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Article

A New Triterpenoid Saponin from *Abrus precatorius* Linn

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Abstract: A new triterpenoid saponin, 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl subprogenin D (**1**), together with six known triterpenoids: subprogenin D (**2**), abrusgenic acid (**3**), triptotriterpenic acid B (**4**), abruslactone A (**5**), abrusogenin (**6**) and abrusoside C (**7**) were isolated from the leaves and stems of *Abrus precatorius*. Their structures were elucidated on the basis of physical and NMR analysis, respectively. Compounds **5** and **6** showed moderate cytotoxicity against MCF-7, SW1990, Hela, and Du-145 cell lines. Compounds **1**, **2** and **4** were isolated from this plant for the first time.

Keywords: *Abrus precarorius* Linn; triterpenoid; saponin; cytotoxicity

1. Introduction

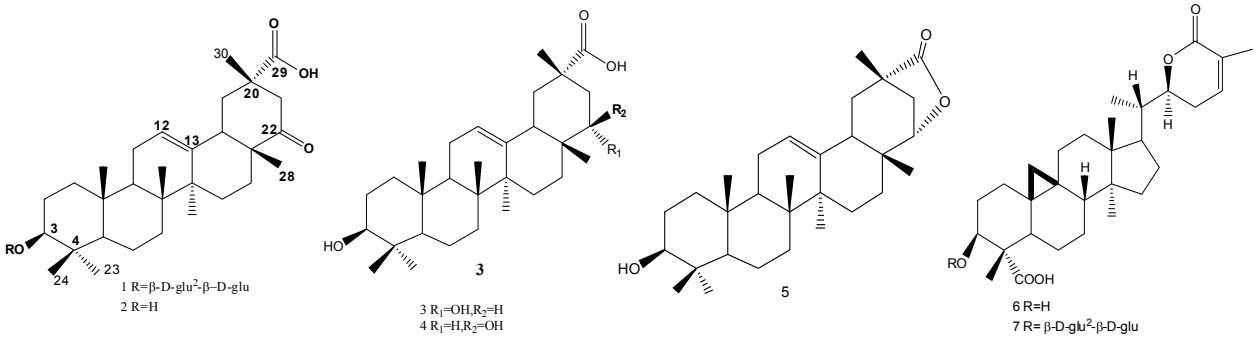
Abrus precatorius Linn belongs to the family Leguminosae. Its seeds, known as Xiang-si-zi, have been used in China as an insecticide and for treatment of some skin diseases since ancient times [1]. Besides, the leaves and roots are sweetish and traditionally used to cure fever, stomatitis, asthma and bronchititis [2]. Several groups of biologically active secondary compounds including alkaloids [3], flavones [4], triterpenoids [5] and isoflavano-quinones [6] have been isolated from this plant, some of

which possess anti-inflammatory [7], antibiosis [8], antiplatelet [9], and anti-implantation [10] properties. In our research on bioactive compounds from *Abrus precatorius* collected from the mangrove wetlands of Hainan Island, China, a new 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl subprogenin D (**1**), as well as six known ones (compounds **2**–**7**) were obtained. The structure of the new compound was elucidated using 1D, 2D NMR and MS experiments, while the configuration of **1** was defined by NOESY spectroscopy. Compounds **2**–**7** were identified as subprogenin D (**2**) [11], abrusgenic acid (**3**) [12], triptotriterpenic acid B (**4**) [13], abruslactone A (**5**) [14], abrusogenin (**6**) [15] and abrusoside C (**7**) [15], respectively, by comparison of their spectroscopic data with those reported in the literature. Compounds **1**, **2** and **4** were isolated from this plant for the first time.

2. Results and Discussion

The aqueous EtOH extract of the *Abrus precatorius* was suspended in water, and then partitioned with petroleum ether, EtOAc, and *n*-butanol by liquid-liquid extraction respectively. The EtOAc and *n*-butanol fractions were successively subjected to repeated silica gel column, Sephadex LH-20 to yield compounds **1**–**7** (Figure 1).

Figure 1. Structures of compounds **1**–**7**.



