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ENTADA SAPONIN-III, A SAPONIN ISOLATED FROM THE BARK OF *ENTADA PHASEOLOIDES*

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Key Word Index—*Entada phaseoloides*; Leguminosae; echinocystic acid; structure determination; triterpenoid saponin; Entada saponin (ES)-III.

Abstract—The structure of Entada saponin (ES)-III, one of the main saponins of *Entada phaseoloides* bark, was established to be 3-*O*-[β-D-xylopyranosyl (1 → 2)-α-L-arabinopyranosyl (1 → 6)] [β-D-glucopyranosyl (1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-28-*O*-[β-D-apiofuranosyl (1 → 3)-β-D-xylopyranosyl (1 → 2)] [(2-*O*-acetoxyl)-β-D-glucopyranosyl-(1 → 4)] (6 – *O*(*R*) (–)2,6-dimethyl-2-*trans*-2,7-octadienoyl)-β-D-glucopyranosyl echinocystic acid.

INTRODUCTION

The bark of a subtropical woody climber, *Entada phaseoloides* (L) Merrill, has been used as a washing agent which suggests the presence of saponins. Although several workers have reported saponins in this plant, nothing is known of their structure [1–3]. This paper describes the isolation and the structure elucidation of the main saponin named Entada saponin-III.

RESULT AND DISCUSSION

From the methanolic extracts of the bark of *E. phaseoloides* saponins were obtained by HPLC on an ODS column and named Entada saponins (ES)-I ~ VI by the sequence of spots on an ODS-TLC plate. The pure saponins ES-II (**1**), -III (**2**) and -IV (**3**), were isolated by HPLC using Aquasil [4] and ODS columns.

Compound **2** on reflux with 2 N sulphuric acid in ethanol afforded the sapogenin **4**. Compound **4** was identified as echinocystic acid from its physical data (mp, TLC, IR, ^{1}H NMR and ^{13}C NMR).

ES-III (**2**) had an IR spectrum which indicated the presence of many hydroxyl groups and ester groups. The ^{13}C NMR spectrum of **2** showed a signal due to the C-28 carbonyl carbon of the sapogenin, which was shifted from δ180.0 to 175.9, and a signal at δ89.6 indicated a sugar moiety attached to the C-3 hydroxyl group of sapogenin. Therefore, compound **2** was a bisdesmosidic saponin. Alkaline hydrolysis of **2** with 20% potassium hydroxide in ethanol afforded a prosapogenin (**5**), a powder, $C_{54}H_{87}NO_{22}$. After hydrolysis of **5** with 2 N sulphuric acid, the hydrolysate in the aqueous layer, examined by TLC and GC, showed the presence of glucose, arabinose, xylose and an unidentified component giving a positive ninhydrin reaction. All the ^{13}C NMR signals of **5** are shown in Table 1, which reveals the presence of three characteristic signals at δ23.8, 58.1 and 170.2. On the other hand, the presence of one nitrogen atom in the molecule of **5** was proved by the elementary analysis, and this was ascribable to an NH group by the ^{1}H NMR signal at δ8.27, and by the IR spectral absorptions at 1645 and 1550 cm^{-1} for a CONH group. Thus the presence of an amino sugar moiety in **5** was confirmed.

Amino sugars are usually identified as their hydrochloride [5]. By the hydrolysis of **5** with 2 N hydrochloric acid, D-glucosamine hydrochloride was obtained and identified by comparing its R_f value on HP-TLC (cellulose) and its $[\alpha]_D$ with those of authentic sample. However, a singlet methyl signal at δ2.08, and the one proton of the NH group at 8.27 were seen in the ^{1}H NMR spectrum. Also, a methyl carbon signal at δ23.8, a carbonyl carbon signal at 170.2 and a characteristic signal at 58.1 due to C-2 carbon of *N*-acetyl-β-D-glucosamine were observed in the ^{13}C NMR spectrum of **5**. Therefore, the aminosugar component in **5** is *N*-acetyl-D-glucosamine.

Hence, compound **5** consists of echinocystic acid, *N*-acetyl-D-glucosamine, glucose, arabinose and xylose. The fast atom bombardment mass spectrum (FAB-MS) of **5** gave an $[M+H]^+$ ion at *m/z* 1102, and an $[M+K]^+$ ion at *m/z* 1140 to confirm the molecular weight of 1101 for $C_{54}H_{87}NO_{22}$ (**5**).

Partial hydrolysis of **5** with 0.5 N sulphuric acid afforded compound **6**, $C_{38}H_{61}NO_9$, compound **7**, $C_{44}H_{71}NO_{14}$ and compound **8**, $C_{49}H_{79}NO_{18}$, which were hydrolysed again with 2 N hydrochloric acid to give the molecular fragments as indicated in Table 2.

