-25 (δ 36.6) moved to the low field and the chemical shifts of C-26 (δ 67.9) moved to the high field. The chemical shifts of C-26 (δ 67.9) and C-25 (δ 36.6) suggested that C-26 was linked with a hydroxyl group. Starting from the two anomeric protons, the exact identity of the monosaccharides and the sequence of the disaccharide chain were also determined by analysis of a combination of DEPT, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra. The connectivity of the two sugars was mainly based on the HMBC correlations: $H_{Glc\ I}$-1 (δ 4.94, 1H, d, J = 7.7 Hz) with C-3 (δ 78.6) of the aglycone, $H_{Rha\ I}$-1 (δ 5.91, 1H, br. s) with $C_{Glc\ I}$-4 (δ 78.7) (Fig. 2). The anomeric configuration of glucose was determined to be β on the basis of the J value of the anomeric proton in glucose (J = 7.7 Hz), the α-configuration of rhamnopyranosyls was determined by the two sets of chemical shifts: $C_{Rha\ I}$-3 (δ 73.2), $C_{Rha\ I}$-5 (δ 70.8) based on the literature [15]. The two sugars, D-glucose and L-rhamnopyranosyls were identified by GC analysis after derivatization.

The NOESY correlations (Fig. 3) of Me-19/H-1β, H-1α/H-3, Me-18/H-16, H-20 indicated the α-orientation of H-3 and OH-16. The absolute configuration of 25*R* in **1** was established by the chemical shift of H_2-26

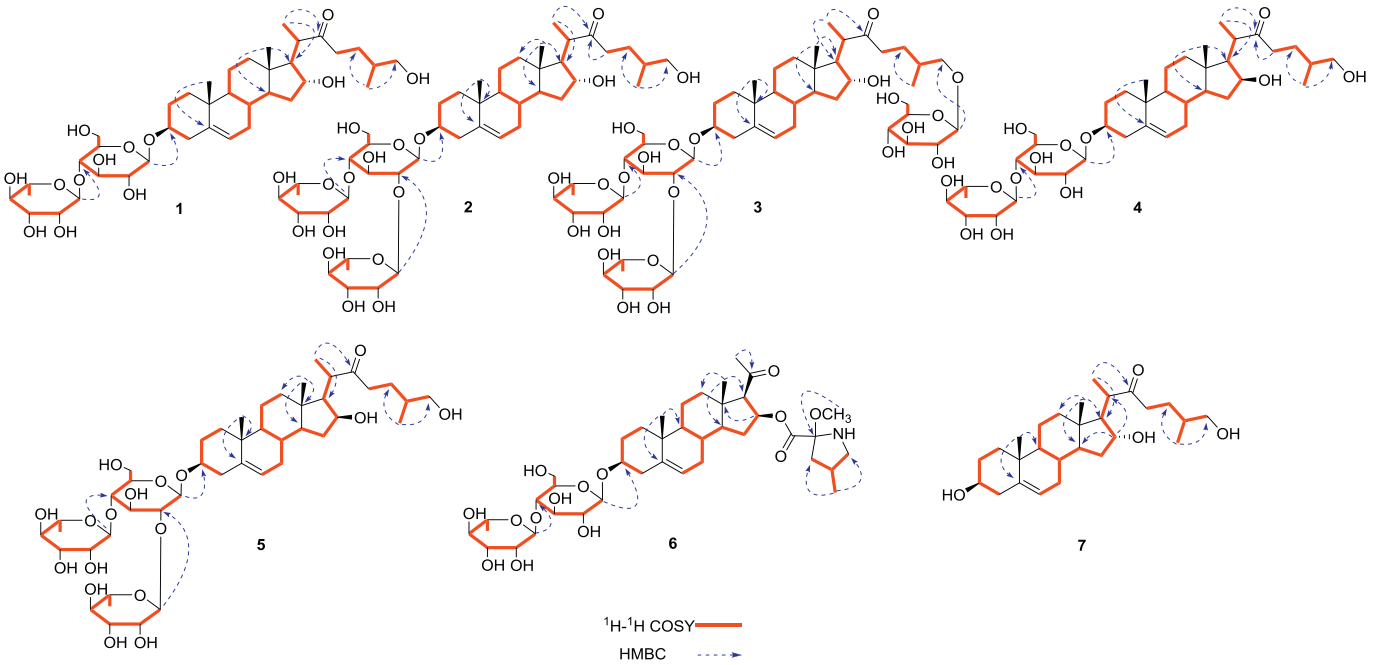

Fig. 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1–7**.

