Medicinal Plants of Thailand. I Structures of Rheedeiosides A—D and *cis*-Entadamide A β-D-Glucopyranoside from the Seed Kernels of *Entada rheedei*

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Four new oleanane-type triterpene oligoglycosides, named rheedeiosides A, B, C and D, and one new thioamide glycoside, *cis*-entadamide A β-D-glucopyranoside, were isolated from the seed kernels of a Thai medicinal plant, *Entada rheedei* SPRENGEL. The rheedeiosides were found to contain *N*-acetylglucosamine as a sugar component. Their structures were elucidated based on spectral and chemical evidence.

Key words *Entada rheedei*; Fabaceae; rheedeioside; entadamide; Thai medicinal plant

Entada rheedei SPRENGEL is cultivated throughout the tropics. Its seeds have various medicinal uses including topical application as an ointment for the treatment of jaundice.[1] Through phytochemical studies on *Entada* species, several triterpene glycosides have been isolated. The seed kernels of *E. rheedii*,[1] and *E. pursaetha*,[2] and a bark of *E. phaseoloides*[3,4] were reported to contain a number of *N*-acetyl-D-glucosamine-containing entagenic acid saponins. Despite these investigations, the chemical constituents of *E. rheedei* as well as their biological activities have not yet been fully characterized. In this paper, we describe the isolation and structure elucidation of four new oleanane-type triterpene saponins, named rheedeiosides A—D, and a new thioamide glycoside, *cis*-entadamide A β-D-glucopyranoside, from the seed kernels of *E. rheedei*. The structures of the new compounds were elucidated on the basis of spectral and physicochemical evidence.

Isolation and Structural Elucidation Finely chopped seed kernels of *E. rheedei* were extracted with *n*-hexane and then the residue was extracted with MeOH. The MeOH was evaporated off under reduced pressure to yield a white residue. An aliquot of the residue was subjected to Diaion HP-20 column chromatography (CC) to give H_2O-, MeOH-, and acetone-eluted fractions. The MeOH-eluted fraction was then subjected to normal- and reversed-phase silica gel CC, and repetitive HPLC separation, which gave five new compounds, rheedeiosides A (**1**, 190 mg, 0.027%), B (**2**, 6.6 mg, 0.00093%), C (**3**, 22.1 mg, 0.0030%), and D (**4**, 44.5 mg, 0.0063%), and *cis*-entadamide A β-D-glucopyranoside (**5**, 4.4 mg, 0.0034%), together with two known compounds, entadamide A β-D-glucopyranoside[5] (**6**, 878 mg, 0.67%) and phenylpropanol β-D-glucopyranoside[6] (**7**, 9.2 mg, 0.0071%). Their structures were elucidated by extensive spectroscopic analyses, which yielded 1D- and 2D-NMR spectral data and electrospray ionization (ESI)-MS spectra, and by acid and alkaline hydrolyses.

Rheedeioside A (**1**) was isolated as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{23}$ −27.1). The IR spectrum of **1** showed absorption bands at 3395, 1729, 1647 and 1078 cm^{-1}, ascribable to hydroxyl, ester carbonyl, amide carbonyl and ether functional groups. The molecular formula, $C_{72}H_{115}NO_{37}$, of **1** was determined by high-resolution (HR)-ESI-MS analysis. The ^{1}H-NMR spectrum of **1** showed seven singlet methyl signals at δ_H 0.84, 0.87, 0.94, 1.07, 1.09, 1.10 and 1.74 (Table 1), and two fairly deshielded singlet methyl signals at δ_H 1.90 and 2.10 (Table 1). A typical signal for an axial proton attached to a hydroxylated carbon at δ_H 3.20 (dd, J=11, 4 Hz) (Table 1), and seven anomeric protons for sugar moieties [δ_H 4.75 (d, J=8 Hz), 4.98 (d, J=8 Hz), 5.16 (d, J=7 Hz), 5.32 (d, J=7 Hz), 5.40 (1H, d, J=8 Hz), 6.03 (d, J=7 Hz), and 6.11 (d, J=3 Hz)] were also observed (Table 1). The ^{13}C-NMR spectral data indicated the presence of one terminal apiofuranose and three terminal xylopyranoses, and the aglycone moiety showed good resemblance to those of entagenic acid (**8**) isolated from *Entada phaseolloides* (Table 2).[3,4] Judging from the downfield shift of C-3 (δ_C 89.6) and the upfield shift value observed for C-28 (δ_C 175.8) (Table 2), **1** was presumed to be a bisdesmosidic glycoside of entagenic acid with sugar linkages at C-3 through a glycosidic bond and at C-28 through an ester bond. Alkaline hydrolysis of **1** liberated a monodesmoside (**1a**) and a triose, whose sugar composition was analyzed to be D-apiose, D-xylose and D-glucose. The NMR spectral data of **1a** showed that the monodesmoside (**1a**) possessed two terminal xylopyranoses and two inner sugars (Table 2). Acid hydrolysis of **1a** gave entagenic acid as an aglycone (**1b** = **8**). and sugar analysis of the hydrolyzate revealed the presence of D-xylose, D-glucose and D-glucosamine hydrochloride. The presence of an *N*-acetylglucosamino group was substantiated by the ^{1}H-NMR at δ_H 2.10 (3H, s) and the D_2O exchangeable proton at δ_H 8.91 (d, J=8 Hz, NH), and the ^{13}C-NMR resonances at δ_C 23.5 and 170.4, together with the heteronuclear single quantum correlation (HSQC) spectroscopic data for C-2′ of the hexopyranose moiety (δ_H 4.40 on δ_C 57.4), confirmed the presence of the 2-(acetylamino)-2-deoxyglucopyranose (GlcNAc) unit.[7] The connectivities of the sugar chains were confirmed by heteronuclear multiple bond correlation (HMBC) experiments (Fig. 1). The cross peak between δ_H 4.98 (GlcNAc-H-1′) and δ_C 89.5 (C-3) showed that the GlcNAc moiety was linked with the hydroxy group at C-3 of the aglycone. Further correlation was also observed between H-1″ (δ_H 5.40, J=8 Hz) of xylopyranose and C-3′ (δ_C 80.7) of GlcNAc, indicating the location of it at C-3′. The HMBC correlations between H-1‴ [δ_H 5.16 (d, J=7 Hz)] of glucopyranose and C-6′ (δ_C 68.1) of GlcNAc, and between H-1⁗ [δ_H 4.75 (d, J=8 Hz)] of xylopyranose and C-3‴ (δ_C 83.2) of glu-