The configuration of each sugar component was determined by NMR spectroscopy as shown in Table 3. These results suggested the equatorial anomeric conformation of each sugar component of **5**. The molecular rotation differences between **4** and **6**, **6** and **7**, **7** and **8**, **8** and **5** were –113.5, –14, –100.4 and –27°, respectively. By the Klyne rule [6], the above results showed that *N*-acetylglucosamine, glucose and xylose were in the β-D-form, and arabinose was the α-L form.

Table 1. ^{13}C NMR chemical shift values (δ) of compound **5**.

Carbon	Aglycone moiety	Sugar moiety
1	39.0 *d*	23.8 *u*
2	26.6 *d*	58.1 *u*
3	89.7 *u*	62.7 *d*
4	39.4 —	66.1 *d*
5	56.2 *u*	67.4 *d*
6	18.8 *d*	68.3 *d*
7	33.8 *d*	68.8 *u*
8	40.2 —	71.0 *u*
9	47.5 *u*	71.9 *u*
10	37.3 —	73.3 *u*
		73.7 *u*
11	24.0 *u*	75.0* *u*
12	122.6 *u*	75.2 *u*
13	145.2 —	76.2 *u*
14	42.3 —	78.1 *u*
15	36.4 *d*	78.2 *u*
16	74.9* *u*	78.6 *u*
17	49.2 —	81.4 *u*
18	41.7 *u*	82.7 *u*
19	47.5 *d*	103.4 *u*
20	31.1 —	104.3 *u*
		104.6 *u*
21	36.4 *d*	107.4 *u*
22	32.8 *d*	170.2 —
23	28.4 *u*	
24	17.2 *u*	
25	15.7 *u*	
26	17.7 *u*	
27	27.4 *u*	
28	180.1 —	
29	33.4 *u*	
30	25.1 *u*	

u: up $-\overset{|}{C}H$, $-CH_3$; d: down $-\underset{|}{C}H_2$; —: $-\overset{|}{\underset{|}{C}}-$.

INEPT: Insensitive Nuclei Enhanced by Polarization Transfer.

*The assignments of the asterisked signals are ambiguous and might have to be reversed.

Table 2. Products by acid hydrolysis of prosapogenin **5**

A	B
Echinocystic acid (4)	4
6	4 + GlcN.HCl
7	4 + GlcN.HCl + Glc
8	4 + GlcN.HCl + Glc + Ara
Unchanged **5**	4 + GlcN.HCl + Glc + Ara + Xyl

A: Products obtained by hydrolysis of the materials with 0.5 N H_2SO_4.

B: Products obtained by hydrolysis of A with 2 N HCl.

Table 3. ^{1}H NMR chemical shift values (δ) and $J_{H_1H_2}$ (Hz) of anomeric protons of the sugar components in compounds **6**, **7**, **8** and **5**

Compound	Chemical shift	$J_{H_1H_2}$ (Hz)
6	5.04	7.3
7	4.99	7.9
	5.03	7.9
8	4.91	6.7
	5.00	7.8
	5.18	7.6
5	4.82	7.3
	5.09	8.6
	5.19	6.4
	5.41	7.9

^{13}C NMR chemical shift values (δ) and $J_{C_1H_1}$ (Hz) of anomeric carbons of compound **5**

Chemical shift	$J_{C_1H_1}$(Hz)	Sugar
103.4	164.1	Ara
104.3	162.4	GlcNAc
104.6	164.1	Glc
107.4	160.7	Xyl

The ^{13}C NMR spectra of olean-12-ene type triterpenes have been reported by Tori *et al.* [7] to give a full assignment of signals. In comparison with these data, the ^{13}C NMR data of **6**, **7** and **8** obtained from a proton noise decoupling spectrum and from insensitive nuclei enhanced by polarization transfer (INEPT) experiments [8] revealed the number of CH, CH_2 and CH_3 carbons unequivocally.

As shown in Table 4, by the application of glycosylation shifts [9–12], the sugar moieties were attached to the C-3 hydroxyl group of echinocystic acid in each prosapogenin **6**, **7**, **8** and **5**. Further assignments on the carbon signals of **5** ~ **8** were made to determine the sequence of sugar moieties. The ^{13}C NMR spectrum of **6** was in good accordance with the structure of *O*(3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-echinocystic acid in comparison with the ^{13}C NMR signals of 2-acetamido-2-deoxy-β-D-glucopyranose (**9**), lansioside A (**10**) [13], and *O*(3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-oleanolic acid (**11**) which is an amino sugar containing triterpenoid glycoside isolated from two *Pithecellobium* species [14].

