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Actaticas A–G, Cycloartane Triterpenes From *Actaea asiatica* With Their Antiproliferative Activity

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Phytochemical studies on the rhizomes of *Actaea asiatica* led to the isolation of seven new cycloartane triterpenes, actaticas A–G (**1**–**7**). Their structures were determined by NMR, HRESIMS, and chemical analysis. All the isolates were evaluated for their antiproliferative activity against HT-29 and McF-7 cell lines. The results showed that all the compounds displayed cytotoxicity. All compounds showed significant inhibitory effects with IC_{50} values of 9.2–26.4 μM.

Keywords: Actaea asiatica, cycloartane triterpenes, antiproliferative activity, HT-29 cell lines, MCF-7 cell lines

INTRODUCTION

Actaea asiatica H. Hara, a perennial herb belonging to the family Ranunculaceae, is mainly distributed in the southwest and northwest of China. Its roots have been traditionally used among the Tujia folk in Hubei Province for treating headache, sore throat, rheumatic pain, rubella, measles, pertussis, uterine prolapse, and dog bites (Gao et al., 2006a; Gao et al., 2006b; Fan et al., 2007; Gao et al., 2007). Phytochemical studies indicated that the genus *Actaea* contained cycloartane triterpene glycosides with cytotoxic activities (Kusano et al., 1998; Kusano et al., 1999; Gao et al., 2006b). However, little systematic chemical work on *A. asiatica* has been carried out so far. In order to find the bioactive constituents from *A. asiatica*, chemical research were carried out, resulting in the isolation of seven new cycloartane triterpene glycosides, namely, actaticas A–G (**1**–**7**) (**Figure 1**). Their structures were determined by spectroscopic analysis and chemical methods. Herein, structural elucidation of compounds **1**–**7** was reported as well as their cytotoxic activities.

MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were obtained on a PerkinElmer 341 digital polarimeter. IR spectra were recorded on Shimadzu FTIR-8400S spectrometers. NMR spectra were obtained with a Bruker AV III 600 NMR spectrometer (chemical shift values are presented as δ values with TMS as the internal standard). HR-ESIMS spectra were performed on a LTQ-Obitrap XL spectrometer. Preparative HPLC was performed on a Lumtech K-1001 analytic LC equipped with two pumps of K-501, a UV

