Triterpene Saponins from *Entada phaseoloides*

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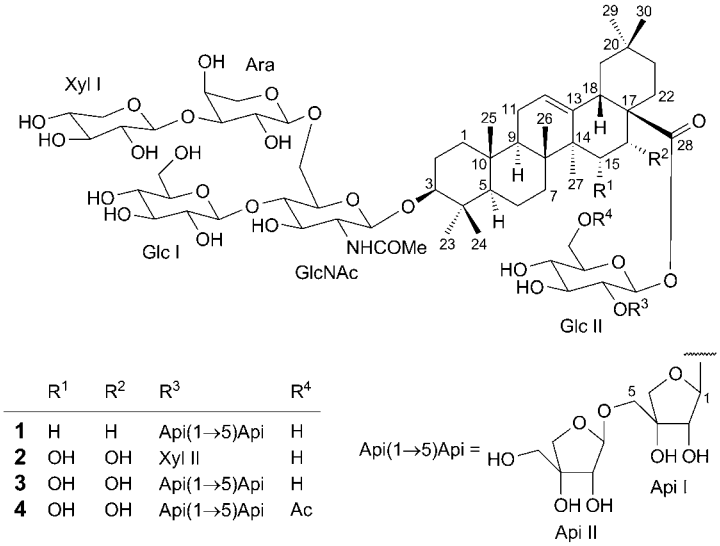
Four oleanane-type triterpene saponins, phaseoloideside A–D (**1**–**4**), were isolated from the seed kernels of *Entada phaseoloides*, along with rheediinoside B. The structures of the four new compounds were established by 2D-NMR spectroscopic methods, HR-ESI-MS analysis, and chemical degradation. Phaseoloideside D (**4**) showed cytotoxic activity against the Eca-109 cell line with an IC_{50} value of 28.0 μM.

Introduction. – The genus *Entada* (Leguminosae) comprises about 30 species and is distributed widely in tropical areas of Africa and America, whereas there exists only one species in China – *Entada phaseoloides* (L.) MERR. It has long been used as a traditional folk medicine for the treatment of jaundice, stomachache, constipation, and hernia by one of China's ethnic minorities, the Dai people living in Yunnan Province, southwest of China. In addition, it is the core constituent of the famous Dai herbal compound formula, 'Qi-Wei-Ke-Teng-Zi-Wan', recorded in the Chinese Pharmacopoeia, which is used to treat stomach disease [1]. Previous chemical investigations on *Entada phaseoloides* (L.) have indicated the presence of natural products, including S-containing amides [2–5], phenylacetic acid derivatives [6–10], and oleanane-type triterpene saponins [2][11–14]. Meanwhile, pharmacological studies on extracts of *Entada phaseoloides* and isolated principles have illustrated a variety of activities, including antiulcer activity and anti-inflammatory activity [3]. Moreover, some triterpene saponins from the genus have been reported to be cytotoxic against several tumor cell lines [15–17]. Further pharmacological study indicated that S-containing amides and phenylacetic acid derivatives are to be considered as the major bioactive components [18]. In our previous studies on *Entada phaseoloides*, a new S-containing amide and two cytotoxic triterpene saponins were reported [19][20].

Results and Discussion. – An active BuOH fraction prepared from the 70% aq. EtOH extract of the seed kernels of *Entada phaseoloides* was subjected to repeated column chromatography (silica gel, *ODS*, and *MCI* gel), followed by semi-prep. HPLC to yield four new compounds, phaseoloideside A–D (**1**–**4**) (*Fig.*), along with the known compound rheediinoside B [14].

Phaseoloideside A (**1**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{70}H_{113}NO_{34}$, deduced from the HR-ESI-MS ($[M+Na]^+$ at



	R^1	R^2	R^3	R^4
1	H	H	Api(1→5)Api	H
2	OH	OH	Xyl II	H
3	OH	OH	Api(1→5)Api	H
4	OH	OH	Api(1→5)Api	Ac

Figure. *Phaseoloidesides A–D* (**1**–**4**), *isolated from* Estada phaseoloides

1534.7037), as well as its NMR spectroscopic data. The FAB-MS (negative-ion mode) showed the quasi-molecular-ion peak at *m*/*z* 1510 ($[M-1]^-$), and further fragments were observed at *m*/*z* 1378 ($[M-132-1]^-$), 1246 ($[M-132-132-1]^-$), 1084 ($[M-132-132-162-1]^-$), 922 ($[M-162-132-132-162-1]^-$), 658 ($[M-162-132-132-162-132-132-1]^-$), and 455 ($[M-132-132-162-162-132-132-203-1]^-$), corresponding to the successive loss of one pentosyl, one pentosyl, one hexosyl, one hexosyl, two pentosyls, and one (acetylamino)deoxyhexosyl moiety, respectively. The ^{1}H-NMR spectrum of **1** (*Tables 1* and *2*) showed signals for seven tertiary Me groups at δ(H) 1.28, 1.19, 1.19, 1.01, 0.93, 0.87, and 0.77 (each *s*) and an olefinic H-atom at δ(H) 5.40 (*t*-like), being characteristic for an oleanolic acid (=(3β)-3-hydroxyolean-12-en-28-oic acid) skeleton. In addition, signals for seven anomeric H-atoms were observed at δ(H) 6.43, 6.14, 5.63, 5.54, 5.25, 5.10, and 4.82, which were correlated with seven anomeric C-atom signals at δ(C) 110.9, 94.7, 110.8, 104.5, 103.5, 104.5, and 107.0, respectively, based on the HSQC experiment. Hydrolysis of compound **1** yielded D-glucose, D-apiose, D-xylose, and L-arabinose in a ratio of 3:2:1:1, as established by GC analysis of derivatives of these monosaccharides, and comparison with those of the reference sugars [2][15–17]. A downfield ^{13}C-NMR chemical shift of C(3) (δ(C) 89.4) and an upfield chemical shift of C(28) (δ(C)176.5) were observed (*Table 1*), suggesting that compound **1** is a bidesmosidic glycoside of oleanolic acid with sugar linkages at C(3) through an acetal bond and at C(28) through an ester bond. The combined analysis of COSY, HSQC, ROESY, HMBC, and HSQC-TOCSY experiments and acid hydrolysis allowed the complete assignment of the sugar residues as one terminal β-D-xylopyranosyl (Xyl), two β-D-apiofuranosyl (Api), two β-D-glucopyranosyl (Glc), one α-L-arabinopyranosyl (Ara), and one 2-(acetylamino)-2-deoxy-β-D-glucopyranosyl unit. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of the