The terminal glucose of **7** was reasonably assigned in comparison with the data of methyl-β-D-glucopyranoside and other numerous glycosides. The signal due to the C-4 carbon of *N*-acetyl-β-D-glucosamine was shifted to lower field by +9.5 ppm (73.5 → 83.0 ppm); the signals due to C-5 and C-3 were shifted to upper field, (78.2 → 76.3 ppm) and (76.4 → 73.7 ppm), respectively. These glycosylation shifts indicated that **7** had a β-D-glucopyranosyl (1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl moiety as a sugar component. The structure of **7** was, therefore, established as *O*(3)-β-D-glucopyranosyl (1 → 4)-2-

Table 4. ^{13}C NMR chemical shift values (δ) of the aglycone moieties of compounds **6**, **7** and **8**

Carbon	6	7	8
1	39.3	39.1	39.1
2	26.8	26.6	26.6
3	89.7	89.7	89.9
4	39.7	39.5	39.5
5	56.5	56.3	56.3
6	19.1	18.9	18.9
7	34.0	33.9	33.9
8	40.5	40.4	40.4
9	47.7	47.6	47.6
10	37.6	37.4	37.4
11	24.2	24.1	24.1
12	122.7	122.6	122.6
13	145.4	145.3	145.2
14	42.6	42.4	42.4
15	36.6	36.4	36.5
16	75.1	74.9	75.0
17	49.6	49.4	49.4
18	42.0	41.8	41.8
19	47.7	47.6	47.6
20	31.3	31.1	31.1
21	36.6	36.4	36.5
22	32.7	32.6	32.6
23	28.7	28.5	28.5
24	17.3	17.1	17.1
25	15.9	15.7	15.7
26	17.9	17.8	17.8
27	27.6	27.5	27.5
28	180.0	179.9	180.0
29	33.5	33.4	33.4
30	25.4	25.2	25.2

acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid.

In the ^{13}C NMR spectrum of **8**, the signal due to the C-6 carbon of either *N*-acetyl-β-D-glucosamine or glucose shifted to lower field at δ68.8, to suggest that the terminal arabinose was attached to the C-6 position of one of them. If arabinose was attached to the C-6 position of glucose, the signal due to C-5 of glucose would shift up-field (-1 ~ -6 ppm), but signals observed at δ78.2 and 78.6 in the ^{13}C NMR spectrum of **8** showed no glycosylation shift in comparison with the corresponding two signals at δ78.4 of **7**, while the signal due to C-5 of *N*-acetyl-β-D-glucosamine shifted up-field (76.3 → 75.2). Therefore, the terminal arabinose was confirmed to be attached to the C-6 position of *N*-acetyl-β-D-glucosamine. Thus, all the signals of **8** were completely assigned to represent the structure of **8** as *O*(3)-[α-L-arabinopyranosyl (1 → 6)] [β-D-glucopyranosyl (1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid.

The signal at δ103.4, one of the four anomeric carbon signals given by **5**, was assigned to C-1 of one of the sugars, which is shifted to higher field due to the linkage with terminal xylose at the C-2 position. As shown in Table 5, the glycosylation shifts are reasonably related to the anomeric carbon of arabinose to assign all the signals given by **5**. The T_1-values of each anomeric carbon of **5** were measured, and the partially relaxed Fourier transform (PRFT) spectra of **5** also agreed with this sequence of sugar linkages. Accordingly, the structure of **5** obtained by alkaline hydrolysis from Entada saponin-III (**2**) is *O*(3)-[β-D-xylopyranosyl (1 → 2)-α-L-arabinopyranosyl (1 → 6)] [β-D-glucopyranosyl (1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid. The sequence of sugar components of **5** was also confirmed by methylation analysis.

Table 5. ^{13}C NMR chemical shift values (δ) of the sugar moieties of compounds **6**, **7**, **8** and **5**

Carbon		6	7	8	5
GlcNAc	1	104.7	104.2	104.3	104.3
	2	58.9	58.2	58.1	58.1
	3	76.4	73.7	73.3	73.3*
	4	73.5	83.0	82.1	82.7
	5	78.2	76.3	75.2*	75.2*
	6	63.7	62.9*	68.8	68.3
Me CONH-		23.9	23.7	23.7	23.8
Me CONH-		170.2	170.2	170.1	170.2
Glc	1		105.1	104.9	104.6
	2		75.0	75.0*	75.0*
	3		78.4	78.2	78.2*
	4		71.9	72.1	71.9
	5		78.4	78.6	78.6
	6		62.7*	62.8	62.7
Ara	1			105.3	103.4
	2			72.6	81.4
	3			74.6	73.7*
	4			69.4	68.8
	5			66.7	66.1
Xyl	1				107.4
	2				76.2
	3				78.1*
	4				71.0
	5				67.4

* The assignments of the asterisked signals are ambiguous and might have to be reversed.

