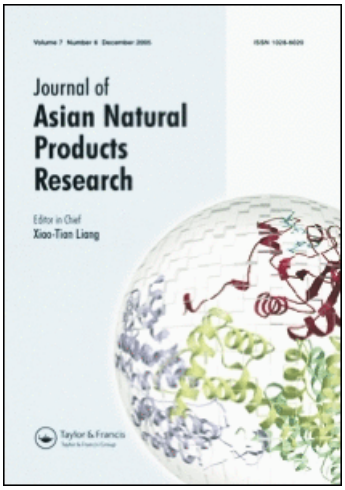

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Two new triterpenoid saponins from *Symplocos Chinensis*

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Two new triterpenoid saponins, symplocososides X (**1**) and Y (**2**) have been isolated from the roots of *Symplocos chinensis*, and their structures elucidated as 21β-*O*- cinnamoyl-22α-*O*-(2-methylbutanoyl)-15α, 16α, 28-trihydroxyolean-12-ene-3β-*O*-[β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 4)-β-D-glucuronopyranoside (**1**) and 21β-*O*-cinnamoyl-22α-*O*-(2-methylbutanoyl)-15α, 16α, 28-trihydroxyolean-12-ene-3β-*O*-(3-*O*-acetyl)-[β-D-glucopyranosyl (1 → 2)]-α-L-arabinofuranosyl (1 → 4)-β-D-glucuronopyranoside (**2**) by spectral and chemical methods. Their antitumor activities have also been tested.

Keywords: Symplocososides X and Y; *Symplocos chinensis*; Triterpenoid saponins

1. Introduction

Symplocos chinensis is widely distributed in the south of China and has been used as folk medicine to treat cold, fever, malaria and cough. It is a toxic folk medicine. When the dose is too large it causes dizziness, chest oppression and disorders of gastrointestinal tract [1]. The EtOAc- and n-BuOH soluble fractions of the alcohol extract of the roots exhibit significant inhibitions on the A549 cell line. During our previous work, a new triterpene with strong cytotoxic activities was isolated along with three known triterpenes from the EtOAc-soluble fraction [2]. We report herein two new triterpenoid saponins, symplocososides X (**1**) and Y (**2**), which have been isolated from the EtOAc-soluble fraction (figure 1). Their structures have been determined by chemical and spectral evidences. Compound **1** shows cytotoxic activity (see table 3).

2. Results and discussion

Symplocososide X (**1**) was obtained as white amorphous powder. It shows an $[M - H]^-$ ion at *m/z* 1189 and an $[M + Na]^+$ ion at *m/z* 1213 in the negative and positive ESIMS spectra respectively. HESIMS analysis suggests that the molecular formula of **1** is $C_{61}H_{90}O_{23}$. Its 1D NMR spectra reveals seven tertiary methyl groups between δ 0.77 and 1.81 and a double