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Table 1. ^{1}H-NMR Spectral Data for Rheedeiosides A—D (**1**—**4**) (600 MHz, Pyridine-d_5) δ in ppm and J in Hz

H	**1**	**2**[a)]	**3**	**4**
1	0.89 m	0.85 m	0.92 m	1.10 m
	1.47 br d 13	1.45 m	1.47 br d 13	1.64 br d 13
2	1.70 br d 13	1.67 m	1.70 br d 13	1.84 br d 13
	2.07 m	2.10 m	2.08 m	2.24 dd 13, 4
3	3.20 dd 11, 4	3.24 dd 11, 4	3.22 dd 12, 4	3.35 dd 13, 4
5	0.77 d 12	0.79 m	0.78 d 12	0.86 d 12
6	1.27 br d 12	1.25 m	1.28 br d 11	1.32 br dd 12, 3
	1.50 br dd 12, 2	1.49 m	1.50 br dd 11, 3	1.53 br dd 12, 3
7	1.93 m	1.96 m	1.95 m	2.00 m
	2.12 m	2.13 m	2.13 br d 10	2.16 br d 11
9	1.68 d 9	1.73 m	1.71 d 9	1.76 d 10
11	1.94 2H m	1.99 2H m	1.97 2H m	2.02 2H m
12	5.62 br s	5.64 br s	5.64 br s	5.62 br s
15	4.47 d 3	4.50 m	4.50 d 3	4.52 m
16	4.95 br s	5.01 m	4.97 m	4.99 m
18	3.41 br d 11	3.43 m	3.39 dd 13, 5	3.40 dd 13, 5
19	1.26 m	1.32 m	1.27 dd 13, 5	1.30 dd 13, 5
	2.69 dd 13, 13	2.74 m	2.72 dd 13, 13	2.72 dd 13, 13
21	1.20 dd 12, 2	1.13 m	1.23 br d 12	1.22 m
	2.34 dd 12, 5	2.39 m	2.37 dd 12, 4	2.38 dd 13, 5
22	2.16 br d 12	2.20 m	2.17 br d 13	2.19 br d 13
	2.28 dd 12, 5	2.24 m	2.33 dd 13, 5	2.31 dd 14, 4
23	1.10 s	1.13 s	1.09 s	1.14 s
24	0.87 s	0.89 s	0.88 s	0.94 s
25	0.84 s	0.76 s	0.84 s	0.89 s
26	1.07 s	1.11 s	1.10 s	1.12 s
27	1.74 s	1.79 s	1.79 s	1.78 s
29	0.94 s	0.99 s	0.96 s	0.97 s
30	1.09 s	1.08 s	1.11 s	1.11 s
1′	4.98 d 8	5.04 d 7	5.02 d 8	5.00 d 8
2′	4.40 dd 9, 8	4.41 m	4.35 dd 9, 8	4.51 m
3′	4.72 m	4.77 m	4.74 dd 9, 8	4.48 m
4′	4.06 m	4.10 m	4.07 m	4.35 m
5′	3.96 m	4.02 m	4.00 m	3.93 m
6′	4.55 dd 11, 3	4.62 m	4.59 dd 11, 3	4.57 dd 11, 2
	4.65 dd 11, 5	4.72 m	4.70 dd 11, 5	4.62 dd 11, 5
1″	5.40 d 8	5.48 d 8	5.43 d 8	
2″	3.89 m	3.86 m	3.94 m	
3″	4.23 m	4.23 m	4.21 m	
4″	3.94 m	4.00 m	4.08 m	
5″	3.53 br d 11	3.55 br d 12	3.65 dd 11, 2	
	4.33 dd 11, 5	4.66 m	4.66 dd 11, 6	
1‴	5.16 d 7	5.23 d 8	5.19 d 7	5.10 d 5
2‴	4.37 m	4.40 m	4.44 m	4.47 m
3‴	4.38 m	4.48 m	4.46 m	4.69 m
4‴	4.17 m	4.21 m	4.21 m	4.35 m
5‴	4.14 m	4.28 m	4.20 m	3.70 dd 11, 2
				4.24 dd 11, 5
6‴	4.58 dd 11, 3	4.27 m	4.62 m	
	4.66 dd 11, 5	4.43 m	4.69 dd 10, 5	
1⁗	4.75 d 8	4.84 d 8	4.78 d 8	4.96 d 7
2⁗	3.95 m	4.05 m	3.98 m	3.98 m
3⁗	4.20 m	4.21 m	4.25 m	4.17 m
4⁗	4.16 m	4.20 m	4.09 m	3.97 m
5⁗	3.40 br d 11	3.48 br d 13	3.41 br d 11	3.54 dd 12, 2
	4.59 dd 11, 5	4.30 m	4.27 dd 11, 4	4.08 dd 12, 5
1⁗′	6.03 d 8	6.15 d 7	6.06 d 8	6.07 d 8
2⁗′	4.15 m	4.22 m	4.20 m	4.22 m
3⁗′	4.04 m	4.06 m	4.04 m	4.08 m
4⁗′	4.14 m	4.16 m	4.19 m	4.09 m
5⁗′	3.86 m	3.92 m	4.21 m	4.00 m
6⁗′	4.21 dd 11, 5	4.33 m	4.24 dd 12, 4	4.64 dd 12, 2
	4.42 m	4.45 m	4.44 dd 12, 5	4.69 dd 12, 6
1⁗″	5.32 d 7	5.38 d 7	5.40 d 7	5.35 d 8
2⁗″	3.99 m	4.08 m	4.06 m	4.02 m
3⁗″	4.20 m	4.19 m	4.23 m	4.17 m
4⁗″	4.24 m	4.10 m	4.12 m	4.15 m
5⁗″	3.60 br d 11	3.62 br d 13	3.60 br d 10	3.60 dd 12, 2
	4.17 dd 11, 5	4.18 m	4.18 m	4.18 dd 12, 5
1⁗‴	6.11 d 3	6.17 d 3	5.16 d 8	6.16 d 3
2⁗‴	4.70 d 3	4.68 d 3	3.96 m	4.74 d 3
3⁗‴	—	—	4.10 m	
4⁗‴	4.68 d 10	4.74 d 10	3.94 m	4.69 d 10
	4.26 d 10	4.30 d 10		4.29 d 10
5⁗‴	4.12 d 13	4.12 2H br s	3.63 dd 11, 2	4.13 d 13
	4.09 d 13		4.38 dd 11, 4	4.11 d 13
NHCOC$\underline{H}_3$	2.10 s	2.11 s	2.11 s	2.10 s
N$\underline{H}$COCH$_3$	8.91 d 8	8.86 d 10	8.90 d 9	8.74 d 7
OCOC$\underline{H}_3$	1.90 s			1.92 s