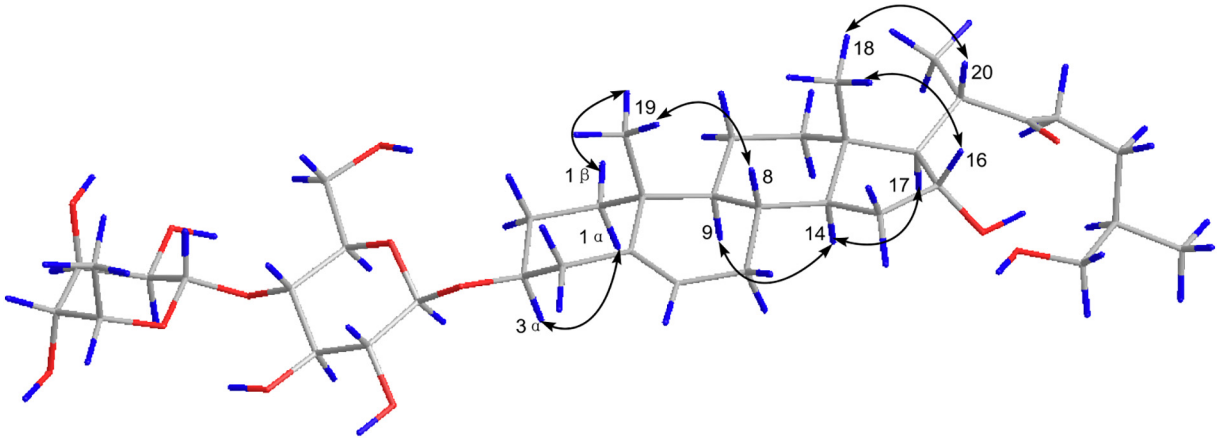

Fig. 3. Key NOESY correlations of compound **1**.

(δ 3.75 and 3.68 ppm, $^{\Delta}\delta = 0.07$) ($^{\Delta}\delta \leq 0.57$ ppm) [16]. Thus, **1** was inferred as (25*R*) 3*β*, 16*α*, 26-trihydroxy-5-en-cholestan-22-one-3-O-*α*-L-rhamnopyranosyl- (1 → 4)-*β*-D-glucopyranoside, and named Abutiloside P.

Compound **2** was obtained as a white amorphous powder with a molecular formula of $C_{45}H_{74}O_{17}$ as determined by the HRESIMS (*m/z* 909.4825 $[M+Na]^+$ calcd. for 909.4824). The NMR spectra of **2** (Tables 1 and 2) were nearly identical to those of **1**, with one major difference due to the presence of one more sugar in **2** as indicated by the addition of anomeric proton and the carbon signal at δ 4.83 (1H, d, J = 1.5 Hz, $H_{Rha\ II}$-1) and δ 103.0 ($C_{Rha\ II}$-1), respectively. This suggested that **2** contained a diglycosidic moiety. The attachment of the rhamnopyranosyl units to C-2 of the glucose was confirmed by the HMBC correlations observed between δ 4.83 (1H, d, J = 1.5 Hz, $H_{Rha\ II}$-1) and δ 79.3 ($C_{Glc\ I}$-2) (Fig. 2). The relative configurations of **2** were determined to be the same as those of **1** by a NOESY experiment. Finally, the structure of **2** was established as (25*R*) 3*β*, 16*α*, 26-trihydroxy-5-en-cholestan-22-one-3-O-*α*-L-rhamnopyranosyl-(1 → 2)-[*α*-L-rhamnopyranosyl-(1 → 4)]-*β*-D- glucopyranoside, and named Abutiloside Q.

Compound **3** was separated as a white amorphous powder. The molecular formula of **3** was established as $C_{51}H_{84}O_{22}$ from its HRESIMS at *m/z* 1071.5344 $[M+Na]^+$ (calcd. for 1071.5352). In the ^{1}H and ^{13}C-NMR spectrum of **3** (Tables 1 and 2), signals due to an aglycone moiety were similar with those of **2**, although the signals due to the sugar moiety were not identical. A comparative study of the ^{13}C-NMR of **2** with that of **3** indicated the presence of an additional glucosyl unit in **3**, which was linked to C-26 hydroxy group of aglycone moiety, because the chemical shifts of C-25 (δ 33.9) moved to the high field and the chemical shifts of C-26 (δ 75.6) moved to the low field. The long-range correlations were observed between the anomeric proton at δ 4.81 (1H, d, J = 7.8, $H_{Glc\ II}$-1) with the carbon at C-26 (δ 75.6) from the HMBC experiment (Fig. 2). The relative configurations of **3** were determined by a NOESY experiment, which were consistent with those of **2**. Consequently, the structure of **3** was determined to be (25*R*) 26-O-*β*-D-glucopyranoside 3*β*, 16*α*, 26-trihydroxy-5-en-cholestan-22-one-3-O-*α*-L-rhamnopyranosyl-(1 → 2) -[*α*-L-rhamnopyranosyl-(1 → 4)]-*β*-D-glucopyranoside, and named Abutiloside R.