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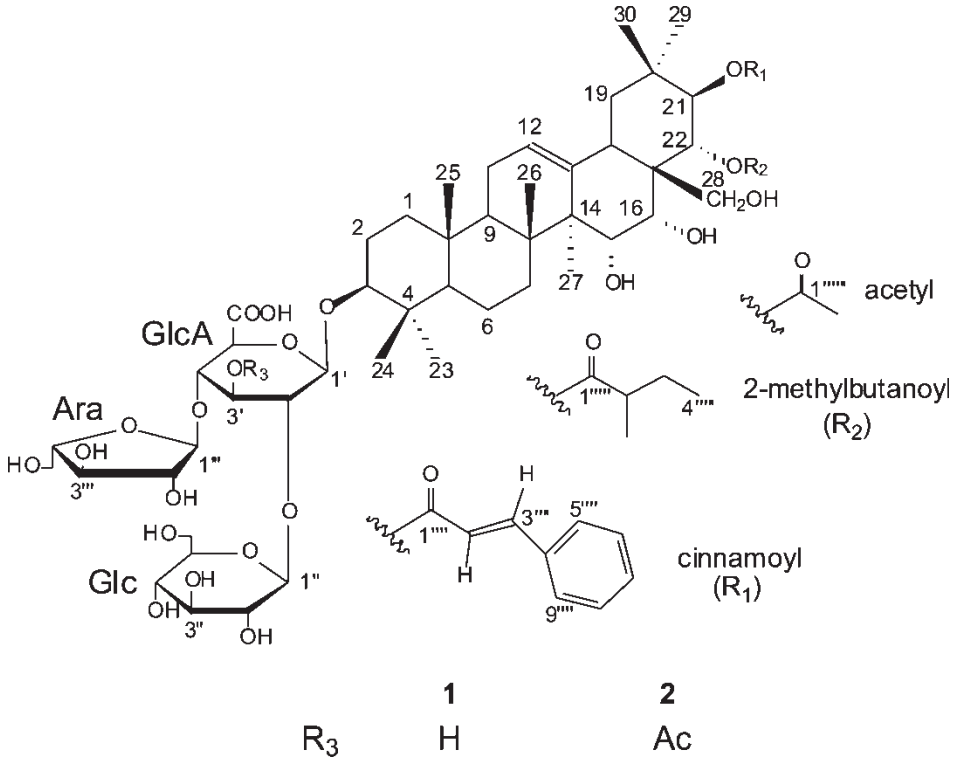

Figure 1. Structures of symplocososides X (**1**), Y (**2**).

bond with typical ^{13}C NMR resonances at δ 125.9 and 143.9, indicating an olean-12-ene triterpene derivative [3].The ^{1}H-NMR spectrum exhibits three anomeric protons [δ 6.05 (1H, br s, H-1″), 5.31 (1H, d, $J = 7.5$ Hz, H-1″) and 4.76 (1H, d, $J = 7.5$ Hz, H-1′)], two coupled doublets of trans olefinic protons [δ 6.83 (1H, d, $J = 16$ Hz, H-2″), 8.02 (1H, d, $J = 16$ Hz, H-3″)] and five aromatic protons [δ 7.33 (3H, m, H-6″ 7″ 8″), 7.59 (2H, d, $J = 6.5$ Hz, H-5″ 9″)] attributed to the cinnamoyl moiety, one methyl doublet [δ 0.95 (3H, d, $J = 7$ Hz, H$_3$-5‴)] and one triplet [δ 0.63 (3H, *t*, $J = 7.5$ Hz, H$_3$-4‴)] attributed to the 2-methylbutanoyl moiety. The ^{1}H and ^{13}C-NMR spectra of the two acyl groups were further assigned by a combination of HMBC and ^{1}H–^{1}H COSY experiments.

The structure of the aglycone has been elucidated as a 21,22-disubstituted olean-12-ene-3,15,16,21,22,28-hexaol on the basis of 1D (^{1}H, ^{13}C and DEPT) and 2D (^{1}H–^{1}H COSY, DQF-COSY, HMQC, HMBC and NOESY) NMR experiments. A 10.3 Hz coupling constant of H-2β-H-3 obtained from a DQF-COSY experiment indicates an equatorial orientation of the 3-hydroxy group. A 10 Hz constant of H-21-H-22 is compatible with a 21-22 diaxial orientation of the hydrogens. β-Axial orientation of H-15, and β-equatorial for H-16, were deduced by combining the 4.8 Hz coupling constant of H-15-H-16 obtained from the DQF-COSY experiment and the NOESY correlations H-16/H-28b, H-15/H-28a, H-15/H$_3$-26 (figure 2) [4]. The above results were confirmed by comparing the ^{13}C-NMR data of **1** and those of R$_1$-barrigenol (3β,15α,16α,21β,22α,28-hexahydroxyolean-12-ene) [5]. Thus, 21,22-disubstituted R$_1$-barrigenol is the aglycone of compound **1**.