Compound **1** had the molecular formula $C_{42}H_{66}O_{14}$ as deduced from HRESI-MS *m/z* 817.4345 $[M+Na]^+$ (calcd for $C_{42}H_{66}O_{14}Na$, 817.4350) and NMR data (Table 1). The IR spectrum exhibited absorption bands at 3458 (OH), 1727(C=O), 1693(C=O) and 1624 (C=C) cm^{-1}. Seven methyl groups (δ_H 1.42, 1.32, 1.27, 1.23, 1.14, 0.91 and 0.86), one oxygenated methine proton (δ_H 3.29, dd, J = 5.0, 11.0 Hz), and one olefinic proton (δ_H 5.31, br s) were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR and DEPT data confirmed the presence of seven methyl carbons (δ_C 28.1, 25.5, 21.6, 20.9, 16.8, 16.7, 15.6), two olefinic carbons (δ_C 124.7, 141.4), one oxygenated methine carbons (δ_C 89.0), a carbonyl carbon (δ_C 214.9) and a carboxylic carbon (δ_C 178.6) (Table 1). The ^{1}H- and ^{13}C-NMR spectra of **1** has the characteristic of Δ^{12} oleanene skeleton [16]. Comparison the ^{13}C-NMR data of **1** with **2** except for the C-3 signal (δ_C 89.0) which shifted down field by 11 ppm, others were in accordance with that of 3*β*-hydroxy-22-oxo-12-oleanen-29-oic acid (**2**) [11]. The locations of carbonyl carbon and carboxylic carbon could be confirmed by the HMBC correlations from δ_H 1.23 (Me-28) to δ_C 214.9, 26.6 (C-16), and δ_H 1.42 (Me-30) to δ_C 178.6, 41.6 (C-19), 46.5 (C-21) (Figure 2). Moreover, the ^{1}H and ^{13}C-NMR spectra of **1** showed two sugar anomeric protons at δ_H 5.26 (1H, d, J = 7.0 Hz) and δ_H 5.03 (1H, d, J = 7.0 Hz) and carbons at δ_C 107.2 and 105.3 (Table 1). The monosaccharides were analysed as *β*-D-glucose with acetylated alditols derivatives by GC using authentic samples as references after

hydrolysis of **1**. This also could be validated by a combination of the coupling constants (J = 7.0 Hz for H-1″ and J = 7.0 Hz for H-1′) and 1D, 2D-NMR experiments. The signal at δ_C 89.0 in the ^{13}C-NMR suggesting that the *β*-D-glucose moieties are linked to the oxygen at C-3 of the aglycon [17]. This deduction and sequence of inter-glycosidic linkages were deduced from the following HMBC correlations: H-1′ (δ_H 5.03) of inner glucose with C-3 (δ_C 89.0) of sapogenin, H-1″ (δ_H 5.26) of terminal glucose with C-2′ (δ_C 83.9) (Figure 2). The relative configuration of the hydroxylated carbon (C-3) was assigned as *β* form mainly on the basis of ^{1}H-NMR coupling (1H, dd, J = 5.0, 11.0 Hz, H-3) [17] and by comparison with **2**. In NOESY spectrum, the key NOE correlations of H-28/H-21β, H-28/H-18 and H-30/H-18, showed that H-30, H-28, H-21β, H-18 were on the same face, so the relative stereochemistry were determined (Figure 3).

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **1** (in Pyr-d_5, δ in ppm, J in Hz).

No.	δ_C	δ_H	Key HMBC (H to C)
1	38.7 CH_2	1.40 (1H, m, H-1a) 0.82 (1H, m, H-1b)	C-2,C-3
2	27.3 CH_2	2.24 (2H, m, H-2)	C-1,C-3
3	89.0 CH	3.29 (1H, dd, J = 5.0, 11.0 Hz, H-3)	C-1′, 23, 24
4	39.5 qC		
5	55.6 CH	0.71 (1H, br d, J = 11.5 Hz, H-5)	C-23,24,25
6	18.4 CH_2	1.64 (1H, m, H-6a), 1.46 (1H, m, H-6b)	C-24
7	32.8 CH_2	1.48 (1H, m, H-7a), 1.29 (1H, m, H-7b)	
8	39.9 qC		
9	47.6 CH	1.56 (1H, m, H-9)	
10	36.8 qC		
11	23.8 CH_2	1.84 (2H, m, H-11)	
12	124.7 CH	5.31 (1H, br s, H-12)	
13	141.4 qC		
14	41.9 qC		
15	25.5 CH_2	1.68 (1H, m, H-15a), 0.98 (1H, m, H-15b)	
16	26.6 CH_2	1.89 (1H, m, H-16a), 1.28 (1H, m, H-16b)	C-28
17	48.2 qC		
18	47.0 CH	2.54 (1H, m, H-18)	
19	41.6 CH_2	2.88 (1H, t, J = 13.5 Hz, H-19a), 1.97 (1H, br d, J = 12.0 Hz, H-19b)	C-30
20	44.6 qC		
21	46.5 CH_2	3.46 (1H, d, J = 14.5 Hz, H-21a), 2.71 (1H, br d, J = 14.0 Hz, H-21b)	C-30
22	214.9 qC		
23	28.1 CH_3	1.32 (3H, s, Me-23)	C-3