Compound **4** possessed a molecular formula of $C_{39}H_{64}O_{13}$ on the basis of the HRESIMS with $[M+Na]^+$ at *m/z* 763.4255 (calcd. for 763.4245). The NMR spectroscopic data of **4** (Tables 1 and 2) were closely related to those of **1**. A comparison of the ^{13}C-NMR data of these

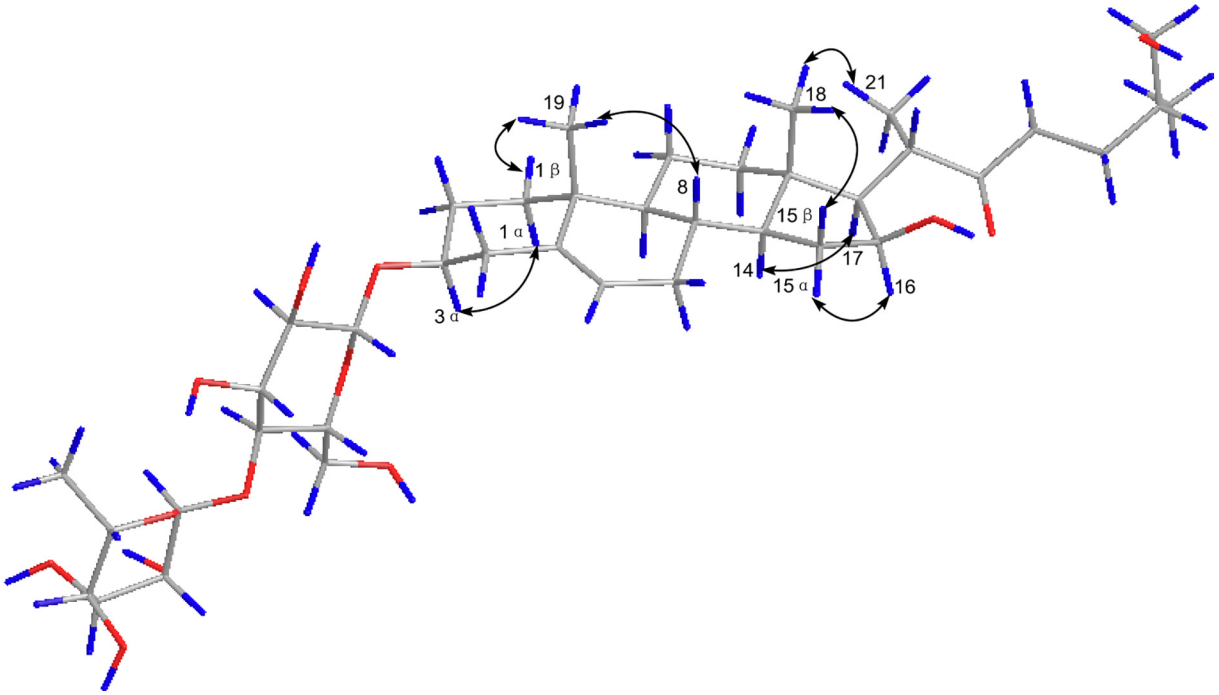

Fig. 4. Key NOESY correlations of compound **4.**

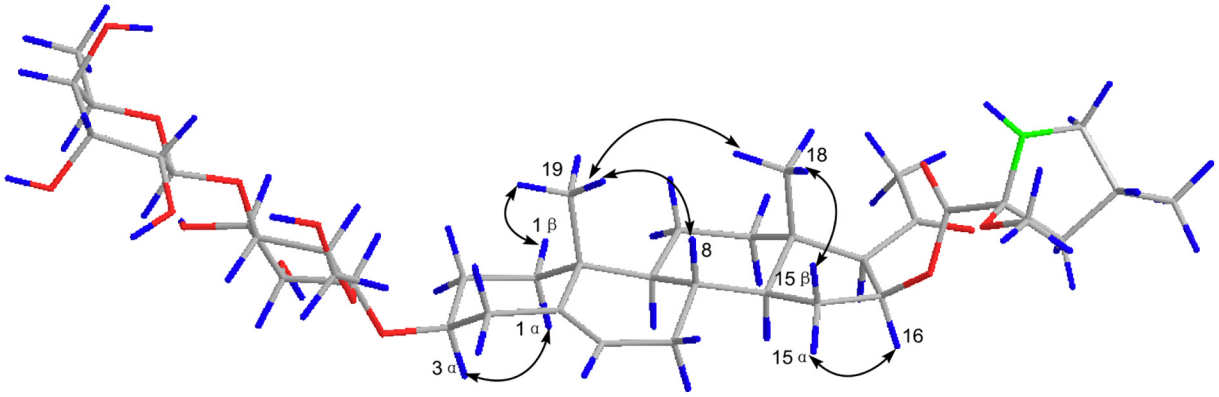

Fig. 5. Key NOESY correlations of compound **6.**

two compounds indicated that they have same planar structures. The key differences were the signals from carbons around C-16, which suggested that these two compounds should be different orientations of C-16. The orientation of H-16 was deduced by the NOESY observed for Me-18/Me-21, H-15β and H-15α/H-16 (Fig. 4), indicating a β-orientation of OH-16. The rest of the configurations of **4** were determined to be the same as those of **1**. Thus, compound **4** was determined to be (25*R*) 3β,16β,26-trihydroxy-5-en-cholestan-22-one-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, and named Abutiloside S.

Compound **5** was obtained as a white amorphous powder. Its molecular formula was deduced as $C_{45}H_{74}O_{17}$ by HRESIMS at (*m/z* 909.4846 $[M+Na]^+$ calcd. for 909.4824). Comparison of the NMR data (Tables 1 and 2) of **5** with those of **4** showed the close similarity between the structures of both compounds, except for the presence of one additional sugar in **5** as indicated by the addition of an anomeric proton and the carbon signal at δ 6.39 (1H, br. s, $H_{Rha\ II}$-1) and δ 102.0 ($C_{Rha\ II}$-1), respectively. The attachment of the rhamnopyranosyl units to the C-2 of the glucose was