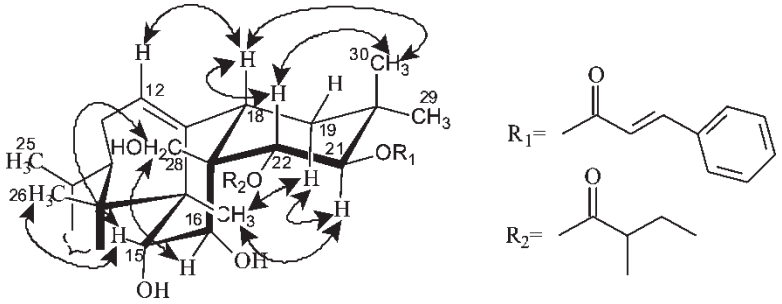

Figure 2. Important NOESY correlations for **1**.

Acid hydrolysis of **1** on HPTLC plates afforded a mixture of glucose, arabinose and glucuronic acid, which correspond to three anomeric carbons (δ 109.0, 105.2, 105.2) in the ^{13}C-NMR spectrum and three anomeric protons [δ 60.5 (br s), 5.31 (d, $J = 7.5$ Hz), 4.76 (d, $J = 7.5$ Hz)] in the ^{1}H-NMR spectrum. The ^{1}H and ^{13}C-NMR data of the sugar part were completely assigned on the basis of the ^{1}H–^{1}H COSY, DQF-COSY, TOCSY, HMQC and HMBC spectra and by a comparison of their NMR data with those of tarasaponin IV [6].

In the HMBC experiment on **1**, long-range correlations occur between H-4′ of glucuronic acid and C-1″ of arabinose, between H-1″ of glucose and C-2′ of glucuronic acid and between H-2′ of glucuronic acid and C-1″ of glucose; thus the sequence of the trisaccharide chain was determined as [β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 4)-β-D-glucuronopyranoside.

Correlation between H-1′ of glucuronic acid and C-3 of the aglycone and reverse correlation between H-3 of the aglycone and C-1′ of glucuronic acid show that the trisaccharide is attached to C-3 of the aglycone. The anomeric configurations of the sugar moieties were defined from their chemical shifts and the J_{H-H} of the anomeric protons.

Finally, two cross peaks between H-21 of the aglycone and C-1″ of the cinnamoyl moiety and between H-22 of the aglycone and C-1‴ of the 2-methylbutanoyl moiety show these two moieties are linked to the aglycone at positions 21, 22 respectively. Hence, compound **1** is 21β-*O*-cinnamoyl-22α-*O*-(2-methylbutanoyl)-15α,16α,28-trihydroxyolean-12-ene-3β-*O*-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl (1 → 4)-β-D-glucuronopyranoside, named symplocososide X (**1**).

Symplocososide Y (**2**) was obtained as a white amorphous powder. It shows an $[M - H]^-$ ion at *m/z* 1231, HESIMS analysis reveals the molecular formula as $C_{63}H_{92}O_{24}$. Comparison of the NMR data for **1** and **2** (tables 1 and 2) indicates that **2** has the same aglycone and the same sugar chain at C-3 of the aglycone as **1**. Significant differences are the downfield shift of H-3′ of glucuronic acid (δ 5.76), the upfield shift of C-2′ of glucuronic acid (δ 77.3) and an additional acetyl group [δ_H 2.27 (3H, s, H$_3$-2‴); δ_C 22.0, C-2‴; 171.3, C-1‴], suggesting the replacement of the hydroxyl of the C-3′ of glucuronic acid in **1** with an acetoxy group in **2**; this is further confirmed by the HMBC correlation between H-3′ of glucuronic acid and the C-1‴ of the acetyl group. Consequently, **2** is 21β-*O*-cinnamoyl-22α-*O*-(2-methylbutanoyl)-15α,16α,28-trihydroxyolean-12-ene-3β-*O*-(3-*O*-acetyl)-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinofuranosyl(1 → 4)-β-D-glucuronopyranoside, named symplocososide Y (**2**).

The pharmacological activities of **1** and **2** (table 3) have been evaluated. Compound **1** demonstrates cytotoxic activities against A549, Bel7402, HCT-8 cell lines.