a) At 500 MHz. m: multiplet or overlapped signals.

copyranose indicated the location of them at C-6′ and C-3‴, respectively. In addition, the correlation observed between H-1⁗′ [δ_H 6.03 (d, J=8 Hz)] of glucopyranose and the signal of the carbon at δ_C 175.8 (C-28) in the HMBC spectrum confirmed the ester linkage at C-28. Further correlations observed in the HMBC spectrum between the signal at H-1 [δ_H 5.32 (d, J=7 Hz)] of xylopyranose and δ_C 79.9 (C-2⁗), and between H-1⁗″ [δ_H 6.11 (d, J=3 Hz)] of apiofuranose and C-3 (δ_C 85.0) of glucopyranose established the sugar linkage of the ester moiety. The position of acetyl group was determined to be at the hydroxy group at C-6‴, as judged from the significant downfield shift of the methylene protons (H_2-6‴, δ_H 4.58, 4.66), and the HMBC correlation peaks between H_2-6‴ and the carbonyl carbon (δ_C170.7) of acetyl group. Acetylation of **1** with acetic anhydride, pyridine and *N*,*N*-dimethylaminopyridine (DMAP) gave a nonadecaacetate (**1c**), and protons assignable to H-3′, H-6′, H-3‴, H-2⁗′ and H-3⁗′ remained intact in the ^{1}H-NMR spectrum. The ^{1}H–^{1}H correlation spectroscopy (COSY) and HMBC spectral data for **1c** also supported ramification of the sugar chains. The mode of linkage of all sugars was determined to be β from the coupling constants of their anomeric protons. Thus, the structure of **1** was elucidated to be entagenic acid 3-*O*-β-D-xylopyranosyl-(1→3)-6-*O*-acetyl-β-D-glucopyranosyl(1→6)-*O*-[β-D-xylopyranosyl(1→3)]-2-acetylamino-2-deoxy-β-D-glucopyranoside 28-*O*-[β-D-xylopyranosyl(1→2), β-D-apiofuranosyl(1→3)]-β-D-glucopyranosyl ester (rheedeioside A), as shown in Chart 1.

Rheedeioside B (**2**) was also isolated as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{23}$ −28.5). The IR spectrum of **2** showed absorption bands at 3395 and 1077 cm^{-1}, suggestive of a glycosidic structure, together with absorption bands at 1647 and 1559 cm^{-1} due to an amide function. NMR spectral data were essentially the same as those of **1**, except for the absence of an acetyl group. The elemental composition ($C_{70}H_{113}NO_{36}$), determined by HR-ESI-MS, was also 42 mass units less (CH_2CO) from that of **1**. Mild alkaline hydrolysis of **1** gave deacetylated **1**, whose spectroscopic data were identical with those of **2**. Consequently, the structure of **2** was elucidated to be entagenic acid 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl(1→6)-*O*-[β-D-xylopyranosyl(1→3)]-2-acetylamino-2-deoxy-β-D-glucopyranoside 28-*O*-[β-D-xylopyranosyl(1→2), β-D-apiofuranosyl(1→3)]-β-D-glucopyranosyl ester, as shown in Chart 1.

Rheedeioside C (**3**) was also obtained as an amorphous powder exhibiting negative optical rotation ($[\alpha]_D^{23}$ −20.5). The molecular formula, $C_{72}H_{115}NO_{37}$, of **3** was determined by positive-ion HR-ESI-MS. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of **1**, exhibiting seven singlet methyls, and seven anomeric proton and carbon signals. The ^{13}C-NMR spectrum exhibited 30 signals for the aglycone of bisdesmoisde, and four terminal xylopyranoses. On acid hydrolysis of **3**, the presence of D-xylose, D-glucose and D-glucosamine was confirmed. Thus, the apiofuranose found in **1** was replaced by xylopyranose. The HMBC experiment showed that there was a long-range correlation between H-1⁗″ (δ_H 5.16) and C-3⁗′. On the basis of the above mentioned evidence, the structure of **3** was elucidated to be entagenic acid 3-*O*-β-D-xylopyranosyl-(1→3)-6-acetyl-β-D-glucopyranosyl-(1→6)-*O*-[β-D-xylopyranosyl-(1→3)]-2-acetylamino-2-deoxy-β-D-glucopyranoside, 28-*O*-β-D-xylopyr-

Table 2. ^{13}C-NMR Spectroscopic Data for Rheedeiosides A—D (**1**—**4**), Entagenic Acid (**8**), and **1a** (150 MHz, Pyridine-d_5)