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of the Aglycon Moieties of Compounds* **1**–**4**[a]). δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.45 (d, J = 12.5), 0.90–0.92 (m)	39.0	1.45 (d, J = 15.0), 0.90–0.93 (m)	39.0	1.44 (d, J = 15.0), 0.87–0.89 (m)	39.0	1.46–1.52, 0.83–0.89 (2m)	37.2
CH_2(2)	2.00–2.16 (m)	28.2	1.63–1.69, 2.00–2.10 (2m)	26.2	1.62–1.65, 2.09–2.16 (2m)	26.5	1.68–1.74, 2.16–2.19 (2m)	26.3
H–C(3)	3.28 (*dd*, J = 11.4, 4.4)	89.4	3.30 (*dd*, J = 11.4, 4.4)	89.5	3.30 (br. d, J = 11.5)	89.7	3.30 (*dd*, J = 11.4, 4.4)	89.7
C(4)	–	39.3	–	39.2	–	39.3	–	39.3
H–C(5)	0.76[b])	55.5	0.76[b])	56.0	0.81[b])	55.6	0.81[b])	55.6
CH_2(6)	1.70–1.76 (m)	19.5	1.40 (d, J = 15.0), 1.70 (d, J = 15.0)	19.0	1.33 (d, J = 15.0), 1.56 (d, J = 15.0)	19.0	1.38 (d, J = 15.0), 1.58 (d, J = 15.0)	19.0
CH_2(7)	1.47–1.52 (m)	33.5	1.54–1.57 (m)	39.1	1.99[b]), 2.20 (br. s)	37.1	1.99[b]), 2.22 (br. s)	39.1
C(8)	–	42.1	–	41.6	–	42.1	–	41.6
H–C(9)	1.72–1.75 (m)	47.0	1.65 (*t*-like, J = 15.0)	47.0	1.70 (*t*-like, J = 15.0)	47.5	1.65 (br. d, J = 15.0)	47.5
C(10)	–	37.0	–	37.1	–	37.2	–	37.2
CH_2(11)	1.90 (br. d, J = 5.0)	22.5	2.00 (br. d, J = 5.0)	24.2	1.99[b])	24.1	1.99[b])	23.8
H–C(12)	5.40 (br. s)	122.8	5.65 (*t*-like)	124.8	5.40 (*t*-like)	124.8	5.40 (*t*-like)	124.8
C(13)	–	144.1	–	144.6	–	144.7	–	144.5
C(14)	–	50.0	–	47.7	–	47.9	–	48.9
CH_2(15) or H–C(15)	1.25–1.27, 2.05–2.09 (2m)	27.7	4.28 (d, J = 4.9)	68.4	4.25 (d, J = 5.0)	68.9	4.25 (d, J = 5.0)	68.4
CH_2(16) or H–C(16)	0.87–0.91 (m)	37.0	5.00[b])	79.1	5.02 (*d*-like, J = 5.0)	79.1	5.02 (*d*-like, J = 5.0)	78.9
C(17)	–	46.9	–	48.6	–	48.7	–	49.5
H–C(18)	3.13 (d, J = 10.0)	42.0	3.45 (d, J = 10.0)	41.6	3.37 (d, J = 10.0)	42.1	3.45 (d, J = 10.0)	42.1
CH_2(19)	1.16–1.20 (m)	46.0	1. 25–1.29 (m), 2.75 (*t*-like, J = 15.0)	46.8	2.74 (*t*-like, J = 15.0)	46.7	2.78 (br. d, J = 15.0)	46.8
C(20)	–	30.8	–	30.8	–	30.9	–	30.9
CH_2(21)	1.10 (d, J = 2.5)	35.5	1.10 (d, J = 10.0)	36.0	1.28 (d, J = 10.0), 2.38–2.45 (m)	36.0	1.32 (d, J = 10.0), 2.42 (*dd*, J = 10.0, 5.0)	36.0
CH_2(22)	1.30–1.34 (m)	33.5	1.28–1.30 (m)	32.4	2.22–2.28 (m)	31.9	2.26–2.29 (m)	31.9
Me(23)	1.19 (s)	28.1	1.08 (s)	28.1	1.14 (s)	28.2	1.14 (s)	28.2

Table 1 (cont.)