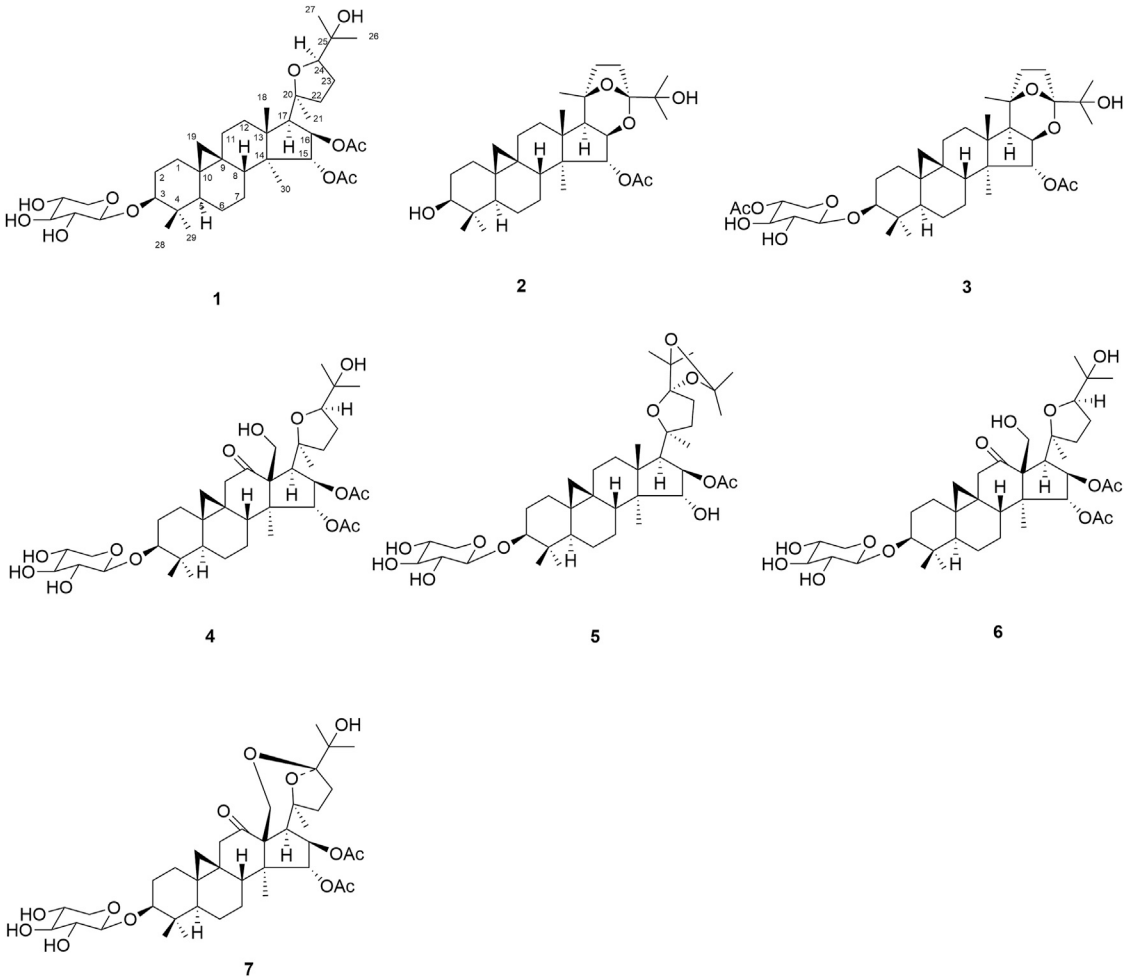

FIGURE 1 | Structures of compounds **1–7**.

detector of K-2600, and an YMC Pack C_{18} column (250 × 10 mm, i.d., 5 μm, YMC Co. Ltd., Japan) eluted with CH_3OH-H_2O at a flow rate of 2 ml/min. C_{18} reversed–phase silica gel (40–63 μm, Merk, Darmstadt, Germany), MCI gel (CHP 20P, 75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and silica gel (100–200 mesh, Qingdao Marine Chemical plant, Qingdao, the People's Republic of China) were used for column chromatography. Pre-coated silica gel GF254 plates (Zhi Fu Huang Wu Pilot Plant of Silica Gel Development, Yantai, the People's Republic of China) were used for TLC. All solvents used were of analytical grade (Beijing Chemical Works).

Plant Material

The plants of *A. asiatica* were collected at Jinfuo Mountain in Chongqing province, the People's Republic of China, in November 2016, and were authenticated by Professor Sirong Yi. The voucher specimen (CS161108) has been deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

Extraction and Isolation

The air-dried powdered rhizomes *A. asiatica* (6.8 kg) was extracted with 95% EtOH (20 L) three times (each time for 2 h). Removal of the EtOH under reduced pressure yielded the extract (879 g). The residue was suspended in H_2O (1.5 L) and partitioned with petroleum ether (3 × 1 L), EtOAc (3 × 1 L), acetone (3 × 1 L), and *n*-BuOH (3 × 1 L) successively. The EtOAc fraction (510 g) was subjected to CC over silica gel (100–200 mesh, 8 × 100 cm) eluting with a stepwise gradient of CH_2Cl_2-MeOH (from 1:0 to 0:1) to afford six fractions A–F. Fraction B (29.4 g) was subjected to MCI column chromatography (4 × 80 cm) elution with MeOH-H_2O (40: 60; 60:40; 70:30; 80:20; 100:0, v/v) giving five subfractions (Fr. B1–B5). Subfraction B3 (911 mg) was chromatographed by semi-preparative HPLC using acetonitrile–H_2O (75: 25, v/v) to yield compound 1 (9.4 mg, t_R = 26.3 min) and **7** (7.2 mg, t_R = 29.5 min). Subfraction B4 (503 mg) was purified through preparative HPLC elution using an acetonitrile–H_2O (65: 35, v/v) system to give compound 2 (12.1 mg, t_R = 23.0 min).

TABLE 1 | ^{1}H NMR Spectroscopic Data (600 MHz, in pyridine-d_5) for compounds **1**–**7**.