Table 1. *Cont.*

No.	δ_C	δ_H	Key HMBC (H to C)
24	15.6 CH_3	0.86 (3H, s, Me-24)	C-3
25	16.7 CH_3	0.91 (3H, s, Me-25)	
26	16.8 CH_3	1.14 (3H, s, Me-26)	
27	25.5 CH_3	1.27 (3H, s, Me-27)	C-13
28	20.9 CH_3	1.23 (3H, s, Me-28)	C-22,C-16
29	178.6 qC		
30	21.6 CH_3	1.42 (3H, s, Me-30)	C-29,C-19,C-21
glu			
1′	105.3 CH	5.03 (1H, d, *J* = 7.0 Hz, H-1′)	C-3
2′	83.9 CH	4.33 (1H, t, *J* = 8.0 Hz, H-2′)	C-1′,C-1″,C-4′
3′	77.7 CH	4.40 (1H, br d, *J* = 8.0 Hz, H-3′)	
4′	73.1 CH	4.64 (3H, m, H-4′, 2″, 3″)	
5′	74.7 CH	4.20 (1H, br d, *J* = 9 Hz, H-5′)	
6′	61.3 CH_2	4.44 (2H, m, H-6′)	
glu			
1″	107.2 CH	5.26 (1H, d, *J* = 7.0 Hz, H-1″)	C-2′,C-3″
2″	74.9 CH	4.64 (3H, m, H-4′, 2″, 3″)	C-4″
3″	77.4 CH	4.64 (3H, m, H-4′, 2″, 3″)	
4″	69.5 CH	4.73 (1H, m, H-4″)	
5″	76.9 CH	4.09 (1H, t, *J* = 6.1 Hz, H-5″)	
6″	61.3 CH_2	4.67 (2H, m, H-6″)	

Figure 2. Key HMBC and COSY correlations of **1**.

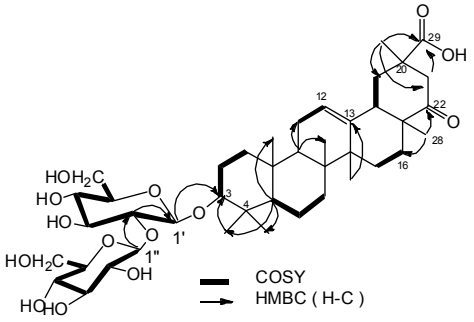
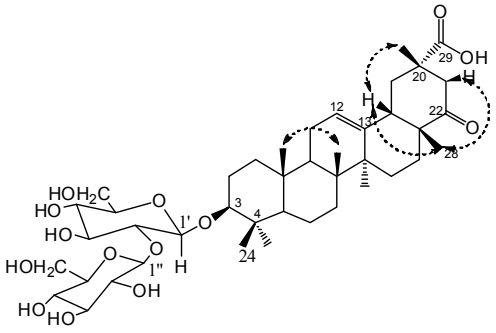


Figure 3. Key NOESY correlations of **1**.



All the above data identified **1** as 3-O-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl subprogenin D. The structures of known compounds **2**–**7** were confirmed by detailed NMR data comparison with those in the literature [11-15].

The cytotoxicity of **1**–**7** against MCF-7, SW1990, Hela, Du-145 cancer cell lines were evaluated with 5-FU (5-Fluorouracil) and DOX (doxorubicine) as positive controls. Compound **5** showed moderate cytoxicity against SW1990, Hela, Du-145 cancer cell lines, and compound **6** showed moderate cytoxicity against MCF-7, SW1990, Du-145 cancer cell lines, whereas other compounds had no significant activity (Table 2).

Table 2. Cytotoxicity of **1**–**7** against four cancer cell lines.

	Cytotoxicity (IC_{50} [μg/mL])(mean ± SD%)			
	MCF-7	**SW1990**	**Hela**	**Du-145**
1	-[a]	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	5 ± 0.32	10 ± 0.89	5 ± 0.40
6	4 ± 0.18	2 ± 0.09	-	2 ± 0.08
7	-	-	-	-
DOX	1 ± 0.06		2 ± 0.16	1 ± 0.05
5-Fu		10 ± 0.95		

[a] No significant activity at 10 μg/mL.

3. Experimental

3.1. General

1D and 2D NMR spectra were recorded on a Bruker-AV-500 spectrometer with TMS as internal standard. HRESIMS were measured with MAT 95XP mass spectrometer. IR were recorded on FT-IR Nicolet 6700. UV spectra were obtained on a Beckman DU-640 UV spectrophotometer. Optical rotations were measured with a Perkin-Elmer 341 plus. GC were run on a QP2010PLUS (Shimadzu Corporation) equipped with an ACQ mass spectrometer. For column chromatography (CC), silica gel (200–300 mesh) and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, China.