On the other hand, the signals, at δ12.6, 20.7, 24.2, 28.4, 41.6, 72.4, 111.7, 127.9, 144.0, 146.6, 167.4 and 170.3 as well as, signals due to the sapogenin part and sugar moieties, were observed in the ^{13}C NMR spectrum of ES-III (**2**). The ^{13}C NMR chemical shifts of the above ten signals, except for the two signals at δ20.7 and 170.3, were very similar to those of the (*S*) (+)2,6-dimethyl-6-hydroxyl-2-*trans*-2,7-octadienoic acid moiety in the molecule of Gleditsia saponin C isolated from the fruit of *Gleditsia japonica* [15].

Hydrolysis of **2** with 1% potassium hydroxide in dioxane afforded two oily substances, **12** and **13**, which were separated by silica gel column chromatography. By comparing their TLC, ^{1}H NMR, ^{13}C NMR and mass spectra **12** and **13** were identified as 2,6-dimethyl-6-hydroxyl-2-*trans*-2,7-octadienoic acid and its transformed product [15], respectively.

The vinyl proton signal at δ6.89 given by **12** was assigned to a *trans*-β-vinyl proton at C-3, and the stereochemistry at C-6 of **12** was assigned to be *R* by comparing its optical activity, $[\alpha]_D^{25}$ −24.6°, with that of (*S*) (+)2,6-dimethyl-6-hydroxyl-2-*trans*-2,7-octadienoic acid, $[\alpha]_D^{25}$ +17.8°.

Two signals at δ20.7 and 170.3 given by **2** were not shown in the ^{13}C NMR spectrum of desacyl-ES-III (**14**) derived from **2** by hydrolysis with 1% potassium hydroxide, and ^{1}H NMR signal at δ2.00 given by **2** was not seen in the ^{1}H NMR spectrum of **14**. Therefore, the presence of an acetyl group, in addition to **12**, in the molecule of **2** was indicated. It was also confirmed by hydrolysing **2** with 0.025% potassium carbonate to give compound **15** which was proved to be desacetyl-ES-III by comparing its ^{1}H NMR and ^{13}C NMR spectra with those of **2** and **14**. The ^{13}C NMR signals at δ20.7 and 170.3 given by **2** were not observed in the spectra of **14** and **15**, and signals at 12.6, 24.2, 28.4, 41.6, 72.4, 111.7, 127.9, 144.0, 146.6 and 167.4 given by **2** were also observed in the spectra of **15**, but not in that of **14**. Furthermore, ^{1}H NMR signal at δ2.00 given by **2** was not observed in the spectra of **14** and **15**. The FAB-MS (exact mass by negative-ion mode) of **2** gave an $[M-1]^-$ ion at *m/z* 1897, while that of **15** gave the $[M-1]^-$ ion at *m/z* 1855 ($\Delta-42$). This result showed that **15** was the desacetyl derivative of **2**.

The ^{13}C NMR spectra of **2** showed eight anomeric carbon signals. The sugar components attached to the C-28 carboxyl group of the sapongenin were examined by TLC and GC to prove the occurrence of glucose (2 mol), xylose (1 mol) and apiose (1 mol). Compound **14** was methylated by Hakomori's method [16] to afford a permethylate (**16**). On reduction with lithium aluminium hydride, **16** afforded compounds **17** and **18**, and the latter was proved to be the prosapogenin part.

The ^{1}H NMR spectrum of **17** indicated the presence of eleven *O*-methyl groups and three anomeric protons [δ4.30 (1H, *d*, J = 8 Hz), 4.88 (1H, *d*, J = 8 Hz), 5.44 (1H, *d*, J = 2 Hz)].

On methanolysis with 5% hydrogen chloride in dry methanol, **17** afforded methyl 2,3,4,6-tetra-*O*-methyl glucopyranoside, methyl 2,3,5-tri-*O*-methyl apiofuranoside, methyl 2,4-di-*O*-methyl xylopyranoside and 3,6-di-*O*-methyl glucitol (unidentified). On the other hand, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl apitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol, 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl xylitol and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl glucitol were detected by GC/MS analysis of the partially methylated alditol acetates obtained from **17** by hydrolysis followed by sodium borohydride reduction and acetylation.

The formation of 3,6-di-*O*-methyl glucitol and 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl glucitol from **17** indicated that the oligosaccharide moiety of **2** links to the C-28 carboxyl group of the sapogenin with a hemi-acetal hydroxyl group of glucose. The above observations indicated that one of the two glucose moieties and apiose must be the terminal sugars.