determined by the HMBC correlations observed between δ 6.39 (1H, br. s, $H_{Rha\ II}$-1) and δ 78.5 ($C_{Glc\ I}$-2) (Fig. 2). The relative configurations of **5** were established to be the same as those of **4** by a NOESY experiment. Therefore, the structure of **5** was determined to be (25*R*) 3β, 16β, 26-trihydroxy-5-en-cholestan-22-one-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside, and named Abutiloside T.

Compound **6** was obtained as an amorphous powder and its molecular formula was $C_{40}H_{63}NO_{14}$ according to the HRESIMS at *m/z* 804.4138 $[M+Na]^+$ (calcd. for 804.4146). The ^{1}H-NMR spectrum (Table 3) of **6** showed signals due to two tertiary methyl groups (δ 1.38, 3H, s; δ 0.89, 3H, s), two secondary methyl groups (δ 1.72, 3H, d, J = 6.2 Hz; δ 0.73, 3H, d, J = 6.6 Hz), one acetyl group (δ 2.27, 3H, s), one methoxyl group (δ 3.39, 3H, s), and one olefinic proton (δ 5.30, 1H, br.d, J = 5.1 Hz). The ^{13}C-NMR spectrum (Table 3) of **6** showed 40 carbon signals including two carbonyl carbons (δ 206.5, 167.4), two olefinic carbons (δ 140.9, 121.8), one nitrogen- and oxygen-bearing carbon (δ 97.2), and two anomeric carbons (δ 102.7, 102.4), suggesting **6** to be a disaccharide of a steroidal alkaloid. The ^{1}H- and ^{13}C-NMR signals of aglycone moiety of **6** were superimposable on those of abutiloside O, which was obtained previously from the underground parts of *Solanum sodomaeum* [17]. The ^{1}H- and ^{13}C-NMR signals of the sugar moiety were very similar to those of **1**. In the HMBC spectrum, key correlations of **6** were observed as illustrated in (Fig. 2). The stereochemistry of C-16 and C-17 was determined by analysis of the coupling