No	Compounds						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	1.09 m; 1.52 m	1.22 m; 1.58 m	1.20 m; 1.58 m	1.10 m; 1.35 m	1.01 m; 1.52 m	1.06 m; 1.49 m	1.24 m; 1.37 m
2	1.92 m; 2.34 m	1.83 m; 2.40 m	1.96 m; 2.25 m	1.90 m; 2.35 m	1.92 m; 2.34 m	1.90 m; 2.34 m	1.12 m; 2.26 m
3	3.47dd (12.0, 3.6)	3.52 dd (12.0, 4.2)	3.36 dd (11.4, 3.0)	3.47 dd (12.0, 3.6)	3.49 dd (12.0, 3.6)	3.48 dd (12.0, 3.6)	3.58 dd (12.0, 3.6)
5	1.27 m	1.29, m	1.28, m	1.20 m	1.27 m	1.24, m	1.28 m
6	0.59 q (12.0); 1.38 m	0.71 q (6.6)	0.72 m; 1.41 m	0.52 q (12.0); 1.38 m	0.57 q (12.0); 1.33 m	0.63 q (12.0); 1.38 m	0.84 m; 1.42 m
7	1.06 m; 1.31 m	1.09 m; 2.09 m	2.02 m; 1.08 m	1.10 m; 1.29 m	1.05 m; 1.29, m	1.06 m; 1.38, m	1.21 m; 1.39 m
8	1.73, m	1.77 dd (4.8)	1.72, m	1.63 m	1.72, m	1.77 m	1.78 m
11	2.03 m; 1.10, m	2.02 m; 1.21 m	2.00 m; 1.08 m	2.04 d (10.8); 1.98 d (10.8)	2.02 m; 1.10m	2.43 d (10.8); 1.99 d (10.8)	2.25 d (10.8); 1.92 d (10.8)
12	2.37 m; 1.76, m	1.55 m; 2.30 m	2.32 m; 1.71 m	—	2.36 m; 1.76 m	—	—
15	5.49 d (4.8)	5.70 d (3.0)	5.67 d (3.0)	5.88 d (4.8)	5.54 d (4.8)	1.47 m; 2.50 d (8.4)	5.50 d (4.8)
16	5.82 dd (4.8, 1.2)	4.47 q (3.0)	4.30 dd (8.4, 3.0)	5.90 dd (9.6, 4.8)	5.95 dd (9.6, 4.8)	5.47 t (8.4)	5.78 dd (9.6, 4.8)
17	2.76, s	2.00, m	2.00 m	2.01 m	2.80 d (10.2)	2.92 d (9.6)	3.02 t (8.4)
18	3.29, s	1.72, m	1.89, s	3.52 d (12.0); 4.18 d (12.0)	1.49 s	3.51 d (11.4); 4.03 d (11.4)	3.54 d (11.4); 4.13 d (11.4)
19	0.18 d (4.2); 0.47 d (4.2)	0.53, d (4.2); 0.32 d (4.2)	0.21 d (4.2); 0.42 d(4.2)	0.18 d (4.2); 0.39 d (4.2)	0.26 d (4.2); 0.51 d (4.2)	0.12 d (4.2); 0.44 d (4.2)	0.28 d (4.2); 0.41 d (4.2)
21	1.47 s	1.61 s	1.58 s	1.30 s	1.74 s	1.30 s	1.32 s
22	2.37 m; 1.76 m	2.02 m; 1.78 dd (4.8)	2.03 m; 1.80 m	2.36 m; 1.77 m	2.33 m; 1.76 m	2.39 m; 1.77 m	2.24 m; 1.63 m
23	2.29 m; 1.90, m	2.02 m; 1.57 m	2.02 m; 1.73 m	2.18 m; 1.90 m	2.29 m; 1.90 m	2.18 m; 1.90 m	2.12 m; 1.92 m
24	3.83 t (7.2)	—	—	3.72 t (7.2)	—	3.72, t (7.2)	—
26	1.32 s	1.66 s	1.66 s	1.15 s	1.33 s	1.23 s	1.24 s
26′	—	—	—	—	1.31 s	—	—
27	1.36 s	1.49, s	1.48 s	1.55 s	1.42 s	1.53 s	1.51 s
27′	—	—	—	—	1.45 s	—	—
28	1.35 s	1.40, s	1.38 s	1.55 s	1.17 s	1.55 s	1.52 s
29	1.02 s	1.10, s	1.07 s	1.00 s	1.04 s	1.02 s	1.01 s
30	1.09, s	1.23, s	1.12 s	1.31 s	1.25 s	1.22 s	1.20 s
15-Ac	2.11 s	2.07 s	2.06 s	1.96 s	2.06 s	—	2.01 s
16-Ac	2.13 s	—	—	1.91 s	2.09 s	1.97 s	2.03 s
1′	4.86 d (7.2)[a]	—	4.81 d (7.8)[b]	4.85 d (7.2)	4.88 d (7.2)	4.86 d (7.2)	4.86 d (7.2)
2′	4.02 t (8.4)	—	3.97 m	4.02 t (8.4)	4.04 t (8.4)	4.02 t (8.4)	4.02 t (8.4)
3′	4.15 t (9.0)	—	4.18 m	4.15 t (9.0)	4.16 t (9.0)	4.15 t (9.0)	4.15 t (9.0)
4′	4.23 m	—	4.54 m	4.22 m	4.23 m	4.23 m	4.24 m
5′	3.73 t (8.0); 4.35 dd (4.8)	—	4.34 m; 3.72 m	3.72 m; 4.35 m	3.74 m; 4.37 m	3.72 m; 4.35 m	3.72 m; 4.35 m
4′-Ac	—	—	2.07 s	—	—	—	—

[a]*Xylose.*
[b]*4′-acetylxylose.*

Fraction D (5.8 g) was loaded on an ODS C_{18} column (2 × 80 cm) eluted with MeOH-H_2O (40:60; 60:40; 70:30; 80:20; 100: 0, v/v) to give five subfractions (Fr. D1–D5). Subfraction D3 (503 mg) was chromatographed by semi-preparative HPLC using acetonitrile–H_2O (70: 30, v/v) to yield compounds 3 (6.1 mg, t_R = 18.5 min), 4 (8.7 mg, t_R = 21.4 min), and 5 (7.0 mg, t_R = 28.3 min). Fraction F (6.7 g) was fractioned on an MCI-gel column chromatography eluted with MeOH–H_2O (40:60; 60:40; 70:30; 80:20; 100:0, v/v) to give five subfractions (Fr. F1–F5). Subfraction F3 (223 mg) was chromatographed by preparative HPLC using acetonitrile–H_2O (75: 25, v/v) to yield compounds 6 (5.8 mg, t_R = 22.7 min).