On treatment with 0.2% hydrogen chloride in dry methanol, **17** afforded methyl 2,3,5-tri-*O*-methyl apiofuranoside and an *O*-methylated oligosaccharide (**19**). The ^{1}H NMR spectrum of **19** showed the presence of two anomeric protons [δ4.27 (1H, *d*, J = 7.9 Hz), 4.52 (1H, *d*, J = 7.3 Hz)] and eight *O*-methyl groups. On methanolysis with 0.5% hydrogen chloride in dry methanol, **17** afforded methyl 2,3,5-tri-*O*-methyl apiofuranoside, methyl 2,4-di-*O*-methyl xylopyranoside and *O*-methylated oligosaccharide (**20**). The ^{1}H NMR spectrum of **20** showed the presence of one anomeric proton [δ4.28 (1H, *d*, J = 7.6 Hz)] and six *O*-methyl groups. Compound **20** was methylated to afford an *O*-methylated oligosaccharide (**21**). From the ^{1}H NMR spectral data and the optical activities, **21** was identical to the compound (**22**) which was derived from D-(+)-cellobiose (4-*O*-β-D-glucopyranosyl-D-glucopyranose) by reduction with sodium borohydride followed by permethylation. The ^{1}H NMR spectrum of **21** showed the presence of nine *O*-methyl groups (δ3.35, 3.38 × 2, 3.42, 3.48, 3.52, 3.558, 3.564, 3.62) and one anomeric proton [δ4.39 (1H, *d*, J = 7.9 Hz)]. On methanolysis with 5% hydrogen chloride in dry methanol, **21** afforded methyl 2,3,4,6-tetra-*O*-methyl glucopyranoside and 1,2,3,5,6-penta-*O*-methyl glucitol (unidentified).

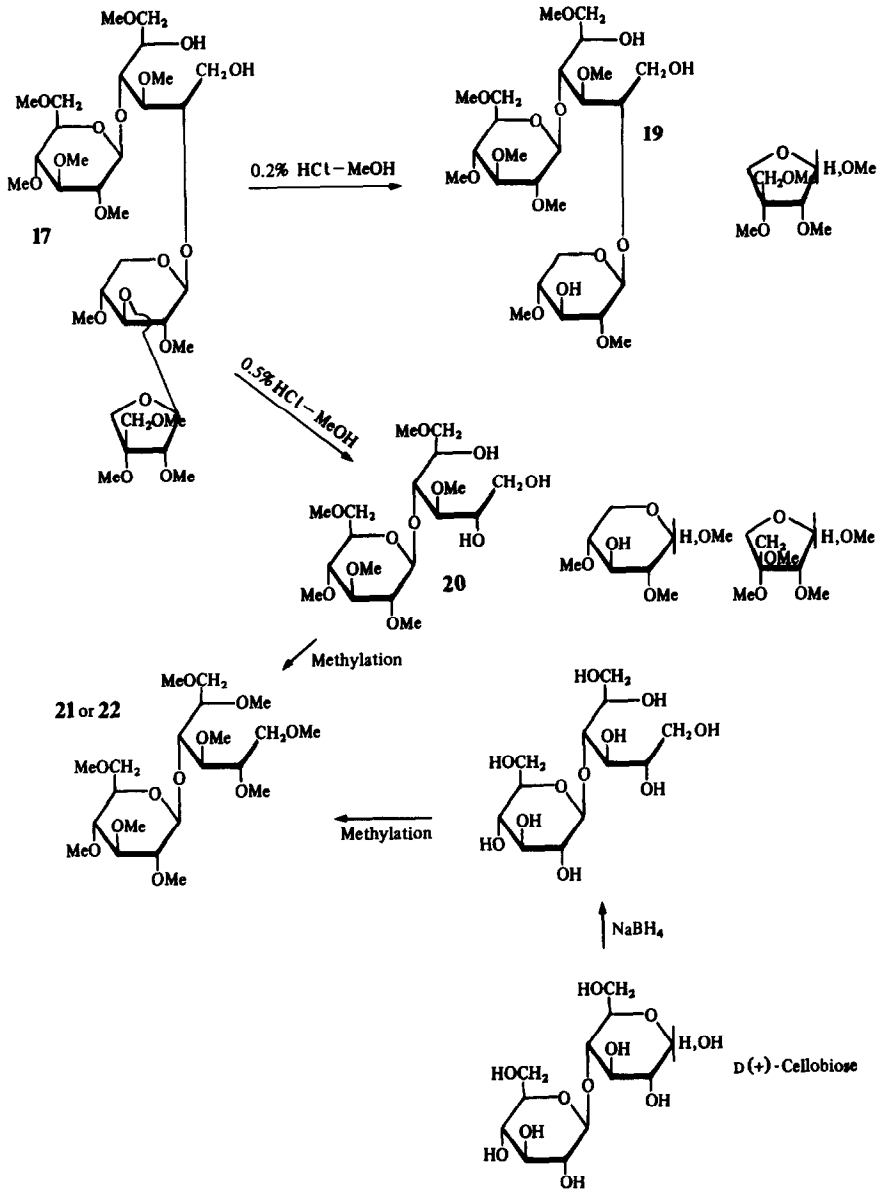
The results showed that the terminal D-glucosyl moiety was attached to the C-4 hydroxyl group of a D-glucose, which is substituted at the C-2 hydroxyl group. The configuration of the terminal D-apiose was assigned as the β-form from the molecular optical rotation difference between **17** and **19** ($\Delta[M]_D = -239°$, methyl 2,3,4-tri-*O*-methyl-α-D-apiofuranoside: $[M]_D = +239°$; methyl 2,3,4-tri-*O*-methyl-β-D-apiofuranoside: $[M]_D = -163°$) [17]. Similarly, D-xylose was assigned as the β-form ($\Delta[M]_D = -86°$, methyl 2,4-di-*O*-methyl-β-D-xylopyranoside: $[M]_D = -158°$) [18]. Therefore, desacyl-ES-III (**14**) was established to be 3-*O*-[β-D-xylopyranosyl (1 → 2)-α-L-arabinopyranosyl (1 → 6)] [β-D-glucopyranosyl (1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-28-*O*-[β-D-apiofuranosyl (1 → 3)-β-D-xylopyranosyl (1 → 2)] [β-D-glucopyranosyl (1 → 4)]-β-D-glucopyranosyl echinocystic acid.