C	**1**	**1a**	**2**[a]	**3**	**4**	**8**[b]	C	**1**	**1a**	**2**[a]	**3**	**4**
1	38.8	38.8	38.9	39.1	39.0	39.5	1′	104.3	104.3	104.4	104.32	104.8
2	26.3	26.4	26.5	26.4	26.6	28.3	2′	57.4	57.6	57.7	57.6	57.8
3	89.5	89.5	89.6	89.5	89.1	78.6	3′	80.7	80.8	81.0	80.8	75.3
4	39.0	39.1	39.2	38.9	39.2	39.4	4′	73.6	73.0	73.3	73.7	72.5
5	55.6	55.7	55.8	55.7	55.7	56.0	5′	76.2	76.3	76.5	76.4	76.0
6	18.8	18.8	18.8	18.8	18.8	19.4	6′	67.9	68.0	68.1	68.0	69.44
7	36.9	36.9	37.1	37.1	37.0	37.2	1″	104.2	104.3	104.5	104.34	
8	41.3	41.2	41.5	41.1	41.5	41.6	2″	74.7	74.5	74.79	75.0	
9	47.6	47.3	47.4	47.4	47.4	47.8	3″	77.9	77.7	78.0	78.3	
10	37.0	37.1	37.0	37.0	37.1	37.9	4″	70.2	70.7	70.9	70.80	
11	23.8	23.9	24.0	23.9	24.0	34.2	5″	66.1	66.1	66.2	66.2	
12	124.8	124.0	124.8	124.9	124.9	124.3	1‴	103.2	103.2	103.4	103.3	102.2
13	144.4	145.4	144.7	144.4	144.5	145.8	2‴	73.0	73.5	73.8	73.1	80.1
14	47.3	47.8	47.3	47.7	47.7	48.2	3‴	82.9	83.0	83.2	83.1	72.9
15	68.1	68.3	68.3	68.2	68.2	68.6	4‴	68.6	68.6	68.6	68.7	67.4
16	78.7	78.0	79.0	78.8	78.8	79.7	5‴	77.7	79.0	78.8	77.8	64.2
17	48.5	48.4	48.6	48.6	48.6	48.8	6‴	64.0	62.3	62.4	64.1	
18	41.7	42.0	41.8	41.8	41.8	42.4	1⁗	107.4	107.5	107.7	107.6	106.2
19	46.3	46.7	46.6	46.4	46.4	47.1	2⁗	75.6	74.8	75.1	75.7	75.8
20	30.7	30.9	30.8	30.8	30.8	31.0	3⁗	78.1	78.3	78.7	78.4	78.3
21	35.8	36.1	36.0	35.9	35.9	36.4	4⁗	70.7	71.4	70.7	70.83	70.4
22	31.8	32.8	32.1	31.9	31.9	32.8	5⁗	67.2	67.3	67.5	67.3	67.2
23	28.0	28.0	28.1	28.1	28.1	28.9	1⁗′	93.1		93.5	93.2	93.2
24	16.9	17.0	17.0	17.0	17.0	16.5	2⁗′	79.9		80.6	80.1	80.3
25	15.6	15.5	15.7	15.7	15.8	15.9	3⁗′	85.0		85.2	87.2	85.0
26	17.9	18.0	18.1	18.0	18.0	18.3	4⁗′	71.4		71.6	71.5	70.8
27	20.8	20.8	20.8	20.6	20.7	20.7	5⁗′	78.2		78.5	78.3	77.8
28	175.8	179.6	175.8	175.9	175.9	180.0	6⁗′	62.1		62.2	62.3	64.1
29	33.1	33.3	33.3	33.2	33.2	33.4	1⁗″	105.4		105.7	105.3	105.6
30	24.4	24.5	24.5	24.5	24.5	25.0	2⁗″	74.5		74.77	74.5	74.9
							3⁗″	78.1		78.1	78.0	78.3
							4⁗″	69.2		69.4	69.0	69.36
							5⁗″	66.9		67.1	67.2	67.0
							1⁗‴	111.1		111.5	106.0	111.3
							2⁗‴	77.6		77.8	74.7	77.7
							3⁗‴	80.2		80.4	78.0	80.4
							4⁗‴	74.9		75.2	70.3	75.1
							5⁗‴	65.2		65.6	66.9	65.4
							NHCO$\underline{C}$H$_3$	23.5	23.4	23.7	23.6	23.6
							NH$\underline{C}$OCH$_3$	170.4	170.2	169.9	170.1	170.3
							OCO$\underline{C}$H$_3$	20.5			20.7	20.6
							O$\underline{C}$OCH$_3$	170.7			170.7	170.6

a) At 125 MHz. *b*) Data taken from ref. 4.

anosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl ester.

Rheedeioside D (**4**) was also obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{25}$ −24.0). The IR spectrum of **4** showed absorption bands at 3388, 1730, 1648, and 1079 cm^{-1}, ascribable to hydroxy, ester carbonyl, amide and ether functional groups. The molecular formula, $C_{66}H_{105}NO_{32}$, of **4** was determined by positive-ion HR-ESI-MS. Acid hydrolysis of **4** liberated entagenic acid together with D-apiose, D-glucose, D-glucosamine, D-xylose and L-arabinose, which were identified by HPLC analysis. The ^{1}H- and ^{13}C-NMR spectra of **4** showed signals assignable to one terminal apiofuranoside [δ_H 6.16 (d, J=3 Hz)], two terminal xylopyranosides [δ_H 4.96 (d, J=7 Hz) and 5.35 (d, J=8 Hz)], and GlcNAc [H-1′: δ_H 5.00 (d, J=8 Hz), C-2′: δ_C 57.8, and NHCOCH$_3$: δ_C 23.6 and 170.3 and δ_H 2.10 (3H) and 8.74 (D_2O exchangeable)], together with one acetyl group. The remaining anomeric proton resonating at δ_H 5.10 (d, J=5 Hz) was assigned as that of arabinopyranose, and that resonating at 6.07 (d, J=8 Hz) as that of ester linked glucopyranose. In the HMBC spectrum, the anomeric proton of GlcNAc, showed a correlation peak with with C-3, the anomeric proton of arabinopyranose with C-6′ (δ_C 69.44) of GlcNAc and the anomeric proton of one of the xylopyranoses (δ_H 4.96) with C-2‴ (δ_C 80.1), thus establishing the sugar linkage was at the C-3 position. The ^{13}C-NMR spectral data for the ester linked oligosaccharide were superimposable on those of **1**—**3**, except for the downfield shift of the H_2-6⁗ protons, as well as H_2-6⁗ protons showing a correlation peak with the acetyl carbonyl carbon (δ_C 170.6). Consequently, the structure of **4** was determined to be entagenic acid 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetylamino-2-deoxy-β-D-glucopyranoside, 28-*O*-β-D-xylopyranosyl-(1→2)-[β-D-apiofuranosyl-(1→3)]-6-acetyl-β-D-glucopyranosyl ester.