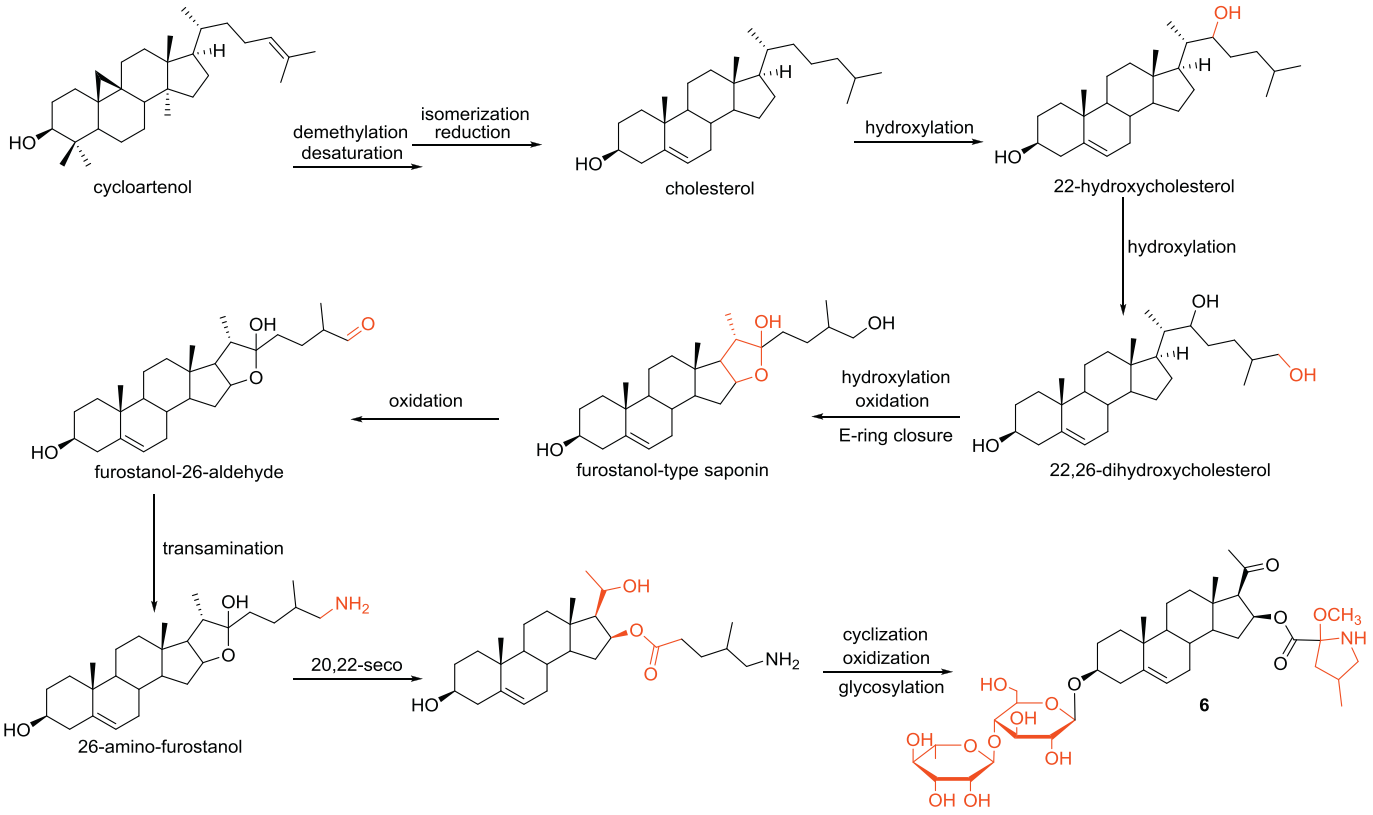

Fig. 6. Hypothetical biogenetic pathway of compound **6.**

Table 4
NO inhibitory activities of compounds **1-10** in RAW 264.7 cell line.

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	12.6 ± 1.3	**7**	59.5 ± 4.7
2	26.8 ± 2.3	**8**	58.8 ± 3.7
3	> 80	**9**	37.4 ± 3.1
4	26.2 ± 2.6	**10**	> 80
5	38.2 ± 2.9	Indomethacin[a]	39.6 ± 2.2
6	52.8 ± 4.2		

[a] Positive control.

constant values of the signals due to H-16 (δ 5.69, 1H, ddd, J = 4.8, 8.2, 8.2 Hz) and H-17 (δ 2.43, 1H, d, J = 8.2 Hz), which were similar to those of 16β-[(4S)-5-(β-D-glucopyranosyloxy)-4-methyl-1-oxopentyl] oxy]-3β-[(O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranosyl)oxy]pregn-5-en-20-one [17]. The correlations of Me-19/H-1β, H-1α/H-3, Me-18/H-15β, H-15α/H-16α observed in the NOESY spectrum (Fig. 5) further confirmed the β-orientation of C-3 and C-16. However, the configurations of C-23 and C-25 have not been confirmed. Consequently, **6** was determined to be 3β,16β-dihydroxy-pregn-5-en-20-one-16-O-(2,5-epimino-2-methoxy-4-pentanoicacid)-ester-3-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, and named Abutiloside U.