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Me(24)	0.93 (*s*)	17.0	0.86 (*s*)	17.0	0.93 (*s*)	17.1	0.94 (*s*)	17.1
Me(25)	0.77 (*s*)	15.8	0.74(*s*)	15.7	0.80 (*s*)	15.8	0.80 (*s*)	15.8
Me(26)	1.01 (*s*)	17.4	1.14 (*s*)	18.1	1.16 (*s*)	18.1	1.17 (*s*)	18.1
Me(27)	1.28 (*s*)	26.2	1.81 (*s*)	20.8	1.80 (*s*)	20.8	1.81 (*s*)	20.8
C(28)	–	176.5	–	175.8	–	175.7	–	175.7
Me(29)	0.87 (*s*)	33.2	1.05 (*s*)	33.2	0.99 (*s*)	33.4	1.00 (*s*)	33.4
Me(30)	1.19 (*s*)	26.2	1.13 (*s*)	24.6	1.05 (*s*)	24.6	1.13 (*s*)	24.6

[a]) Assignments were confirmed by HMBC, HSQC, HSQC-TOCSY, and ROESY experiments. [b]) Overlapped signals are reported without designated multiplicity.

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of the Sugar Moieties of Compounds* **1**–**4**[a]). δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
GlcNAc:								
H–C(1)	5.10 (*d*, *J* = 7.9)	104.5	5.10 (*d*, *J* = 7.9)	104.5	5.10 (*d*, *J* = 7.9)	104.5	5.10 (*d*, *J* = 7.9)	104.5
H–C(2)	4.42 (*t*-like, *J* = 9.0)	58.6	4.44 (*t*-like, *J* = 9.0)	57.5	4.45 (*t*-like, *J* = 9.0)	57.6	4.45 (*t*-like, *J* = 8.5)	57.6
H–C(3)	4.14 (*dd*, *J* = 9.4, 8.7)	74.0	4.10 (*dd*, *J* = 9.5, 9.0)	73.9	4.14 (*t*-like, *J* = 9.0)	73.9	4.14 (*dd*, *J* = 9.0, 8.0)	73.9
H–C(4)	4.76 (*d*, *J* = 5.0)	80.8	4.80 (*t*-like, *J* = 9.5)	80.9	4.85 (br. *d*, *J* = 9.7)	81.0	4.85 (*dd*, *J* = 9.2, 8.4)	80.8
H–C(5)	4.32–4.34 (*m*)	75.3	4.26–4.28 (*m*)	75.1	4.27–4.31 (*m*)	75.1	4.30–4.32 (*m*)	75.1
CH_2(6)	4.60 (*dd*, *J* = 11.6, 2.6), 4.23 (*dd*, *J* = 11.7, 3.0)	68.2	4.70 (br. *d*, *J* = 11.6), 4.33 (br. *d*, *J* = 12.0)	67.6	4.50–4.54 (*m*)	68.2	4.50–4.53 (*m*)	68.2
MeC=O	2.15 (*s*)	23.7, 170.0	2.16 (*s*)	24.6, 170.0	2.15 (*s*)	23.7, 170.0	2.13 (*s*)	23.7, 170.2
Glc I:								
H–C(1)	5.54 (*d*, *J* = 7.9)	104.5	5.52 (*d*, *J* = 7.9)	104.5	5.53 (*d*, *J* = 7.9)	104.5	5.53 (*d*, *J* = 7.9)	104.5
H–C(2)	4.02 (*dd*, *J* = 9.0, 8.0)	75.1	3.96 (*dd*, *J* = 9.0, 8.0)	75.7	3.98 (*t*-like, *J* = 9.6)	74.8	3.98 (*t*-like, *J* = 9.4)	74.8
H–C(3)	4.26 (br. *d*, *J* = 10.0)	78.9	4.02 (br. *d*, *J* = 9.0)	78.6	4.26–4.28 (*m*)	78.8	4.28 (*t*-like, *J* = 8.0)	78.7
H–C(4)	3.95–3.99 (*m*)	71.2	4.24 (br. *d*, *J* = 10.0)	70.1	4.10 (*t*-like, *J* = 10.0)	71.3	4.10 (*t*-like, *J* = 9.6)	71.5
H–C(5)	4.20–4.23 (*m*)	78.9	4.19–4.22 (*m*)	78.6	4.20–4.25 (*m*)	78.7	4.22–4.25 (*m*)	78.7
CH_2(6)	4.32–4.34 (*m*), 4.23 (*dd*, *J* = 11.4, 4.0)	62.3	4.48 (br. *d*, *J* = 12.0), 4.32 (br. *d*, *J* = 11.5)	62.3	4.48 (*dd*, *J* = 11.7, 3.0), 4.32 (br. *d*, *J* = 12.0)	62.3	4.48 (br. *d*, *J* = 12.0), 4.32 (br. *d*, *J* = 11.5)	62.3
Ara:								
H–C(1)	5.25[b])	103.5	5.25 (*d*, *J* = 7.0)	103.4	5.25[b])	103.5	5.25 (*d*, *J* = 6.5)	103.5
H–C(2)	4.50 (*dd*, *J* = 9.0, 8.0)	73.9	4.42 (*dd*, *J* = 9.0, 8.5)	73.2	4.48 (*dd*, *J* = 9.0, 8.0)	73.2	4.48 (*dd*, *J* = 9.0, 8.6)	73.2
H–C(3)	4.48 (br. *d*, *J* = 9.0)	83.4	4.50 (br. *d*, *J* = 9.2)	83.1	4.49–4.54 (*m*)	83.4	4.53 (*t*-like, *J* = 8.5)	83.3
H–C(4)	4.23 (br. *d*, *J* = 2.0)	69.2	4.22 (br. *d*, *J* = 2.4)	68.9	4.23 (br. *d*, *J* = 2.5)	68.5	4.23 (br. *d*, *J* = 3.0)	68.9
CH_2(5)	3.60 (*d*-like, *J* = 9.0), 4.20 (*d*-like, *J* = 10.0)	67.5	4.18 (br. *d*, *J* = 14.0)	67.5	3.52 (*d*-like, *J* = 9.0), 4.20 (*d*-like, *J* = 10.0)	66.4	3.52 (*dd*, *J* = 11.6, 2.0), 4.20 (*d*-like, *J* = 12.0)	66.4
Xyl I:								
H–C(1)	4.82 (*d*, *J* = 7.4)	107.0	4.81 (*d*, *J* = 7.5)	107.8	4.82 (*d*, *J* = 7.4)	107.0	4.82 (*d*, *J* = 7.4)	107.8
H–C(2)	4.04 (*dd*, *J* = 8.5, 7.0)	76.7	4.04 (*t*-like, *J* = 8.5)	74.8	4.03–4.07 (*m*)	76.7	4.04 (*t*-like, *J* = 8.8)	76.5
H–C(3)	4.13 (*t*-like, *J* = 9.0)	78.9	4.12 (*t*-like, *J* = 8.5)	78.6	4.09–4.14 (*m*)	78.9	4.13 (*t*-like, *J* = 9.0)	78.0
H–C(4)	4.28 (br. *d*, *J* = 11.0)	71.0	4.26 (*dd*, *J* = 12.0, 10.0)	71.0	4.28 (br. *d*, *J* = 10.0)	71.0	4.28 (*t*-like, *J* = 10.5)	71.0
CH_2(5)	3.50 (*t*-like, *J* = 12.0), 4.60–4.64 (*m*)	67.5	3.50 (*t*-like, *J* = 12.0), 3.64 (br. *d*, *J* = 11.0)	67.5	3.50(*dd*, *J* = 10.0, 9.0), 4.60 (*dd*, *J* = 11.0, 5.6)	67.5	3.50 (*t*-like, *J* = 10.5), 4.60 (*dd*, *J* = 11.0, 5.0)	67.5