Compound **5** was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{24}$ −12.0 in MeOH). The molecular formula, $C_{12}H_{21}NO_7S$, of **5** was determined by positive-ion HR-ESI-MS. The IR spectrum showed absorption bands at 3335 cm^{-1} (br, OH and NH), 1638 cm^{-1} (CO–N),

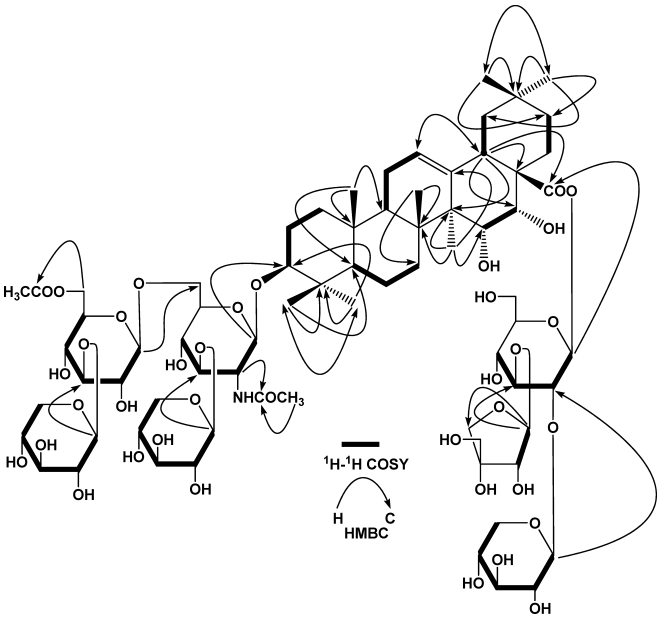

Fig. 1. Significant ^{1}H–^{1}H and HMBC Correlations of Rheedeioside A (**1**)

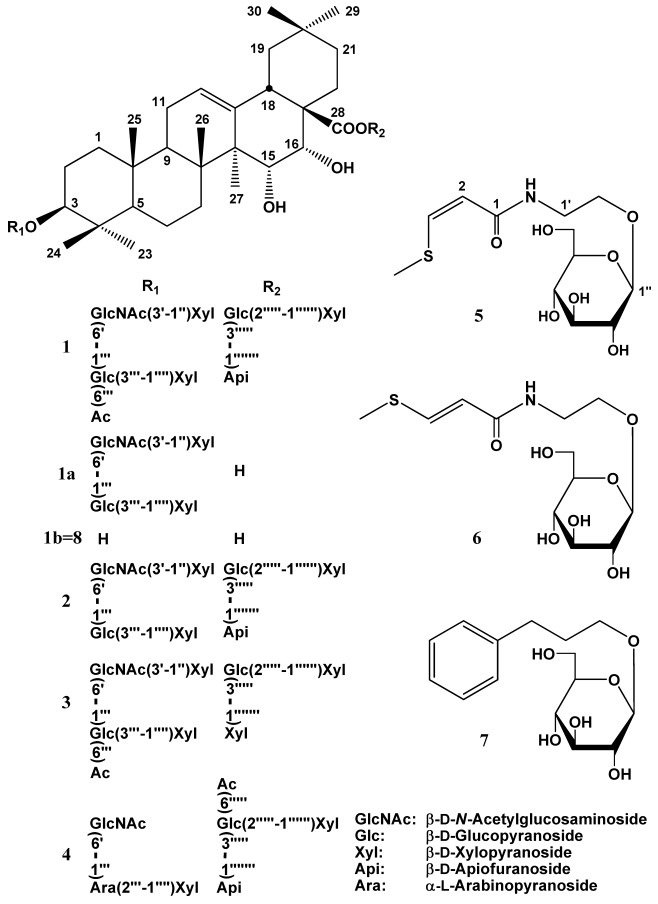

Chart 1.

and 1576 cm^{-1} (C=C). In the UV spectrum of **5**, an absorption maximum was observed at 266 (log ε 4.87) nm. This compound exhibited UV, IR, ^{1}H- and ^{13}C-NMR spectral data similar to those of entadamide A-β-D-glucopyranoside (**6**),[5] which co-occurred in this plant, except for the coupling constant of the olefinic bond. The ^{1}H-NMR spectrum indicated

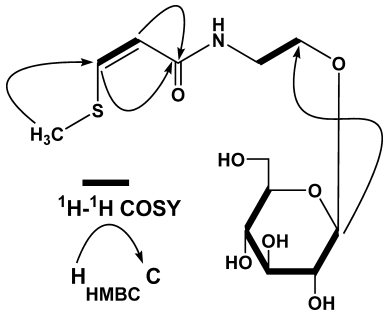

Fig. 2. Significant ^{1}H–^{1}H and HMBC Correlations of *cis*-Entadamide A β-D-Glucopyranoside (**5**)

the geometry of the disubstituted double bond of **5** to be in a *cisoid* form [δ_H 6.93 and 5.89 (each 1H, both d, J=10 Hz)], and then the structure of **5** was determined to be *cis*-entadamide A β-D-glucopyranoside (**5**).

In conclusion, four oleanane-type triterpene bisbesmosides, rheedeiosides A (**1**), B (**2**), C (**3**), and D (**4**), and a thioamide glycoside (**5**), together with two known compounds, were isolated from the seed kernels of *E. rheedei* collected in Thailand.