As previously mentioned, ES-III (**2**) had an acetyl group and a monoterpene moiety in its molecule. For determination of the positions of these two acyl groups, the ^{13}C NMR spectrum of **2** was compared with those of **14** and **15**. Each spectrum gave eight anomeric carbon signals which showed a well separated profile (Table 6). The anomeric carbon signals of the sugar moieties of **2** were almost superimposable with those of **14** and **15** with the exception of a signal at δ102.4.

An upfield shift of an anomeric carbon (β-carbon) signal would also be caused by the substitution of one of the two acyl groups at a hydroxyl at the neighboring C-2 (α-carbon) position of one of the two glucose moieties. On the other hand, as shown in Table 7, one of the three carbon signals due to the C-6 carbons of glucose in the ^{13}C NMR spectrum of **14** is shifted to lower value than in the spectra of **2** and **15**. Consequently the locations of two acyl groups in **2** were confirmed by comparative analysis of the ^{13}C NMR spectra of **2**, **14** and **15**, to prove that an acetyl group is attached to the C-2 hydroxyl of one of the glucose moieties and a monoterpene group is attached to the C-6 hydroxyl of one of those sugars.

In addition to the ^{13}C NMR spectral analysis, methylation analysis using GC/MS was also applied to determine the locations of two acyl groups in the molecule of **2**. Compound **2** was methylated by Kuhn's method [19] to afford a methylate (**23**). On reduction with lithium aluminium hydride, **23** afforded compounds **24** and **25**. By the methylation analysis of partially methylated alditol acetates of the sugar moieties, two acyl groups in **2** were proved to be located in the sugar moiety attached to the C-28 carboxyl group of the sapogenin.

Compound **25** was analysed to give 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl apitol, 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-xylitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl glucitol and 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl glucitol. Accordingly the two acyl groups were proved to be attached to the hydroxyl group at C-2 of the terminal



Scheme 1.

Table 6. ^{13}C NMR chemical shifts (δ) of the anomeric carbon atoms of the sugar moieties of desacyl -ES-III (**14**), desacetyl-ES-III (**15**) and ES-III (**2**)

14	15	2
111.4	111.7	111.4 Api
106.7	107.0	106.8 Xyl
105.1	105.1	105.2 Xyl
104.7	105.0	102.4 Glc
104.4	104.7	104.5 Glc
104.1	104.2	104.5 GlcNAc
103.0	103.3	103.1 Ara
93.4	93.6	93.2 Glc