Actatica A (1): $C_{39}H_{62}O_{11}$, white amorphous powder; [α] 20 D + 21.6 (c = 0.18, MeOH); IR (KBr) ν_{max}: 3,440, 3,397, 2,924, 1,733, 1,457, 1,044 cm^{-1}; UV (MeOH) λ_{max} (log ε): 200 nm; for ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS m/z: 729.4233 (calcd for $C_{39}H_{62}O_{11}Na$ [M + Na]$^+$, 729.4184).

Actatica B (2): $C_{32}H_{50}O_6$, white amorphous powder; [α]20 D + 19.0 (c = 0.15, MeOH); IR (KBr) ν_{max}: 3,376, 2,957, 1,738, 1,373, 1,032 cm^{-1}; UV (MeOH) λ_{max} (log ε): 201 nm; for ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic, data see **Tables 1**, **2**; HR-ESIMS m/z: 553.3533 (calcd for $C_{32}H_{50}O_6Na$, 553.3500).

Actatica C (3): $C_{39}H_{60}O_{11}$, white amorphous powder; [α]20 D+ 35.1 (c = 0.31, MeOH); IR (KBr) ν_{max}: 3,493, 2,928, 1,730, 1,375, 1,044 cm^{-1}; UV (MeOH) λ_{max} (log ε): 201 nm; for ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS m/z: 727.4100 (calcd for C_{39} $H_{60}O_{11}Na$, [M + Na]$^+$, 727.4088).

Actatica D (4): $C_{40}H_{62}O_{13}$, white amorphous powder; [α]20 D + 22.4 (c = 0.22, MeOH); IR (KBr) ν_{max}: 3,439, 2,934, 1,734, 1,264, 1,033, 1,033, 962 cm^{-1}; λ_{max} (log ε): 201 nm; for ^{1}H NMR

TABLE 2 | ^{13}C NMR Data (150 MHz, in pyridine-d_5) for compounds **1–7** (δ_H in ppm, *J* in Hz).

Position	Compounds						
	1	2	3	4	5	6	7
1	32.6	33.0	32.6	32.8	32.6	32.9	32.8
2	30.9	31.7	30.9	32.3	30.1	31.1	32.3
3	88.8	82.8	86.7	88.8	88.7	88.8	88.7
4	41.7	40.5	40.5	41.7	41.7	41.7	41.7
5	47.7	47.8	47.8	47.7	47.6	47.7	47.4
6	21.2	21.7	21.4	21.1	21.1	21.4	20.8
7	26.5	26.5	26.4	26.5	26.1	26.7	26.5
8	48.3	48.3	48.2	48.5	48.0	50.3	49.0
9	19.9	20.1	20.1	20.2	19.8	20.7	20.2
10	26.7	27.1	26.7	26.9	27.1	26.9	26.9
11	26.3	26.6	26.5	31.2	27.0	31.9	31.2
12	37.5	25.9	26.4	216.6	39.1	216.6	216.6
13	48.6	50.3	50.3	59.3	48.3	59.0	59.1
14	47.6	47.0	47.0	42.4	46.4	42.7	42.4
15	86.4	80.3	76.7	86.1	85.7	42.7	82.4
16	79.8	78.3	80.3	80.1	79.8	77.3	79.1
17	56.1	51.6	51.6	52.7	59.0	53.2	56.5
18	21.7	14.1	33.5	64.4	26.1	64.5	64.7
19	30.5	31.2	30.4	26.1	30.5	26.2	26.5
20	85.2	86.7	82.9	86.6	87.3	86.6	87.2
21	27.6	25.1	25.1	27.7	22.3	24.4	24.7
22	33.7	41.5	41.4	39.0	39.1	39.1	32.3
23	27.0	29.0	29.0	29.7	30.5	31.2	30.9
24	83.8	111.2	111.2	81.7	115.2	81.6	114.7
25	70.9	72.4	72.4	71.7	82.8	71.7	71.6
26	27.6	25.9	25.8	26.1	30.3	26.1	26.4
27	27.6	26.5	25.9	21.7	22.3	21.5	26.3
28	26.1	25.7	25.6	21.7	26.1	21.5	26.3
29	15.8	15.3	15.6	14.5	15.7	15.8	15.7
30	13.8	14.1	14.1	13.7	13.9	14.5	15.2
15-Ac	170.9	170.7	170.6	171.5	171.2		171.5
	21.2	22.0		21.5	21.7		21.6
16-Ac	171.2			171.1	170.2	171.8	171.1
	22.0			21.6	21.8	24.4	21.4
3-Ac						76.0	
1′	108.1[a]		105.1[b]	108.1[a]	107.9[a]	108.0[a]	108.1[a]
2′	76.0		71.7	76.0	76.0	76.0	76.0
3′	79.1		76.0	79.4	79.1	79.1	79.1
4′	71.6		89.0	71.1	71.7	71.7	71.7
5′	67.6		67.6	67.6	67.6	67.6	67.6
4′-Ac			170.6				
			21.9				
26′					30.3		
27′					27.4		

[a]*Xylose.*
[b]*4′-acetylxylose.*

(600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS *m/z*: 759.3974 (calcd for $C_{40}H_{62}O_{13}Na$ [M + Na]$^+$, 759.3926).