Experimental

The following instruments were used to obtain physical and spectroscopic data: mp, Yanagimoto micromelting point apparatus (uncorr.); optical rotations, JASCO P-1030 digital polarimeter; IR spectra, Shimadzu FT-710 spectrophotometer; HR-ESI mass spectra, LTQ Orbitrap XL; ^{1}H-NMR spectra, JEOL JNM-LA500 (500 MHz) and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as internal standard and run at 35 °C; and HPLC detector, Shimadzu RID-6A refractive index detector. Highly-porous synthetic resin Diaion HP-20 was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel CC was performed on silica gel 60 [(E. Merck, Darmstadt, Germany), 70—230 mesh]. Reversed-phase octadecyl silanized (ODS) open CC (RPCC) was performed on Cosmosil 75C$_{18}$-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H$_2$O]. HPLC was performed on an ODS-3 column (Inertsil; GL Science, Tokyo, Japan; Φ=10 mm, L=25 cm), and the eluate was monitored with a refractive index monitor. Precoated silica gel 60 F$_{254}$ plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, with visualization by spraying of a 10% solution of H_2SO_4 in ethanol and heated at around 150 °C on a hotplate. Authentic D-apiose [$[\alpha]_D^{23}$ +9.4° (c=0.84, H$_2$O)] was obtained by chromatographic separation of the hydrolyzate of apiin, isolated from commercial parsley (*Petroselinum crispum*). D-Apiose was identified by NMR spectroscopy.[8]

Plant Material The seed kernels of *Entada rheedei* were purchased at a Thai market in 2009 and identified by Prof. Sorasak Lhieochaiphant of Faculty of Pharmacy, Chiang Mai University, Thailand.

Isolation of Compounds 1—7 The seed kernels of *E. rheedei* (1.86 kg) were finely chopped and extracted with hexane (4 l) for three days at room temperature. Evaporation of the solvent under reduced pressure provided a hexane-soluble extract (102 g, 5.49%). The dried-up residue was extracted three times with MeOH (4 l) at room temperature for 3 d each and then evaporated, providing a MeOH-soluble extract (248 g, 13.4%). The MeOH-soluble extract (133 g) was subjected to a Diaion HP-20 CC [2.0 kg, H$_2$O (15 l)→MeOH (15 l)→acetone (10 l)] to give H$_2$O (98.1 g, 9.86%), MeOH (30.0 g, 3.01%) and acetone (3.2 g, 0.32%) eluates.

The MeOH eluate (29.0 g) was subjected to normal-phase silica gel CC (900 g) {CHCl$_3$ (4 l)→CHCl$_3$: MeOH [9 : 1 (6 l)→4 : 1 (6 l)→7 : 3 (6 l)→3 : 2 (6 l)]→CHCl$_3$: MeOH : H$_2$O (6 : 4 : 1) (20 l)→MeOH (6 l)} to give ten fractions [Fr. 1 (274 mg), Fr. 2 (888 mg), Fr. 3 (6.1 g), Fr. 4 (859 mg), Fr. 5 (791 mg), Fr. 6 (871 mg), Fr. 7 (1.7 g), Fr. 8 (3.5 g), Fr. 9 (11.3 g), and Fr. 10 (908 mg)]. Fraction 3 (5.3 g) was separated by RPCC [120 g, MeOH : H$_2$O (1 : 9→3 : 7→1 : 1→7 : 3→MeOH)] to yield 11 fractions [Fr. 3-1 (37.9 mg), Fr. 3-2 (4.9 g), Fr. 3-3 (67.4 mg), Fr. 3-4 (9.6 mg), Fr. 3-5 (9.6 mg), Fr. 3-6 (20.1 mg), Fr. 3-7 (10.2 mg), Fr. 3-8 (51.7 mg), Fr. 3-9 (1.2 mg), Fr. 3-10 (20.6 mg), and Fr. 3-11 (8.4 mg)]. Fraction 3-2 (880 mg) was purified by

HPLC [MeOH–H_2O (1 : 4, v/v)] to give *cis*-entadamide A β-D-glucopyranoside (**5**, 4.4 mg), entadamide A β-D-glucopyranoside (**6**, 878 mg), and phenylpropanol β-D-glucopyranoside (**7**, 9.2 mg).

Fr. 5 (791 mg), Fr. 6 (871 mg), and Fr. 7 (1.70 g) were combined and subjected to RPCC [120 g, MeOH : H_2O (1 : 9→3 : 7→1 : 1→7 : 3→9 : 1→MeOH)] to give 22 fractions [Fr. 5,6,7-1 (527 mg), Fr. 5,6,7-2 (282 mg), Fr. 5,6,7-3 (789 mg), Fr. 5,6,7-4 (254 mg), Fr. 5,6,7-5 (53.0 mg), Fr. 5,6,7-6 (21.2 mg), Fr. 5,6,7-7 (103 mg), Fr. 5,6,7-8 (43.7 mg), Fr. 5,6,7-9 (17.6 mg), Fr. 5,6,7-10 (22.1 mg), Fr. 5,6,7-11 (8.0 mg), Fr. 5,6,7-12 (44.8 mg), Fr. 5,6,7-13 (20.0 mg), Fr. 5,6,7-14 (14.6 mg), Fr. 5,6,7-15 (16.6 mg), Fr. 5,6,7-16 (6.0 mg), Fr. 5,6,7-17 (24.4 mg), Fr. 5,6,7-18 (478 mg), Fr. 5,6,7-19 (78.6 mg), Fr. 5,6,7-20 (55.0 mg), Fr. 5,6,7-21 (20.0 mg), and Fr. 5,6,7-22 (800 mg)]. Fr. 5,6,7-18 (450 mg) was separated by HPLC [MeOH–acetone–H_2O (3 : 2 : 5, v/v/v)] to give rheedeiosides A (**1**, 190 mg), B (**2**, 6.6 mg), C (**3**, 44.5 mg), and D (**4**, 21.2 mg).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H- and ^{13}C-NMR, and MS) with the reported values.