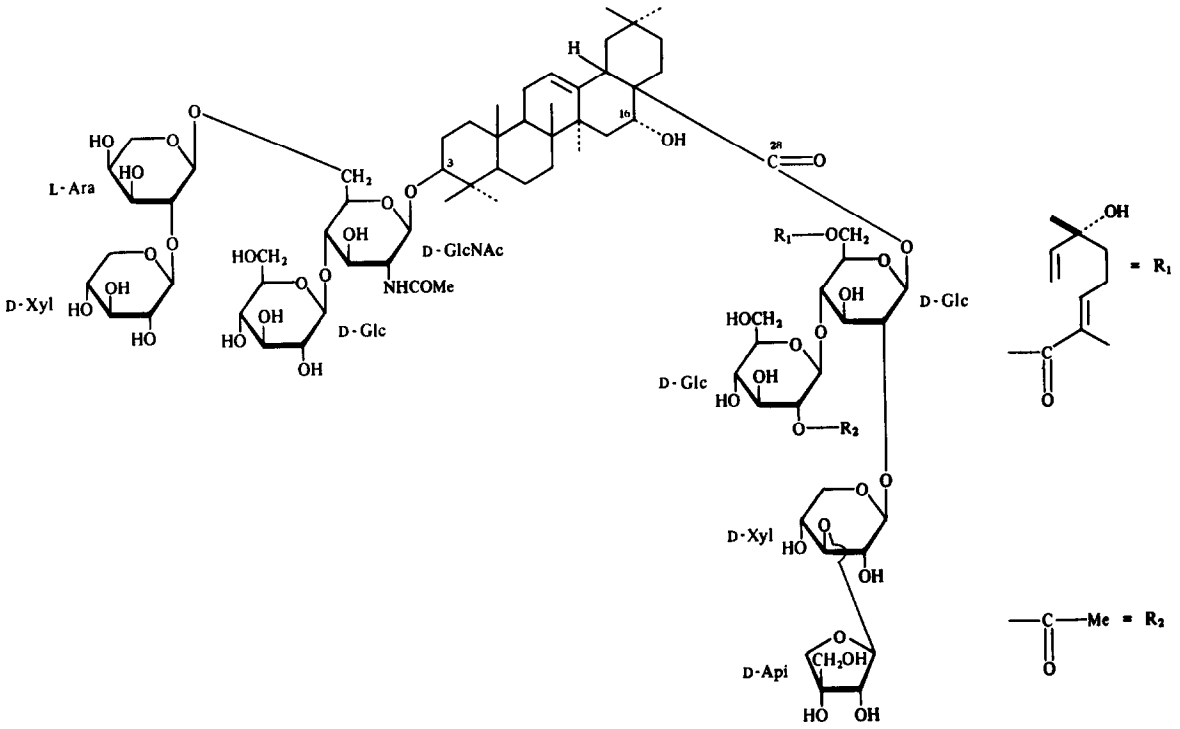
glucose, and C-6 of the inner glucose, respectively.

Thus the structure of one of the major saponins of the bark of *E. phaseoloides*, ES-III (**2**), was established on the basis of spectral and chemical evidence.

Table 7. ^{13}C NMR chemical shifts (δ) of the C-6 carbons of the glucosyl moieties in desacyl-ES-III (**14**), desacetyl-ES-III (**15**) and ES- III (**2**)

14	15	2
62.6	62.9	62.8
62.5	61.9	62.6
61.7	64.8	63.4

Entada saponin (ES) – III (2)



Scheme 2.

Since earlier workers noted an *in vitro* cytotoxic activity in the saponin of Entada [1], the cytotoxicity has been tested using ES-II (1), -III (2) and -IV (3) on L-5178Y tumor cells which showed positive results. The IC_{50} values determined for ES-II, ES-III and ES-IV were 3.10, 0.83 and 0.96 $\mu g/\mu l$, respectively.

The structures of ES-II (1) and IV (3) which possess the same sugar moieties as 2 attached to oleanolic acid and entagenic acid, respectively, have been established by the same procedure as described in this paper. The detailed results will be reported elsewhere.

EXPERIMENTAL

Mps are uncorr. MS(EI) were measured at 70 eV. FAB and FD-MS were measured with a JEOL DX-300/FAB FD ion source xenon atom beam source. The detection method employed for HPLC was refractive index (RI).

Isolation of saponins. The crushed bark (426 g) of *Entada phaseoloides* which is available on the market in Manila was refluxed with MeOH (3 × 3 l) for 6 hr. The combined extracts were concentrated *in vacuo*, to dryness (59.6 g). The dried extracts were subjected to HPLC on a prep. PAK-500/C_{18} column (30 cm × 50 mm i.d.) using MeOH–H_2O (7:3) as the solvent. Six saponins separated were named ES-I ~ VI by the sequence of R_f value (C_{18}-TLC). The saponin mixture was further separated and purified by HPLC on an Aquasil column (30 cm × 20 mm I. D.) using $CHCl_3$–MeOH–H_2O (30:20:4) as the solvent to obtain chromatographically pure major saponins, ES-II (1) (2.7 g), -III (2) (3.2 g) and -IV (3) (4.0 g). ES-III (2), mp. 211–214° (dec.), $[\alpha]_D^{25}$ −27.0° (MeOH; *c* 0.33). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1640, 1550 (CONH). ^1H NMR: δ0.89, 0.93, 0.97, 0.98, 1.08, 1.17, 1.74 (21 H, *s*, 7 × Me), 1.42 (3H, *s*, C-6-CH_3 of 12), 2.00 (3H, *s*, CH_3COO), 2.06 (3H, *s*, $\underline{CH}_3CONH$), 8.22 (1H, NH). FAB-MS (*m/z*) (exact mass by negative-ion mode): 1897 $[M-1]^-$. Found: C, 52.23 H, 7.43 N, 0.69. $C_{88}H_{139}NO_{43}\cdot 6\frac{1}{2}H_2O$ requires: C, 52.42 H, 7.60 N, 0.69%.