Table 2 (cont.)

	1		**2**		**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Glc II:								
H–C(1)	6.14 (*d*, *J* = 7.9)	94.7	6.20 (*d*, *J* = 7.9)	93.6	6.14 (*d*, *J* = 7.5)	94.5	6.14 (*d*, *J* = 7.5)	94.2
H–C(2)	4.28 (*dd*, *J* = 9.0, 8.0)	78.9	4.25 (*dd*, *J* = 8.0, 8.0)	80.9	4.27–4.32 (*m*)	80.1	4.25 (*t*-like, *J* = 8.6)	78.9
H–C(3)	4.25 (*t*-like, *J* = 8.8)	78.1	4.25 (*t*-like, *J* = 8.0)	78.9	4.20 (*t*-like, *J* = 9.0)	78.6	4.20 (*t*-like, *J* = 8.8)	78.7
H–C(4)	4.24 (*dd*, *J* = 9.0, 9.0)	70.9	4.04–4.10 (*m*)	71.9	4.24 (*t*-like, *J* = 9.2)	70.9	4.24–4.27 (*m*)	70.9
H–C(5)	3.99 (br *d*, *J* = 9.0)	78.2	3.95–4.00 (*m*)	78.1	3.97–4.01 (*m*)	78.2	3.99–4.03 (*m*)	78.2
CH_2(6)	4.36 (*t*-like, *J* = 11.0), 4.28 (*dd*, *J* = 11.7, 2.7)	62.5	4.48 (br. *d*, *J* = 11.0), 4.32 (br. *d*, *J* = 10.0)	62.1	4.36 (*dd*, *J* = 10.0, 9.0), 4.28 (*dd*, *J* = 11.0, 5.0)	62.5	4.36 (*t*-like, *J* = 11.0), 4.28 (*dd*, *J* = 11.5, 2.3)	64.8
MeCO							1.91 (*s*)	20.8, 170.9
	Api I:		Xyl II:		Api I:		Api I:	
H–C(1)	6.43 (*d*, *J* = 3.5)	110.9	5.48 (*d*, *J* = 7.5)	106.6	6.38 (*d*, *J* = 3.6)	110.9	6.38 (*d*, *J* = 3.5)	110.9
H–C(2)	4.76 (*d*, *J* = 3.0)	77.8	4.52 (*t*-like, *J* = 9.0)	76.6	4.76 (*d*, *J* = 3.0)	78.0	4.76 (*d*, *J* = 2.7)	78.0
H–C(3)	–	80.4	4.09–4.14 (*m*)	78.9	–	80.4	–	80.4
CH_2(4)	4.41 (*t*-like, *J* = 9.7), 4.32–4.38 (*m*)	75.3	4.28–4.31 (*m*)	70.5	4.74–4.78, 4.29–4.33 (2*m*)	75.2	4.28–4.32, 4.69–4.72 (2*m*)	75.1
CH_2(5)	4.04 (br. *d*, *J* = 9.6), 4.32 (br. *d*, *J* = 9.6)	71.8	4.04 (br. *d*, *J* = 10.0), 4.32 (br. *d*, *J* = 4.0)	68.1	4.13[b])	71.6	4.13[b])	71.6
	Api II:		–					
H–C(1)	5.63 (*d*, *J* = 2.5)	110.8	–	–	5.60 (*d*, *J* = 2.5)	110.8	5.60 (*d*, *J* = 2.5)	110.8
H–C(2)	4.68 (br. *s*)	77.5	–	–	4.64 (br. *s*)	77.6	4.64 (*d*-like)	77.6
H–C(3)	–	80.4	–	–	–	80.4	–	80.4
CH_2(4)	4.55 (br. *d*, *J* = 9.7)	75.1	–	–	4.55 (*dd*-like, *J* = 9.7)	75.1	4.55 (*dd*-like)	75.1
CH_2(5)	3.60–3.65, 4.13–4.20 (2*m*)	65.5	–	–	3.59–3.65, 4.19–4.23 (2*m*)	65.5	3.59–3.64, 4.19–4.24 (2*m*)	65.5