Actatica E (5): $C_{42}H_{66}O_{12}$, white amorphous powder; [α]20 D + 42.7 (*c* = 0.35, MeOH); IR (KBr) ν_{max}: 3,449, 2,935, 1,741, 1,375, 1,045 cm^{-1}; λ_{max} (log ε): 200 nm; for ^{1}H NMR (600 MHz, pyridine) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS *m/z*: 785.4341 (calcd for $C_{42}H_{66}O_{12}Na$ [M + Na]$^+$, 785.4341).

Actatica F (6): $C_{37}H_{58}O_{11}$, white amorphous powder; [α]20 D + 23.3 (*c* = 0.12, MeOH); IR (KBr) ν_{max}: 3,350, 2,820, 1,710, 1,534, 952 cm^{-1}; λ_{max} (log ε): 202 nm; for ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS *m/z*: 701.3904 ([M + Na]$^+$, calcd for $C_{37}H_{58}O_{11}Na$, 701.3926).

Actatica G (7): $C_{39}H_{58}O_{13}$, white amorphous powder; [α]20 D + 22.6 (*c* = 0.13, MeOH); IR (KBr) ν_{max}: 3,298, 2,781, 1,716, 1,422, 950 cm^{-1}; for ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C-APT (150 MHz, pyridine-d_5) spectroscopic data, see **Tables 1**, **2**; HR-ESIMS *m/z*: 757.3765 ([M + Na]$^+$, calcd for $C_{39}H_{58}O_{13}Na$, 757.3770).

Hydrolysis of Compounds

Acid hydrolysis of **1** and **4–7**: A solution of compounds **1, 4, 5, 6, and 7** (5 mg) in 2 M HCl (1 ml) was heated at reflux for 24 h. The reaction mixture was neutralized with 2 M NaOH and extracted by partition with EtOAc (5 × 1 ml). 10 ml of water was added to the residue and extracted with CH_2Cl_2 three times. Sugars were analyzed by TLC and GC analysis and compared with authentic sample of D-sugars. The spots were visualized by spraying with EtOH–H_2SO_4–anisaldehyde (9:0.5:0.5, v/v), then heated at 150°C. Furthermore, the absolute configurations of the sugars were determined by gas chromatography according to a method previously described (Ma et al., 2016; Li et al., 2017). Compound **3** was dissolved in MeOH (15 ml), then 4% K_2CO_3 (15 ml) was added and each solution was stirred at room temperature overnight. Each solution was neutralized by 10% AcOH, and extracted with EtOAc (2 × 20 ml). EtOAc extract after removal of solvent, was dissolved in MeOH (10 ml) and refluxed with 0.5 N HCl (3 ml) for 4 h (Yin et al., 2010).

Cytotoxic Assay

The cytotoxicity of compounds **1–7** was evaluated using the MTT procedure with HT-29 and McF-7 cancer cell lines. The cells were incubated in DMEM supplemented with 10% fetal bovine serum and cultured at a density of 1.2×10^4 cells/ml in a 96-well microtiter plate. Five various concentrations of each agent dissolved in dimethyl sulfoxide (DMSO) were then put in the wells. Each concentration was evaluated three times. After incubation under 5% CO_2 at 37°C for 48 h, 10 ml of MTT (4 mg/ml) was placed into each well, and the cells were incubated for an additional 4 h. Then, the liquid was taken out, and DMSO (200 ml) was put into the wells. The absorbance was documented with a microplate reader at wave length of 570 nm.

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. Its IR spectrum showed absorptions of hydroxyl group at 3,440 and 3,397 cm^{-1} and carbonyl at 1,733 cm^{-1}. The HRESIMS spectrum showed a pseudo-molecular ion at *m/z* 729.4233 [M + Na]$^+$ in the positive ion mode from which in conjunction with NMR data the molecular formula was established as $C_{39}H_{62}O_{11}$, consistent with nine degrees of unsaturation. In the ^{1}H NMR spectrum (**Table 1**) two cyclopropane–methylene protons as an AX system at δ_H 0.18 and 0.47 (each 1H, d, *J* = 4.0 Hz, H_2-19) together with seven tertiary methyl groups at δ_H 1.49, 1.47, 1.32, 1.36, 1.35, 1.02, and 1.09, indicated a cycloartane triterpenoid structure (Ju et al., 2002a; Mohamed, 2014; Gan et al., 2015; Wu et al., 2017). The ^{1}H NMR