Compound **7** was obtained as a white powder and found to have the molecular formula $C_{27}H_{44}O_4$ as inferred from its HRESIMS m/z 455.3144 $[M+Na]^+$ (calcd. for 455.3137). In the ^{1}H- and ^{13}C-NMR spectrum of **7** (Table 3), signals due to an aglycone moiety were similar to those of **2**. The ^{1}H- and ^{13}C-NMR shifts of C-2, C-3, and C-4 in **2** slightly changed in **7**. A comparative study of ^{1}H- and ^{13}C-NMR found that the disaccharide signals in **2** were absent in **7**. The relative configurations of **7** were determined by a NOESY experiment, which were consistent with those of **2**. Therefore, the structure of **7** was determined to be (25*R*) 3β, 16α, 26-trihydroxy-5-en-cholestan-22-one, which was previously synthesized by Robert et al. [18]. This was the first report of its natural occurrence.

The three known steroids, abutiloside G (**8**) [7], solaviaside B (**9**) [19] and tumacone B (**10**) [20], were also identified by comparison of their spectroscopic data with literature values.

Natural products containing 2,5-epimino-2-methoxy-4-pentanoicacid ester steroidal alkaloids were previously isolated from some *Solanum* plants, exemplified by abutiloside O [17,21] and solasodoside E [22]. Here, the hypothetical biogenetic pathways of **6** have been briefly discussed. Cycloartenol is a key precursor metabolized by demethylation, desaturation, isomerization and reduction reactions to generate cholesterol [23–25]. It is transformed into furostanol steroidal alkaloid through repeated steps of hydroxylation, oxidation and transamination via cholesterol [25], followed by biogenetic route B [26] to generate a steroidal alkaloid with an ester side chain at the 16-position. Finally, the 2,5-epimino-2-methoxy-4-pentanoicacid ester steroidal alkaloid was produced by intramolecular cyclization and oxidization reaction (Fig. 6).

Nitric oxide (NO) is a relevant target of inflammation, and plays a key role in the pathogenesis of inflammation [27]. The inhibition of NO release may be considered a therapeutic agent in the inflammatory diseases [28]. The purified compounds **1-10** were tested for inhibition of NO production induced by LPS in the macrophage cell line RAW 264.7. All isolates exhibited no cytotoxicity against RAW 264.7 cells line tested by the MTT method at the concentration of 80.0 μM. The inhibitory activities against the production of nitric oxide (NO) were summarized in (Table 4). Compounds **1**, **2** and **4-9** exhibited moderate inhibition of NO production with IC_{50} values ranging from 12.6 to 59.5 μM. Compounds **3** and **10** were very weak or inactive (IC_{50} values > 80 μM). Due to the limited numbers of active compounds, only the superficial and primary structure-activity relationships were discussed. Compared with compound **4**, **1** showed better activity, while the activity of **2** was better than that of **5**, which suggested that the α-orientation of OH-16 resulted in higher inhibitory activity. The IC_{50} of **2**

and **5** was higher than that of **1** and **4**, respectively, which demonstrated that the number of glycosyl groups may affect the activity.

In summary, our chemical investigation of the roots of *Solanum melongena* L. resulted in the isolation and identification of ten steroids, including six new steroidal saponins, named Abutilosides P-U (**1-6**) and one new natural product (**7**). These compounds were investigated for inhibitory activities on nitric oxide (NO) production stimulated by lipopolysaccharide (LPS) in the RAW 264.7 cell line. Most of the isolates displayed moderate inhibitory activity, except for inactive compounds **3** and **10**. The present investigation suggested that roots of *Solanum melongena* L. could be a potential source of natural anti-inflammatory agents and their steroidal saponins might be responsible for inhibition of NO production.

Conflict of interest

The authors declare no competing financial interests.

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Appendix B. Supplementary data

The spectroscopic data for compounds **1-6** are available as Supporting information. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2018.04.021.

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