Table 1. ^{1}H and ^{13}C-NMR spectral data for symplocososide X (**1**), (C_5H_5N)[a].

Position	δ_C *(ppm)*[b]	δ_H *(ppm) J(Hz)*[b]	*Position*	δ_C *(ppm)*[b]	δ_H *(ppm) J(Hz)*[b]
1	39.2	a 1.29 m	Cinnamoyl group		
		b 0.73 m	1″	167.7	
2	26.8	a 2.07 m	2″	119.8	6.83 d (16)
		b 1.76 m	3″	145.4	8.02 d (16)
3	89.8	3.11 m	4″	135.3	
4	39.8		5″ 9″	128.9	7.59 d (6.5)
5	55.8	0.69 m	6″ 8″	129.7	7.33 m
6	19.0	a 1.45 m	7″	131.0	7.33 m
		b 1.23 m	2-methylbutanoyl group		
7	36.9	a 2.07 m	1‴	177.3	
		b 1.98 m	2‴	41.7	2.15 m
8	41.9		3‴	27.2	a 1.56 m
9	47.2	1.63 m			b 1.21 m
10	37.2		4‴	12.2	0.63 t (7.5)
11	24.3	1.82 m	5‴	17.2	0.95 d (7)
12	125.9	5.55 br s	Glucuronic acid moiety		
13	143.9		1′	105.2[c]	4.76 d (7.5)
14	48.0		2′	81.3	4.29 m
15	67.8	4.18 m	3′	76.4	4.24 t (8.5)
16	73.4	4.38 m	4′	78.8(Ca.)	4.54 m
17	48.7		5′	77.7(Ca.)	4.40 m
18	41.2	3.09 m	6′	176.1(Ca.)	
19	47.4	a 3.06 m	Glucose moiety		
		b 1.43 m	1″	105.2[c]	5.31 d (7.5)
20	36.8		2″	76.8	3.99 t (8)
21	79.9	6.64 d (10)	3″	77.9	4.16 m
22	73.4	6.33 d (10)	4″	72.0	4.11 m
23	28.2	1.10[c] s	5″	78.4	3.87 br s
24	17.1	1.02 s	6″	63.0	a 4.28 m
25	16.1	0.77 s			b 4.46 d (9.5)
26	17.9	1.00 s	Arabinose moiety		
27	21.5	1.81 s	1″	109.0	6.05 br s
28	63.1	a 3.75 d (10.5)	2″	82.8	4.81 m
		b 3.48 d (10.5)	3″	78.6	4.64 br s
29	29.8	1.10[c] s	4″	86.7	4.83 m
30	20.3	1.33 s	5″	62.8	a 4.13 m
					b 4.04 m

[a] Assignments based on $^{1}H-^{1}H$ COSY, HMQC, HMBC and NOESY experiments.
[b] ^{1}H and ^{13}C-NMR spectra obtained at 500 and 125 MHz respectively.
[c] Coincident signals.

3. Experimental

3.1. General experimental procedures

Mps were determined on an XT-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer digital polarimeter. UV spectra were recorded on a Shimadzu UV-260 spectrometer. IR spectra were determined on a Perkin-Elmer 683 infrared spectrometer in KBr pellets. NMR spectra were taken with TMS as internal standard on an Inova 500 FT-NMR spectrometer. HESIMS were measured on an Bruker FTMS Apex III spectrometer and ESIMS on a Finnigan Lcq-Advantage spectrometer. Column chromatography was performed on silica gel (Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Amersham Pharmacia Biotech AB), silanized Si gel (RP-18, 15–35 μm, Unicorn), HPTLC was conducted on Silica GF_{254} (Marine Chemical Factory, Qingdao, China) and RP-18 F_{254} (Merck) plates.

Table 2. ^{1}H and ^{13}C-NMR spectral data for symplocososide Y (**2**), (C_5H_5N)[a].