3.2. Plant Material

The leaves and stems of *Abrus precatorius* were collected in October 2010 from the mangrove wetlands of Hainan Island, China. The identification of the plant was performed by Professor Si Zhang. A voucher sample (No. 20101001) is maintained in the Key Laboratory of Marine Bio-Resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, China.

3.3. Extraction and Isolation

The air-dried leaves and stems of *Abrus precatorius* (8 kg) were extracted with EtOH (95%, 20 L) three times (7 days each time) at room temperature. The combined extract was evaporated *in vacuo*, suspended in water, and then successively partitioned with petroleum ether, EtOAc, and *n*-butanol (800 mL × 3). The EtOAc and *n*-butanol fractions were concentrated to afford 53.7 g and 108 g of residues, resp. The EtOAc extract was subjected to silica gel CC using a gradient elution of $CHCl_3$-MeOH (100:0–1:1) to afford ten fractions (Frs. **A**–**J**). Compound **3** (11 mg) was crystallizated in the bottle when eluted with the solvent $CHCl_3$-MeOH 90:10 (Frs. **B**) and then purified with MeOH. Frs. **B** (7 g) was subjected to CC and eluted with $CHCl_3$-acetone (30:1, 20:1, 15:1, 10:1, 5:1, each 1500 mL) to give six fractions (**B1**–**B6**), **B3** was purified by Sexphadex LH-20 ($CHCl_3$-MeOH 1:1) to yield **2** (8 mg). Compound **4** (10 mg) was isolated from **B1** by repeated chromatographic on Sephadex LH-20 ($CHCl_3$-MeOH 1:1) and silica gel column ($CHCl_3$-MeOH 80:1). Frs. **C** (4.4 g) was subjected to CC with gradient eluting of $CHCl_3$-acetone (30:1, 20:1, 15:1, 10:1, 5:1, each 1,000 mL) to give six fractions (**C1**–**C6**). Compound **5** (7 mg) was crystallizated from **C2** when eluted with the solvent $CHCl_3$-acetone 20:1, and then recrystallizated with MeOH. **C4** was purified by Sexphadex LH-20 ($CHCl_3$-MeOH 1:1) to give compound **6** (7 mg). Frs. **G** (2.36 g) was subjected to CC with gradient eluting of $CHCl_3$-MeOH (30:1, 20:1, 15:1, 10:1, 5:1, each 500 mL) and purification on Sephadex LH-20 to yield **7** (20 mg). The *n*-BuOH extract (108 g) was subjected to CC on Amberlite XAD using MeOH-H_2O (20%, 40%, 60% and 95%). The 40% extract part (7.23 g) was fractioned on silica gel column eluting with $CHCl_3$-MeOH-H_2O 9:1:0.1 to give ten fraction **H**–**Q**. Fraction **Q** (0.4265 g) was purified by Sexphadex LH-20 ($CHCl_3$-MeOH 1:1) to give compound **1** (5 mg). Yellow powder: $[\alpha]_D^{20} = -10$ (*c* = 0.04, MOH), UV (MeOH) λ_{max} 255 nm, IR (KBr) ν_{max}: 3458, 2946, 1727, 1693, 1624, 1466, 1383, 1211, 1042 cm^{-1}; HRESI-MS *m/z* 817.4345 $[M+Na]^+$ (calcd for $C_{42}H_{66}O_{14}Na$, 817.4350). ^1H and ^{13}C-NMR data see Table 1.

3.4. Acid Hydrolysis of **1**

Compound **1** (2 mg) was added into 3 N HCl (0.5 mL) and refluxed for 5 h in a water bath (100 °C). The solution was neutralized and extracted with EtOAc to afford the aglycon. The aglycon of **1** found to be identical with **2** by TLC. The sugars released were converted into acetylated alditols by reduction with $NaBH_4$ followed by acetylation with acetic anhydride-pyridine mixture. The alditol acetates derivatives obtained were analyzed by GC using a GCMS-QP2010 Plus: The injector temperature was set at 250 °C and the column temperature program was as follows: The initial temperature of 200 °C was held constant for 2.5 min and then increased by 5 °C min to the final temperature of 250 °C. The detector temperature was set at 280 °C. MS-Scan: ACQ mode, event Time: 0.50 s with 1,000 scan speed. Alditol acetates were identified by comparison of their retention times with those of authentic samples [16].

3.5. Cytotoxicity Assays

Cytotoxicity was evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide] method using MCF-7, SW1990, Hela and Du-145 cell lines. Details of the assays were described in a previous report [18].

4. Conclusions

The new compound 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl subprogenin D (**1**) was isolated from *Abrus precatorius*, together with six known triterpenoids. Compounds **5** and **6** showed moderate cytoxicities against MCF-7, SW1990, Hela and Du-145. However, the new compound **1** and the other known ones had no significant activity.

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Sample Availability: Not availbale.