[a]) Assignments were confirmed by HMBC, HSQC, HSQC-TOCSY and ROSEY experiments. [b]) Overlapped signals are reported without designated multiplicity.

alkaline hydrolysis product of rheediinoside B (=(3β,15α,16α)-15,16-dihydroxy-3-{{*O*-β-D-xylopyzanosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}oxy}olean-12-en-28-oic acid 28-[*O*-D-apio-β-D-furanosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl] ester) revealed a high degree of structural similarity between the two compounds, which allowed to determine the oligosaccharide chain at C(3) as an {*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}oxy chain. The sequence within the sugar chain at C(3) was further substantiated by HMBC experiments. The significant glycosidation shift of the aglycone at C(3) (δ(C) 89.4) and the significant HMBC between H–C(1) of GlcNAc (δ(H) 5.10, *d*, *J* = 7.9) Hz and C(3) indicated that the GlcNAc moiety was attached at C(3) of the aglycon. Additional correlations were observed between H–C(1) of Glc I (δ(H) 5.54, *d*, *J* = 7.9 Hz) and C(4) of GlcNAc (δ(H) 80.8), between H–C(1) of Ara (δ(H) 5.25) and C(6) of GlcNAc (δ(C) 68.2), and between H–C(1) of Xyl I (δ(H) 4.82, *d*, *J* = 7.4 Hz) and C(3) of Ara (δ(C) 83.4), indicating the location of Glc I at C(4) of GlcNAc, of Ara at C(6) of GlcNAc, and of Xyl I at C(3) of Ara. In a same manner, the remarkable glycosidation shift of C(28) (δ(C) 176.5) and the HMBC of H–C(1) of Glc II (δ(H) 6.14, *d*, *J* = 7.9 Hz) to C(28) indicated that Glc II was connected to C(28) of the aglycone. The important correlations of H–C(1) of Api I (δ(H) 6.43, *d*, *J* = 3.5 Hz) to C(2) of Glc II (δ(C) 78.9), and of H–C(1) of Api II (δ(H) 5.63, *d*, *J* = 2.5 Hz) to C(5) of Api I (δ(C) 71.8), unambiguously confirmed the oligosaccharide chain at C(28) to be an [*O*-D-apio-β-D-furanosyl-(1 → 5)-*O*-D-apio-β-D-furanosyl-(1 → 2)-β-D-glucopyranosyl]oxy chain. Therefore, compound **1** was determined as 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}oleanolic acid 28-[*O*-D-apio-β-D-furanosyl-(1 → 5)-*O*-D-apio-β-D-furanosyl-(1 → 2)-β-D-glucopyranosyl] ester.

Phaseoloideside B (**2**) was obtained as a white amorphous powder. The HR-ESI-MS (positive-ion mode) of **2** showed an accurate $[M + Na]^+$ ion peak at *m*/*z* 1434.6519, in accordance with the empirical molecular formula $C_{65}H_{105}NO_{32}$. The FAB-MS (negative-ion mode) showed the quasi-molecular-ion peak at *m*/*z* 1410 ($([M-1])^-$; further fragments were observed at *m*/*z* 1394 ($[M-17]^-$), 1278 ($[M-132-1]^-$), 1248 ($[M-162-1]^-$), and 1116 ($[M-132-162-1]^-$). The ^{1}H-NMR data (*Tables 1* and *2*) of the aglycon moiety of **2** showed signals for seven tertiary Me groups at δ(H) 1.81, 1.14, 1.13, 1.08, 1.05, 0.86, and 0.74 (each *s*), a trisubstituted olefinic H-atom at δ(H) 5.65 (*t*-like), two O-bearing CH groups at δ(H) 4.28 (*d*, *J* = 4.9 Hz, H–C(15)) and 5.00 (overlapped, H–C(16)). In comparison to compound **1**, the differences in the aglycon were variations of the chemical shifts of C(15) and C(16). In the ^{13}C-NMR spectrum, the signals of these C-atoms changed to δ(C) 68.4 and δ(C) 79.1, respectively. Furthermore, the β-axial configuration of H–C(15) was deduced from the ROESY correlation H–C(15) (δ(H) 4.28)/Me(26) (δ(H) 1.14), and the cross-peak H–C(15)/H–C(16) was in good agreement with the assigned configuration (both OH groups in α-axial position). Therefore, the aglycon was identified as entagenic acid (=(3β,15α,16α)-3,15,16-trihydroxyolean-12-en-28-oic acid) [11–14]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of rheediinoside B [14] showed a considerable structural similarity. The only difference was the absence of a terminal D-

apio-β-D-furanosyl moiety at C(28). The further evidence of the HMBC key cross-peaks between H–C(1) of GlcII (δ(H) 6.20, *d*, *J* = 7.9 Hz) and C(28) (δ(C) 175.8), and between H–C(1) of Xyl II (δ(H) 5.48, *d*, *J* = 7.5 Hz) and C(2) of Glc II (δ(C) 80.9) allowed the complete assignment of location and sequence of the C(28) sugar chain. Consequently, compound **2** was elucidated as 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}entagenic acid 28-[*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl] ester.