Position	δ_C *(ppm)*[b]	δ_H *(ppm) J(Hz)*[b]	*Position*	δ_C *(ppm)*[b]	δ_H *(ppm) J(Hz)*[b]
1	39.0	a 1.31 m	Cinnamoyl group		
		b 0.72 m	1″	167.4	
2	26.5	2.10 m	2″	119.7	6.83 d (16)
3	90.3	3.07 m	3″	145.1	8.01 d (16)
4	39.7		4″	135.2	
5	55.5	0.69 m	5″ 9″	128.7	7.59 d (6.5)
6	18.9	a 1.52 m	6″ 8″	129.5	7.33 m
		b 1.33 m	7″	130.8	7.33 m
7	36.8	a 2.11 m	2-methylbutanoyl group		
		b 2.05 m	1‴	177.0	
8	41.8		2‴	41.6	2.12 m
9	47.2	1.66 m	3‴	27.1	a 1.55 m
10	37.0				b 1.21 m
11	24.1	a 1.80 m	4‴	12.1	0.64 t (7.5)
		b 1.74 m	5‴	16.9	0.96 d (7)
12	125.6	5.55 br s	Glucuronic acid moiety		
13	143.7		1′	104.8	4.66 d (7.5)
14	47.9		2′	77.3	4.35 t (8.5)
15	67.6	4.19 m	3′	76.1	5.76 t (9.5)
16	73.2	4.41 m	4′	78.9	4.47 t (9)
17	48.6		5′	78.5	4.39 m
18	41.0	3.09 m	6′	175.1	
19	47.0	a 3.12 m	Acetyl group		
		b 1.43 m	1‴	171.3	
20	36.7		2‴	22.0	2.27 s
21	79.7	6.67 d (10)	Glucose moiety		
22	73.2	6.33 d (10)	1″	104.6	4.94 d (7.5)
23	27.8	1.01 s	2″	75.2	3.87 t (8.5)
24	16.9	1.06 s	3″	78.3	4.09 m
25	15.9	0.78 s	4″	72.4	3.96[c] m
26	17.7	0.99 s	5″	78.9	3.96[c] m
27	21.4	1.85 s	6″	63.7	a 4.63 m
28	62.9	a 3.76 d (10.5)			b 4.21 m
		b 3.48 d (10.5)	Arabinose moiety		
29	29.7	1.13 s	1″	110.3	5.92 br s
30	20.2	1.35 s	2″	83.2	4.86 br s
			3″	77.8	4.56 m
			4″	85.8	4.58 m
			5″	62.9	a 4.22 m
					b 4.11 m

[a] Assignments based on $^{1}H-^{1}H$ COSY, HMQC, HMBC and NOESY experiments.
[b] ^{1}H and ^{13}C-NMR spectra obtained at 500 and 125 MHz respectively.
[c] Coincident signals.

Table 3. Evaluation of the anticancer potential of compounds **1** and **2**.

	Cell line[a] IC_{50} *(μmol L*$^{-1}$*)*				
Sample	*A549*	*Bel-7402*	*BGC-823*	*HCT-8*	*Helf*
1	2.06[b]	1.76	> 10	1.72	1.95
2	> 10	> 10	> 10	> 10	> 10

[a] A549 = human lung carcinoma; Bel-7402 = human liver carcinoma; BGC-823 = human stomach carcinoma; HCT-8 = human colon carcinoma; Helf = human embryo lung fibroblast.
[b] Evaluation: strongly active ($IC_{50} < 0.1\ \mu mol\ L^{-1}$); active ($0.1\ \mu mol\ L^{-1} < IC_{50} < 1\ \mu mol\ L^{-1}$); weakly active ($1\ \mu mol\ L^{-1} < IC_{50} < 10\ \mu mol\ L^{-1}$); inactive ($IC_{50} > 10\ \mu mol\ L^{-1}$).