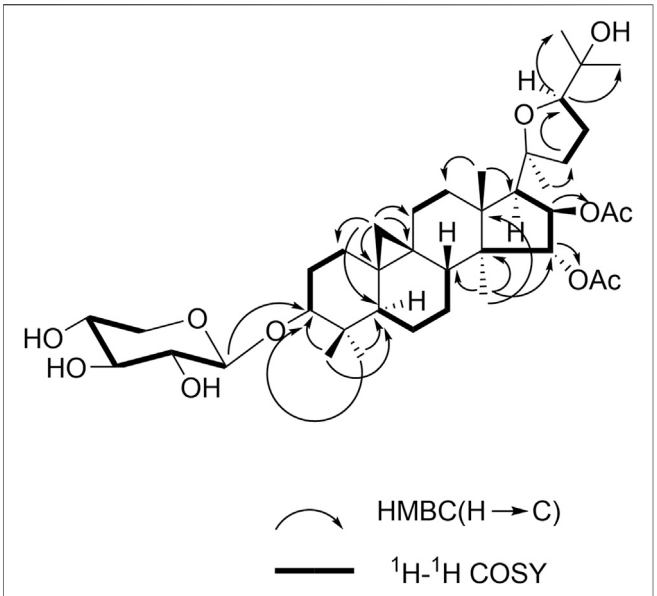

FIGURE 2 | Key HMBC and ^{1}H-^{1}H COSY correlations for 1.

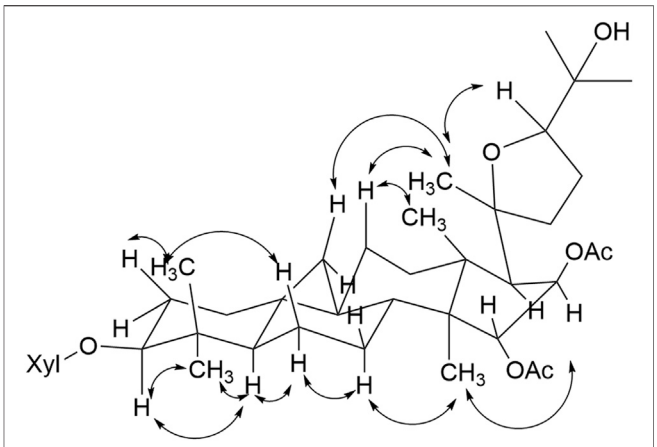

FIGURE 3 | Key NOESY correlations of compound 1.

spectrum also showed two oxygenated proton signals at δ_H 5.49 (d, J = 4.8 Hz) and δ_H 5.82 (d, J = 4.8 Hz), indicating two acetyl groups at C-15 and C-16. Except for sugar carbons, the ^{13}C-NMR spectrum (**Table 2**) of **1** displayed 39 carbon resonances including methylene carbon of cyclopropane ring at δ_C 30.5 (C-19), an oxymethine carbon at δ_C 88.8 (C-3), an oxygenated quaternary carbon at δ_C 85.2 (C-20), and an anomeric carbon at δ_C 108.1, together with acetyl signals at δ_C 170.9, 171.2, 21.2, and 22.0. The ^{1}H and ^{13}C NMR spectroscopic data of **1** confirmed that the compound was a cycloartane triterpene glycoside (Jung et al., 2002; Wu et al., 2017; Wua et al., 2017).

All proton signals were assigned to the corresponding carbons through direct ^{1}H and ^{13}C correlations in the HSQC spectrum. Inspection of the ^{1}H-^{1}H COSY spectrum showed fragments of C-1/C-2/C-3, C-5/C-6/C-7/C-8, C-11/C-12, C-15/C-16/C-17, and C-22/C-23/C-24. In the HMBC spectrum (**Figure 2**), the correlations were observed from H-28/29 to C-3 and C-5, H-19 to C-1, C-5, C-6, C-9, and C-11, and H-18 to C-12 and C-17, H-30 to C-8, C-14, C-16 and C-18, H-21 to C-22, H-22 to C-24, and H-24 to C-26 and C-2 fully confirmed the basic skeleton cycloartane triterpene of compound **1**, which was consistent with the above deduction. The acetyl groups were connected with C-15 and C-16 supported by the correlations from H-15 to δ_C 170.9 (the carbonyl carbon of OAc) and H-16 to δ_C 171.2 (the carbonyl carbon of OAc). The sugar was connected with C-3 based on the key HMBC correlation between H-1′ (δ_H 4.86, d, J = 7.2 Hz) and C-3 (δ_C 88.8), which was identified as D-xylose by TLC in comparison with authentic monosaccharides (visualization with ethanol-5% H_2SO_4 spraying) followed by gas chromatography.

The NOESY experiment and coupling constants established the relative configuration of compound **1** (**Figure 3**), in which correlation of H-3/H-5 showed α-orientation of H-3. The larger coupling constants ($^3J_{1,2}$ > 7.0 Hz) of the anomeric protons indicated the β configuration of the sugar unit. The significant cross peaks from H-15 to H_3-18, H-17α to Me-21, H-16 to H_3-30, and H-24 to Me-21 were observed, which enabled the establishment of OAc-15α and OAc-16β. Until now, all the isolated cycloartane triterpene share the identical absolute configuration with *trans* A/B, B/C, C/D rings. Considering the same cycloartane triterpene skeleton and identical carbon signals at C-20/C-24, compound **1** was established as 20*S* and 24*R* configurations (Ju et al., 2002a). Therefore, the structure of the compound was identified as shown and given the trivial name actatica A.