Phaseoloideside C (**3**) was obtained as a white amorphous powder with the molecular formula $C_{70}H_{113}NO_{36}$, determined by the HR-ESI-MS (positive-ion mode) ($[M+Na]^+$ at *m*/*z* 1566.6930). The FAB-MS (negative-ion mode) showed the quasi-molecular-ion peak at *m*/*z* 1542 ($[M-1]^-$), and further fragments were observed at *m*/*z* 1410 ($[M-132-1]^-$), 1278 ($[M-132-132-1]^-$), and 1116 ($[M-132-132-162-1]^-$). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** (*Tables 1* and *2*) with those of phaseoloideside A (**1**) revealed a high degree of structural similarity. The main spectroscopic differences were that the ^{1}H-NMR spectrum of **3** showed two additional O-bearing CH groups at δ(H) 4.25 (*d*, *J* = 5.0 Hz, H–C(15)) and 5.02 (*d*-like, *J* = 5.0 Hz, H–C(16)), correlated with the C-atom signals at δ(C) 68.9 and 79.1 in the HSQC spectrum, corresponding to a moiety with two vicinal OH groups. The HSQC and HMBC data analysis allowed the construction and confirmation of the entagenic acid skeleton for **3**. The combined analysis of the ^{1}H- and ^{13}C-NMR of compounds **1** and **3** indicated that they possess exactly the same sugar chains at both C(3) and C(28). Accordingly, compound **3** was elucidated as 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}entagenic acid 28-[*O*-D-apio-β-D-furanosyl-(1 → 5)-*O*-D-apio-β-D-furanosyl-(1 → 2)-β-D-glucopyranosyl] ester.

Phaseoloideside D (**4**) was obtained as a white amorphous powder, and had a molecular formula $C_{72}H_{115}NO_{37}$, as deduced from the $[M+Na]^+$ ion at *m*/*z* 1608.7082 in the HR-ESI-MS (positive-ion mode), as well as from its NMR data (*Tables 1* and *2*). The FAB-MS (negative-ion mode) showed the *quasi*-molecular-ion peak at *m*/*z* 1584 ($[M-1]^-$), and further fragments were observed at *m*/*z* 1452 ($[M-132-1]^-$), 1422 ($[M-162-1]^-$), 1320 ($[M-162-132-1]^-$), and 1116 ($[M-162-132-132-42-1]^-$). The detailed comparison of the NMR data of compound **3** and **4** indicated that these two compounds had the same aglycon and sugar chains at the C(3) and C(28), the only difference being the presence of an additional AcO group in **4**, inferred from the signal of a Me group at δ(H) 1.91 (*s*) which correlated with the C-atom at δ(C) 20.8 in the HSQC spectrum, and from the signal of an additional C=O group at δ(C) 170.9. The long range HMBC MeCO (δ(H) 1.91)/C(6) of Glc II (δ(C) 64.8) determined that this AcO group was located at C(6) of Glc II. Thus, compound **4** was elucidated as 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl-(1 → 6)]-*O*-[β-D-glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl}entagenic acid 28-[*O*-D-apio-β-D-furanosyl-(1 → 5)-*O*-D-apio-β-D-furanosyl-(1 → 2)-6-*O*-acetyl-β-D-glucopyranosyl] ester.

Considering the fact that the BuOH fraction of *E. phaseoloides* and the saponins previously isolated from the same genus showed cytotoxic activities against various human-cancer cell lines, compounds **1**–**4** and rheediinoside B were tested against four

human cancer cell lines, HepG-2 (human hepatocellular carcinoma cell line), Eca-109 (human esophageal carcinoma cell line), SW480 (human colon-carcinoma-derived cell line), and HeLa (human epithelial carcinoma cell line) (*Table 3*). Compounds **1**–**3** and rheediinoside B exhibited no *in vitro* cytotoxicity against all four cancer cell lines. Compound **4** showed moderate *in vitro* cytotoxicity against Eca-109 with IC_{50} values of 28.0 μM, while a weak activity was observed against HepG-2 and HeLa with IC_{50} values of 40.7 and 47.7 μM, respectively.

Table 3. *Cytotoxicity of Phaseololidesisdes A–D* (**1**–**4**) *and of Rheediinoside B*

Compound	IC_{50} [μM]			
	HepG-2	SW480	Eca-109	HeLa
1	> 100[a])	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100
3	> 100	121.0	> 100	> 100
4	40.7 ± 3.6	55.6 ± 3.4	28.0 ± 2.0	47.7 ± 3.0
Rheediinoside B	> 100	> 100	> 100	> 100
Cisplatin	6.4 ± 2.3	16.7 ± 2.4	55.1 ± 4.3	2.1 ± 0.65

[a]) '> 100' = inactive.