3.2 Plant material

Roots of *Symplocos chinensis* were collected in November 1998 from Guangxi Province, China. The sample was identified by Professor Shouyang Liu, Department of Pharmacognosy, Guangxi College of Chinese Traditional Medicine. The roots were harvested and air-dried at room temperature in darkness. A voucher specimen has been deposited in the Herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

The dried roots (22 kg) were ground and extracted with EtOH and the resultant extract (2710 g) was successively partitioned with EtOAc, n-BuOH and H_2O. The EtOAc-soluble fraction (195 g) was subjected to chromatography, eluting with $CHCl_3$–CH_3OH (20:1 → 0:1) to yield 12 fractions. Fraction 9 (43 g) was subjected to silica gel chromatography [CH_2Cl_2–CH_3OH–H_2O (5:1:0.1)] to afford fraction A_{1-6}. Then, fraction A_3 was purified by Sephadex LH-20 [CH_3OH–H_2O (40:60)] and repeated ODS [CH_3OH–H_2O (50:50 → 100:0)] chromatography to afford compound **2** (41 mg). Fraction 10 (12.8 g) was subjected to silica gel chromatography [CH_2Cl_2–CH_3OH–H_2O (75:25:8 lower layer)] to afford fraction F_{1-4}. Fraction F_2 was then purified with Sephadex LH-20 [CH_3OH–H_2O (40:60)] and repeated ODS [CH_3OH–H_2O (50:50 → 100:0)] chromatography to afford compound **1** (60 mg).

3.4 Acid hydrolysis of 1

Compound **1** was applied on silica gel G HPTLC plates and left in an HCl atmosphere at 75–80°C for 5 h. HCl vapour was eliminated under hot ventilation and authentic sugars were then applied to the plates. The chromatoplates were developed using EtOAc–CH_3OH–HOAc–H_2O (12:3:3:2) and $CHCl_3$–CH_3OH–H_2O (7:3:0.4) successively and spots were detected by spraying with EtOH–conc.H_2SO_4–anisaldehyde (17:2:1) followed by heating. Three sugars were identified: D-glucose, L-arabinose and D-glucuronic acid [7].

3.5 Alkaline and acid hydrolysis of 2

A solution of **2** (3 mg) in 50% aqueous CH_3OH (1 mL) was treated with 10% aqueous KOH (1 mL) and stirred at 37°C for 1 h. After the mixture was adjusted to pH 6 with 2% aqueous HCl, the solution was concentrated and then hydrolyzed in the same way as for **1**, the same sugars were detected as in **1**.

3.6 Structure and identification

Symplocososide X (1). White amorphous powder, mp 280–282°C; $[\alpha]_D^{19}$ − 30.5 (*c* 0.19, MeOH); ESIMS *m/z*: $[M - H]^-$ 1189, $[M + Na]^+$ 1213; HESIMS *m/z* $[M + Na]^+$ 1213.5760 (calcd for $C_{61}H_{90}O_{23}Na$ 1213.5765); IR (KBr) ν_{max} (cm^{-1}): 3411(br), 2964, 1712, 1637, 1601, 1450, 1282, 1074; UV λ_{max} (MeOH) (nm) 204, 223, 278. ^1H and ^{13}C-NMR see table 1.

Symplocososide Y (2). White amorphous powder; mp 272–274°C; $[\alpha]_D^{19}$ − 26 (*c* 0.25, MeOH); ESIMS *m/z*; $[M - H]^-$ 1231; HESIMS *m/z* $[M + Na]^+$ 1255.5867 (calcd for $C_{63}H_{92}O_{24}Na$, 1255.5871); IR (KBr) ν_{max} (cm^{-1}): 3440(br), 2964, 1716, 1635, 1601, 1450, 1255, 1074; UV λ_{max} (MeOH) (nm): 204, 223, 278. ^1H ^{13}C-NMR see table 2.

3.7 Cell culture and assay for cytotoxic activity

Tumor cells (1×10^4 cells mL^{-1}) were seeded to RPMI 1640 medium supplemented with 10% heat-inactivated FBS and kanamin (0.1 mg mL^{-1}) at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. Test compounds were added to this culture and incubated at 37°C for a further 120 h without medium change. Cell viability was then evaluated by the MTT-reducing test and compared with that of the control culture where the cells were treated without added test compounds.

Acknowledgements

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