Compound **2** was determined to have the molecular formula $C_{32}H_{50}O_6$, by the observation of the ion peak at m/z 553.3533 (calcd for $C_{32}H_{50}O_6Na$, 553.3500). The ^{1}H-NMR spectrum (**Tables 1**, **2**) displayed signals for seven tertiary methyls (δ_H 1.67, 1.62, 1.54, 1.54, 1.58, 1.08, and 1.25), two typical signals at δ_H 0.32 (1H, d, J = 4.2 Hz) and 0.53 (1H, d, J = 4.2 Hz) ascribable to a cyclopropane moiety, indicating that **2** might be a cycloartane-type triterpenoid. The ^{13}C NMR spectrum of 2 displayed 32 carbon signals, three signals attributable to oxygen-bearing quaternary carbons at δ 82.8, 111.2, and 72.4. The NMR data were similar to the reported one (20*S*, 24*S*)-16β, 24; 20, 24-diepoxy-9, 19-cycloeanostane-3β, 15α, 18, 25-tetraol-3-O-β-$_D$-xylopyranoside (Mu et al., 2014). The differences were the absence of the sugar at C-3, and the appearance of acetyl group at C-15 in compound **2**. In the HMBC spectrum, the correlation observed from H-15 to OAc together with the molecular formula confirmed the deduction above. *The α configurations of H-16 and H-17 were confirmed by the NOESY correlations between δ_H 1.86(H-17) and δ_H 1.23 (H3-30), δ_H 4.47 (H-16) and δ_H 2.00 (H-17)*. Taken together with the 2D-NMR spectra data, compound 2 was characterized and named actatica B.

Compound **3**, which was isolated as a white amorphous powder, was assigned as $C_{39}H_{60}O_{11}$, based on its positive HRESIMS ion at m/z 727.4100 (calcd for $C_{39}H_{60}O_{11}Na$, [M + Na]$^+$, 727.4088). The ^{1}H NMR spectrum showed that **3** possesses a cyclopropane ring, seven methyl groups, and an AB-type hydroxymethyl group (H_2-18). The NMR (**Tables 1**, **2**) spectroscopic data for this compound were analogous to **2**, except for the appearance of the anomeric proton at δ_H 4.81 (d, J = 7.8 Hz) and δ_C 105.1, 71.7, 76.0, 89.0, 67.6, 170.6, and 22.0. The

sugar was identified as a 4′-O-β-$_D$- xylose after acid hydrolysis. Inspection of the ^{1}H-^{1}H COSY spectrum showed fragments of C-1/C-2/C-3, C-5/C-6/C-7/C-8, C-11/C-12, C-15/C-16/C-17/C-18, and C-22/C-23. In the HMBC spectrum, the correlation from H-3 (3.36, dd, J = 11.4, 3.0 Hz) to the anomeric carbon signal at δ_C 86.7 supported that the sugar unit was attached to C-3. Thus, the structure of **3** was determined as actatica C.

Compound **4** has a molecular formula of $C_{39}H_{60}O_{13}$ according to the HRESIMS (*m/z* 759.3974 [M + Na] $^+$, calcd for $C_{39}H_{60}O_{13}Na$, 759.3926). Its IR spectrum showed strong hydroxyl (3,439, 1,044 cm^{-1}) and carboxyl (1730 cm^{-1}) absorptions. The ^{1}H and ^{13}CNMR spectra indicated that **4** had two acetoxyl groups. Detailed NMR spectral analysis revealed that **4** possessed a cyclopropane ring, six methyl groups, a hydroxymethyl group at C-18, and a D-xylosyl unit at C-3. The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of beesioside J (Ju et al., 2002b), except for a carbonyl group (C=O) connected to C-12 of **4**, which causes the downfield chemical shift of C-12 (δ_C 216.6). The correlation from δ_H 4.54 (H-11) to δ_C 216.6 (C=O) according to the HMBC supported the above result. Therefore, compound **4** was tentatively determined and named actatica D.

Compound **5** has the molecular formula $C_{42}H_{66}O_{12}$ determined by HR-ESIMS (*m/z* 785.4301, calcd for $C_{42}H_{66}O_{12}Na$ [M + Na] $^+$, 785.4341). In the ^{1}H NMR spectrum (**Table 1**) two cyclopropane–methylene protons as an AX system at δ_H 0.21 and 0.59 (each 1H, d, J = 4.0 Hz, H_2-19) together with nine tertiary methyl groups indicated a cycloartane triterpenoid structure. The ^{1}H NMR and ^{13}C APT data for this compound were analogous to 1, except for the additional NMR signals at δ_C 30.3 and 27.4, and δ_H 1.31 (3H, s), and 1.45 (3H, s). The differences showed that **5** had one more hydroxyisopropyl group connected at C-24. In the HMBC spectrum, the correlations from H-24 to C-26, C-26′, C-27, and C-27′ confirmed the above deduction. Taken together with the NOESY spectra data, compound **5** was established as 24*R* configurations. Thus, compound **5** was established and named actatica E.