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Experimental Part

General. The HPLC-grade MeOH was purchased from *Tedia Company, Inc.* All other chemical reagents were purchased from *Sinopharm Chemical Reagent Co., Ltd.* Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *ODS* (50 μm; *YMC*, Japan), and *MCI* gel *CHP20P* (75–150 μm; *Mitsubish Chemical Industries, Ltd.*). Semiprep. HPLC: *Dionex Ultimate 3000* with VIS-wavelength detector or photodiode-array detector; *Cosmosil* column (*5C18-MS II*, 10.0 × 250.0 mm, 5 μm); t_R in min. GC: *Agilent-6890* GC-MS instrument; *HP-5MS* column (0.25 mm × 30 m × 0.25 μm), hydrogen-flame ionization detector. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-DRX-500* spectrometer; Me_4Si as internal reference. HR-ESI-MS: *Bruker-Apex-IV-FTMS-7.0T* mass spectrometer; in *m*/*z*.

Plant Material. The seed kernels of *Entada phaseoloides* (L.) were collected from the City of Xishuangbanna, Yunnan Province, China, and identified by Prof. *Dingrong Wan*, College of Pharmacy, South-Central University for Nationalities. A voucher specimen (No. EP-200802) was deposited with the Herbarium of the College of Pharmacy, South-Central University for Nationalities.

Extraction and Isolation. Dried seed kernels (8 kg) of *Entada phaseoloides* (L.) MERR. were ground and extracted three times with 70% EtOH at r.t. The extract (518 g) was suspended in 10% MeOH in H_2O, and consecutively extracted with petroleum ether, AcOEt, and BuOH. The BuOH-soluble fraction (129 g) was subjected to CC (SiO_2 (200–300 mesh, 12 × 120 cm, 2500 g), CH_2Cl_2/MeOH 100:0, 97:3, 95:5, 9:1, 8:2, 7:3, 1:1, 3:7, and 0:100): *Fractions 1–15*. *Fr. 12* (26.8 g) was submitted to CC (SiO_2 (200–300 mesh, 4.0 × 50 cm, 500 g), AcOEt/MeOH 90:10 → 10:90): *Fr. 12.2* (collecting 70:30 → 40:60 fractions, 3.0 l) and *Fr. 12.3* (collecting 30:70 fractions, 1.5 l). *Fr. 12.2* was submitted to CC (*ODS* 50 μm, 2.5 × 20 cm, 100 g), H_2O/MeOH gradient): *Fr. 12.2.1* (collecting 70:30 → 60:40 fractions, 1.5 l) and *Fr. 12.2.2* (collecting 40:60 fractions, 0.3 l). *Fr. 12.2.1* was further purified by semi-prep. HPLC (isocratic H_2O/MeCN 73:27, 3.0 ml/min, det. at 212 nm): **2** (20.0 mg, t_R 8.0) and rheediinoside B (41.2 mg, t_R 11.6). *Fr. 12.2.2* was submitted to CC (SiO_2 (200–300 mesh, 2.5 × 20 cm, 40 g), $CHCl_3$/MeOH 0:100 → 70:30)

and further purified by semi-prep. HPLC (isocratic H_2O/MeCN (72:28, 3.0 ml/min, det. at 212 nm): **4** (20.3 mg, t_R 15.0). *Fr. 12.3* was submitted to repeated CC (*MCI* gel (75–150 μm, 2.5 × 20 cm, 40 g), H_2O/MeOH gradient) and further purified by semi-prep. HPLC isocratic H_2O/MeCN 72.5:27.5, 3.0 ml/min, det. at 212 nm): **3** (26.6 mg, t_R 10.6). *Fr. 13* (11.3 g) was submitted to CC (*ODS* (50 μm, 3.0 × 30 cm, 200g), H_2O/MeOH 90:10 → 30:70: *Fr. 13.1* (collecting 60:40 → 40:60 fractions, 1.8 l). *Fr. 13.1* (2.3 g) was submitted to CC (*MCI* gel (75–150 μm, 2.5 × 20 cm, 40 g), H_2O/MeOH gradient): *Fr. 13.1.1* (collecting 90:10 frations, 0.25 l) and *Fr. 13.1.2* (collecting 60:40, 0.25 l). *Fr. 13.1.1* was further purified by semi-prep. HPLC (isocratic H_2O/MeCN 74:26, 3.0 ml/min, det. at 212 nm): **1** (9.0 mg, t_R 33.5).

Phaseoloideside A (*=(3β)-3-{[*O-*β*-D-*Xylopyranosyl-(1 → 3)*-O-*α*-L-*arabinopyranosyl-(1 → 6)*-O-*[β*-D-*glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β*-D-*glucopyranosyl}oxy}olean-12-en-28-oic Acid 28-[*O-D-*Apio-β*-D-*furanosyl-(1 → 5)*-O-D-*apio-β*-D-*furanosyl-(1 → 2)-β-glucopyranosyl Ester*; **1**): White amorphous powder. $[\alpha]_D^{20} = -21.5$ ($c = 0.63$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1510 ($[M-1]^-$), 1378 ($[M-132-1]^-$), 1246 ($[M-132-132-1]^-$), 1084 ($[M-132-132-162-1]^-$), 922 ($[M-162-132-132-162-1]^-$), 658 ($[M-162-132-132-162-132-132-1]^-$), 455 ($[M-132-132-162-162-132-132-203-1]^-$). HR-ESI-MS: 1534.7037 ($[M+Na]^+$, $C_{70}H_{113}NNaO_{34}^+$; calc. 1534.7036).