Rheedeioside A (**1**): Amorphous powder; $[\alpha]_D^{24}$ −27.1 (c=2.41, MeOH); IR (film) ν_{max} 3395, 2944, 1729, 1647, 1559, 1078, 1048 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5): Table 1; ^{13}C-NMR (150 MHz, pyridine-d_5): Table 2; HR-ESI-MS (positive-ion mode): m/z 1608.6993 $[M+Na]^+$ (Calcd for $C_{72}H_{115}NO_{37}Na$: 1608.7040).

Rheedeioside B (**2**): Amorphous powder; $[\alpha]_D^{23}$ −28.5 (c=0.61, MeOH); IR (film) ν_{max} 3395, 2941, 1730, 1647, 1560, 1077, 1049 cm^{-1}; ^{1}H-NMR (500 MHz, pyridine-d_5): Table 1; ^{13}C-NMR (125 MHz, pyridine-d_5): Table 2; HR-ESI-MS (positive-ion mode): m/z 1566.6893 $[M+Na]^+$ (Calcd for $C_{70}H_{113}NO_{36}Na$: 1566.6935).

Rheedeioside C (**3**): Amorphous powder; $[\alpha]_D^{23}$ −20.5 (c=4.30, MeOH); IR (film) ν_{max} 3395, 2941, 1730, 1647, 1560, 1077, 1049 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5): Table 1; ^{13}C-NMR (150 MHz, pyridine-d_5): Table 2; HR-ESI-MS (positive-ion mode): m/z 1608.7003 $[M+Na]^+$ (Calcd for $C_{72}H_{115}NO_{37}Na$: 1608.7040).

Rheedeioside D (**4**): Amorphous powder; $[\alpha]_D^{25}$ −24.0 (c=2.10, MeOH); IR (film) ν_{max} 3388, 2945, 1730, 1648, 1559, 1079, 1047 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5): Tables 2 and 3; ^{13}C-NMR (150 MHz, pyridine-d_5): Table 1; positive-ion HR-ESI-MS: m/z 1446.6471 $[M+Na]^+$ (Calcd for $C_{66}H_{105}NO_{32}Na$: 1446.6512).

cis-Entadamide A β-D-Glucopyranoside (**5**): Amorphous powder; $[\alpha]_D^{23}$ −12.0 (c=0.44, MeOH); IR (film) ν_{max} 3335, 2945, 1748, 1637, 1575, 1074 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): Table 3; ^{13}C-NMR (125 MHz, CD_3OD): Table 3; HR-ESI-MS (positive-ion mode): m/z 346.0927 $[M+Na]^+$ (Calcd for $C_{12}H_{21}NO_7SNa$: 346.0931).

Alkaline Hydrolysis of Rheedeioside A (1) A solution of rheedeioside A (**1**) (38.3 mg) in 50% aqueous 1,4-dioxane (1.0 ml) was treated with 10% aqueous KOH (1.0 ml) and stirred at 80 °C for 3 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and then the resin was removed by filtration. The residue of the reaction mixture was subjected to RPCC [Cosmosil (0.5 g), H_2O–MeOH (100 : 0→4 : 1→2 : 3→0 : 100, v/v)] and on evaporation of the 60% MeOH eluate, entagenic acid monodesmoside (**1a**, 25.2 mg) was obtained. Entagenic acid monodesmoside (**1a**): Amorphous powder; $[\alpha]_D^{23}$ −13.6 (c=0.13, MeOH); IR (film) ν_{max} 3364, 2942, 1718, 1635, 1569, 1050 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.78, 0.90, 1.02, 1.09, 1.13, 1.14, 1.81, 2.11 (3H each, all s, H_3-25, 24, 29, 26, 23, 30, 27 and NHCOC$\underline{H}_3$), 3.22 (1H, dd, J=12, 4 Hz, H-3), 4.79 (1H, d, J=8 Hz, H-1‴), 5.03 (1H, d, J=8 Hz, H-1′), 5.20 (1H, d, J=7 Hz, H-1‴), 5.43 (1H, d, J=8 Hz, H-1″), 5.67 (1H, br s, H-12); ^{13}C-NMR (150 MHz, pyridine-d_5): Table 1; HR-ESI-MS (positive-ion mode): m/z 1140.5555 $[M+Na]^+$ (Calcd for $C_{54}H_{87}NO_{23}Na$: 1140.5561). The residue of the 100% H_2O eluate was heated in 2 M HCl (1.0 ml) under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and then the resin was removed by filtration. The filtrate was extracted with EtOAc and the aqueous layer was subjected to HPLC analysis [column: Shodex Asahipak NH 2P-50 4E, Φ=4.6 mm, L=25 cm; mobile phase: MeCN–H_2O (4 : 1, v/v); detection: optical rotation detector (JASCO 2090$_{Plus}$); and flow rate: 1.0 ml/min] to detect D-apiose, D-xylose and D-glucose, which were identified by comparison of their retention times with those of authentic samples, D-apiose (t_R 4.5 min, positive optical rotation), D-xylose (t_R: 7.5 min, positive optical rotation), and D-glucose (t_R: 10.3 min, positive optical rotation), respectively.

Acid Hydrolysis of Rheedeioside B Monodesmoside (1a) A solution of **1a** (12.5 mg) in 2 M HCl (1.0 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and then the resin was removed by filtration. The filtrate was extracted with EtOAc and the organic layer was evaporated under vacuum to give an aglycone (**1b**, 4.9 mg). The aglycone (**1b**) was crystallized from EtOH–H_2O and identified as entagenic acid (**8**). Entagenic acid (**1b**): Colorless crystals, mp 292—296 °C. $[\alpha]_D^{23}$ +28.6 (c=0.49, EtOH); ^{1}H- (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5); essentially the same as reported for entagenic acid (**8**)[3,4]; HR-ESI-MS (positive-ion mode) m/z: 511.3392 $[M+Na]^+$ (Calcd for $C_{30}H_{48}O_5Na$: 511.3399). The aqueous layer was subjected to HPLC analysis to detect D-glucosamine hydrochloride, D-xylose and D-glucose, which were identified by comparison of their retention times with those of authentic samples, D-glucosamine (t_R: 6.0 min, positive optical rotation).