Compound **6** was determined to have the molecular formula of $C_{37}H_{58}O_{11}$ based on the ^{13}C APT data and by the HRESIMS ion peak at *m/z* 701.3904 ([M + Na] $^+$, calcd for $C_{37}H_{58}O_{11}Na$, 701.3926). The ^{1}H-NMR spectrum (**Table 1**) displayed signals for seven tertiary methyls (δ 1.02, 1.22, 1.23, 1.30, 1.53, and 1.55), two typical signals at δ 0.12 (1H, d, J = 4.2 Hz) and 0.44 (1H, d, J = 4.2 Hz) ascribable to a cyclopropane methylene group, indicating that **6** might be a cycloartane-type triterpenoid. Examination of the ^{1}H and ^{13}C APT data (**Tables 1**, **2**) showed the structure of **6** to be similar to **4**. The NMR spectrum showed that compound 6 has only one set of acetyl group data. On the basis of ^{1}H-^{1}H COSY and HSQC and comparison with related **4**, all signals were assigned as shown in **Tables 1**, **2**. The correlation from H-16 (δ_H 5.47) to acetyl carbon (δ_C 171.8) was observed in HMBC spectrum, which means the acetyl group was connected to C-16. Therefore, compound **6** was clearly determined and named actatica F.

Compound **7** has the molecular formula $C_{39}H_{58}O_{13}$ according to its HRESIMS result (*m/z* 757.3765 [M + Na] $^+$, calcd for $C_{39}H_{58}O_{13}Na$, 757.3770). In the ^{1}H NMR spectrum (**Table 1**) two cyclopropane-methylene protons as an AX system at δ_H 0.28 and 0.41 (each 1H, d, J = 4.2 Hz, H_2-19) together with nine tertiary methyl groups indicated a cycloartane triterpenoid structure. The ^{1}H NMR and ^{13}C APT data were closely related to those of beesioside I (**Tables 1**, **2**) (Sakurai et al., 1990). The differences showed that **7** had a carbonyl group attached to C-12, which caused C-12 to move to a lower field, and the chemical shift is greatly increased to δ_C 216.6). The HMBC spectrum shows that δ_H 4.54 (H-11) is related to δ_C 216.6 (C = O), confirming the above inference. Moreover, in the NOESY spectrum, correlations were also detected between Me-21/H-22*α*/H-23*α*/H-24*α*, H-22*α*/H-22*β*, H-23*α*/H-23*β*, H-22*β*/H-23*β*, and H-24*α*/Me-26/Me-27. Considering the same cycloartane triterpene skeletonand identical carbon signals at C-20/C-24, compound **7** enabled a determination of a 20S*, 24R* configuration (Ju et al., 2016). As a result, the structure of **7** was established and named actatica G.

TABLE 3 | Cytotoxicity of compounds **1–7** against HT-29 and McF-7 cancer cell lines.

Compounds	HT-29 (μM)	McF-7 (μM)
1	10.4 ± 1.9[a]	11.8 ± 2.6
2	24.6 ± 2.6	26.4 ± 1.8
3	21.7 ± 2.3	23.2 ± 1.6
4	12.6 ± 2.8	12.1 ± 1.5
5	11.0 ± 1.1	23.9 ± 3.0
6	17.5 ± 2.2	12.3 ± 0.7
7	9.2 ± 3.0	11.4 ± 1.9
5-FU[b]	3.0 ± 2.1	2.3 ± 1.2

[a]*Value present mean ± SD of triplicate experiments.*
[b]*Positive control substance.*

Bioactive Activity

The cytotoxic of all compounds **1–7** were tested for their inhibitory activity against human HT-29 and McF-7 cancer cell lines using MTT assay. All compounds showed significant inhibitory effects with IC_{50} values of 9.2–26.4 μM (**Table 3**). Compound 7, with an oxygen bridge between C-18 and C-24, showed the best potency among the isolated constituents. With a tetrahydrofuran fragment connected by C-20 and C-24, compounds **1** and **4–7** showed better activity than **2** and **3**.

Seven new 9,19-cycloartane glycosides were isolated from the rhizomes of *A. asiatica* H. Hara. Until now, nearly 200 naturally occurring triterpenes with a 9,19-cycloartane have been reported (Su et al., 2016; Hassan et al., 2020). However, compound **5** with one more hydroxy isopropyl group was first isolated from the genus *Actaea*. All compounds displayed inhibitory activity against human HT-29 and McF-7 cancer cell lines. Further analysis of the data showed that compounds **1** and **4–7** exhibited better protective effect than other compounds, which indicated that the tetrahydrofuran fragment connected by C-20 and C-24 may affect the inhibitory activity regarding HT-29 and McF-7.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HW and XC were responsible for study design. MH and DZ were responsible for compound isolation and cytotoxic activity testing. GM, XX, MH, and DZ were responsible for structure elucidation and validation of compound identities. All authors contributed equally to manuscript writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2021.695456/full#supplementary-material

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