Phaseoloideside B (*=(3β,15α,16α)-15,16-Dihydroxy-3-{[*O-*β*-D-*xylopyranosyl-(1 → 3)*-O-*α*-L-*arabinopyranosyl-(1 → 6)*-O-*[β*-D-*glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β*-D-*glucopyranosyl}oxy}olean-12-en-28-oic Acid 28-[*O-β*-D-*Xylopyranosyl-(1 → 2)-β*-D-*glucopyranosyl] Ester*; **2**). White amorphous powder. $[\alpha]_D^{20} = -16.4$ ($c = 1.40$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1410 ($[M-1]^-$), 1394 ($[M-17]^-$), 1278 ($[M-132-1]^-$), 1248 ($[M-162-1]^-$), 1116 ($[M-162-132-1]^-$). HR-ESI-MS: 1434.6519 ($[M+Na]^+$, $C_{65}H_{105}NNaO_{32}^+$; calc. 1434.6512).

Phaseoloideside C (*=(3β,15α,16α)-15,16-Dihydroxy-3-{[*O-*β*-D-*xylopyranosyl-(1 → 3)*-O-*α*-L-*arabinopyranosyl-(1 → 6)*-O-*[β*-D-*glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β*-D-*glucopyranosyl}oxy}olean-12-en-28-oic Acid 28-[*O-D-*Apio-β*-D-*furanosyl-(1 → 5)*-O-D-*apio-β*-D-*furanosyl-(1 → 2)-β*-D-*glucopyranosyl] Ester*; **3**).White amorphous powder. $[\alpha]_D^{20} = -33.7$ ($c = 1.02$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1542 ($[M-1]^-$), 1410 ($[M-132-1]^-$), 1278 ($[M-132-132-1]^-$), 1116 ($[M-132-132-132-1]^-$). HR-ESI-MS: 1566.6930 ($[M+Na]^+$, $C_{70}H_{113}NNaO_{36}^+$; calc. 1566.6935).

Phaseoloideside D (*=(3β,15α,16α)-15,16-Dihydroxy-3-{[*O-*β*-D-*xylopyranosyl-(1 → 3)*-O-*α*-L-*arabinopyranosyl-(1 → 6)*-O-*[β*-D-*glucopyranosyl-(1 → 4)]-2-(acetylamino)-2-deoxy-β*-D-*glucopyranosyl}oxy}olean-12-en-28-oic Acid 28-[*O-D-*Apio-β*-D-*furanosyl-(1 → 5)*-O-D-*apio-β*-D-*furanosyl-(1 → 2)-6-*O-*acetyl-β*-D-*glucopyranosyl] Ester*; **4**): White amorphous powder. $[\alpha]_D^{20} = -40.8$ ($c = 1.63$, MeOH). ^{1}H-and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 1584 ($[M-1]^-$), 1452 ($[M-132-1]^-$), 1422 ($[M-162-1]^-$), 1320 ($[M-162-132-1]^-$), 1116 ($[M-162-132-132-42-1]^-$). HR-ESI-MS: 1608.7082 ($[M+Na]^+$, $C_{72}H_{115}NNaO_{37}^+$; calc. 1608.7040).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides of Phaseoloidesides A–D (**1**–**4**) *and Rheediinoside B.* Each compound (2.0 mg) was hydrolyzed with 4N aq. CF_3COOH (5 ml) for 2.5 h at 100° in a water bath. The mixture was diluted in H_2O (8 ml) and extracted with CH_2Cl_2 (3 × 5 ml), and then the aq. layer was concentrated to remove CF_3COOH. Subsequently, L-cysteine methyl ester hydrochloride (2.0 mg) was added to the sugar residue, and the mixture was dissolved in anh. pyridine (1.0 ml) and refluxed at 60° in a water bath for 2 h. Then, the mixture was concentrated to dryness. Then, 1-(trimethylsilyl)-1*H*-imidazole (0.2 ml) was added, and the mixture was refluxed at 60° in a water bath for another 1 h. Finally, the mixture was extracted with cyclohexane. The cyclohexane phase was analyzed by GC (column temp. 260°, injection temp. 280°, carrier gas He, flow rate 1.0 ml/min), and the detected sugar derivatives were compared with the derivatives of authentic samples.

MTT Cytotoxicity Assay. HepG-2, Eca-109, SW480, and HeLa cell lines were purchased from the *American Type Culture Collection* (*ATCC*; Rockville, MD, USA). All the cell lines were grown in DMEM (*Dulbecco*'s modified Eagle medium) with 10% fetal bovine serum (*Gibco*), penicillin (100 IU/ml), and streptomycin (100 μg/ml) and cultured at 37° in a humidified atmosphere of 5% CO_2. The DMEM were routinely changed every 2 d. The MTT (3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide; *Sigma–Aldrich*, USA) assay was performed as previously described. Briefly, 1.2 ·

10^4 cells (100 µl) were placed in each well of a flat 96-well plate. After 18 h incubation, the medium containing different concentrations of test compounds and the positive control agent (cisplatin, *Qilu Pharmaceutical Co., Ltd.*) were added, and 0.1% DMSO was used as solvent control. After 48 h, MTT (10 µl; 5 mg/ml) was added into each well and incubated for 4 h. The MTT soln. was discarded, and DMSO (150 µl) was added to dissolve the formazan crystals. The optical density (*OD*) was measured at 492 nm and read on the microplate to express a percentage of cell survival. The experiments were repeated for three times. The inhibition rate was calculated as IC_{50} expressing the cytotoxicity of the test compounds (see *Table 3*).

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