Table 3. NMR Spectroscopic Data for Compound **5** (C: 125 MHz and H: 500 MHz, CD_3OD), δ in ppm and J in Hz

	C	H
1	169.1	—
2	115.9	5.89 d, 10
3	148.8	6.93 d, 10
1′	40.4	3.40 2H, t, 7
2′	69.9	3.62 2H, t, 7
S–CH_3	19.2	2.32 3H, s
1″	104.6	4.26 d, 8
2″	75.1	3.19 dd, 8, 8
3″	77.9	3.34 dd, 8, 8
4″	71.7	3.28 dd, 8, 8
5″	78.0	3.26 ddd, 8, 5, 2
6″	62.7	3.58 dd, 12, 5
		3.78 dd, 12, 2

Acetylation of Rheedeioside A (1) A solution of **1** (17.3 mg) in 2 ml of Ac_2O–pyridine (1 : 1) and DMAP (2.0 mg) was stirred at room temperature for 48 h. The reaction mixture was poured into ice-water and then extracted with EtOAc. The residue of the EtOAc extract was purified by silica gel CC [0.5 g, $CHCl_3$ and then $CHCl_3$: MeOH : H_2O] to give **1c** (19.0 mg). Nonadecaacetate (**1c**): Amorphous powder, $[\alpha]_D^{22}$ −37.0 (c=1.9, $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.62 (1H, br d, J=12 Hz, H-5), 0.68, 0.75, 0.84, 0.85, 0.91, 0.98, 1.33 (3H each, all s, H_3-24, 26, 25, 23, 29, 30 and 27, respectively), 1.85 (3H, s, C$\underline{H}_3$CON–), 1.89 (3H, s), 1.96 (3H, s), 1.97 (6H, s), 1.98 (9H, s), 1.99 (3H, s), 2.01 (3H, s), 2.02 (3H, s), 2.04 (6H, s), 2.05 (6H, s), 2.081 (6H, s), 2.083 (6H, s), 2.09 (3H, s), 2.10 (3H, s) (20×C$\underline{H}_3$COO–), 3.00 (1H, m, H-3), 4.43 (1H, d, J=6 Hz, H-1′), 4.45 (1H, d, J=6 Hz, H-1‴), 4.58 (1H, d, J=8 Hz, H-1), 4.60 (1H, d, J=6 Hz, H-1⁗), 4.69 (1H, d, J=7 Hz, H-1″), 4.97 (1H, br s, H-1⁗⁗), 5.48 (1H, br t, J=4 Hz, H-12), 5.69 (1H, d, J=6 Hz, H-1⁗⁗); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 15.5 (C-25), 16.5 (C-24), 17.3 (C-26), 18.6 (C-6), 20.2 (C-27), 20.4, 20.54, 20.57, 20.59 (×2), 20.62 (×2), 20.65, 20.69 (×2), 20.71 (×3), 20.84, 20.93 (×2), 20.96, 21.1, 21.3, 20.6 (20×C$\underline{H}_3$COO–), 23.2 (C$\underline{H}_3$CON–), 23.4 (C-11), 23.9 (C-30), 25.9 (C-2), 27.9 (C-23), 30.3 (C-20), 30.4 (C-22), 33.2 (C-29), 34.9 (C-7), 35.0 (C-21), 36.9 (C-10), 38.9 (C-1, 4), 40.9 (C-18), 41.3 (C-8), 46.1 (C-14), 46.3 (C-19), 46.9 (C-9), 47.0 (C-17), 55.0 (C-5), 69.6 (C-15), 76.2 (C-16), 90.1 (C-3), 126.4 (C-12), 141.0 (C-13), 172.8 (C-28), 103.4 (C-1′), 101.4 (C-1″), 101.7 (C-1‴), 101.3 (C-1⁗), 93.1 (C-1⁗⁗), 100.5 (C-1), 107.1 (C-1⁗⁗⁗), 169.0, 169.1, 169.3, 169.42, 169.43, 169.5, 169.6, 169.70, 169.74, 169.75, 169.88, 169.93, 170.1, 170.16 (×2), 170.19, 170.3, 170.4, 170.6, 170.7, 171.4 (21×CH_3C$\underline{O}$O–); HR-ESI-MS (positive-ion mode) m/z: 2406.9070 $[M+Na]^+$ (Calcd for $C_{110}H_{153}NO_{56}Na$: 2406.9047).

Mild Alkaline Hydrolysis of Rheedeioside A (1) A solution of rheedeioside A (**1**) (17.3 mg) in 0.5% NaOMe (1.0 ml) was stirred at 20 °C for 18 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and then the resin was removed by filtration. The filtrate was extracted with EtOAc and then the aqueous layer was evaporated under vacuum to give a desacetylrheedeioside A (**1a**, 14.0 mg). The desacyl-rheedeioside A (**1a**) was identified as rheedeioside B (**2**). Desacetylrheedeioside A (=rheedeioside B) (**1a**=**2**): Amorphous powder, $[\alpha]_D^{24}$ −25.1 (c=0.14, MeOH); HR-ESI-MS (positive-ion mode) m/z: 1566.6899 $[M+Na]^+$ (Calcd for $C_{70}H_{113}NO_{36}Na$: 1566.6934).

Acid Hydrolysis of Rheedeiosides B, C and D (2—4) Solutions of **2**, **3** and **4** (1 mg each) were hydrolyzed in the same manner as described previously. HPLC analysis under the same conditions as above revealed the presence of D-glucosamine hydrochloride, D-xylose, D-apiose and D-glucose for **2**, D-glucosamine hydrochloride, D-xylose and D-glucose for **3**, and D-glucosamine hydrochloride, D-apiose, D-xylose and D-glucose for **4**. The sugars were identified by comparison of their retention times with those of authen-

tic samples, L-arabinose (t_R: 7.0 min, positive optical rotation).

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