Isolation of echinocystic acid (4). To the soln of 2 (400 mg) in EtOH (20 ml) was added 2N H_2SO_4 (20 ml), and the mixture was refluxed for 4 hr. The soln was concd to 20 ml *in vacuo* and the pps formed were chromatographed over silica gel using $CHCl_3$–MeOH (50:1) to afford a colourless solid which was recrystallized from EtOH to give echinocystic acid (4) (52 mg). Mp 308–309°, $[\alpha]_D^{25}$ +33.8° (EtOH; *c* 0.44). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1693 (COOH). ^1H NMR: δ0.97, 0.99, 1.04, 1.05, 1.17, 1.18, 1.76 (21 H, 7 × CH_3), 5.13 (1H, *t*-like), 5.61 (1H, *t*-like). MS (*m/z*): 472 $[M]^+$, 454 $[M-H_2O]^+$, 264 $[C_{16}H_{24}O_3]^+$, 246 $[264-H_2O]^+$, 201 $[246-COOH]^+$. High resolution MS: Calc. for $C_{30}H_{48}O_4$: 472.3552, obs. 472.3546. Found: C, 74.87 H, 10.30. Calc. for $C_{30}H_{48}O_4\cdot\frac{1}{2}H_2O$: C, 74.80; H, 10.25%.

Hydrolysis of ES-III (2) *with* 20% KOH. A soln of 2 (600 mg) in 20% KOH (30 ml) and EtOH (30 ml) was refluxed for 2 hr. The reaction mixture was cooled at room temp., neutralized with 10% HCl, concd to 30 ml and dried. The MeOH-soluble fraction was chromatographed on a column of Sephadex LH-20 with MeOH to afford a crude prosapogenin (5). This was further purified by HPLC on an Aquasil column (30 cm × 20 mm i.d.) using $CHCl_3$–MeOH–H_2O (30:20:4) as the solvent to give a colourless powder (140 mg). Mp 225–228° (dec.), $[\alpha]_D^{25}$ −7.9° (MeOH; *c* 1.95). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH), 1645, 1550 (CONH). ^1H NMR: δ0.86, 0.93, 1.03, 1.16, 1.77 (21 H, *s*, 7 × Me), 2.08 (3H, *s*, $C\underline{H}CONH$), 8.27 (1H, *d*, NH). FAB-MS (*m/z*): 1102 $[M+H]^+$, 1140 $[M+K]^+$ with KI. Found: C, 55.92 H, 8.26 N, 1.21. Calc. for $C_{54}H_{87}NO_{22}\cdot 3\frac{1}{2}H_2O$: C, 55.65 H, 8.13 N, 1.20%.

Hydrolysis of 5 *with* 2N H_2SO_4. A soln of 5 (20 mg) in 2N H_2SO_4 (5 ml)–EtOH(5 ml) was refluxed for 3 hr. The soln

was concd to 5 ml and the precipitates formed were collected. The product was identified with echinocystic acid (**4**) by TLC. The aq. layer was neutralized and evaporated *in vacuo*. Glucose, arabinose and xylose were identified by HP-TLC and GC. HP-TLC [silica; $CHCl_3$–MeOH–H_2O (65:35:10) (the lower layer), *n*-BuOH–*iso*-PrOH–H_2O (5:3:1), *iso*-PrOH–*n*-BuOH–H_2O (7:1:2)] colour reagent: 10% H_2SO_4, aniline hydrogen phthalate. GC: column: 3% OV-1 on chromosorb W (DMCS) 60–80 mesh 3.0 m × 2.0 mm glass. Inj. temp. 250°; column temp. programmed 150 to 200° at 2°/min.; N_2 at 1.5 kg/cm^2. Detection: FID. Sample: TMSi derivative.

Hydrolysis of **5** *with* 2N HCl. A soln of **5** (500 mg) in 2N HCl (60 ml) was heated at 100° for 4 hr, and the ppts formed were collected. The filtrate was evapd repeatedly at 40° until the soln indicated neutral reaction. The soln was applied to a prep column of Dowex 50W-X8 (H^+ form) and eluted in the usual manner. The amino sugar fraction was eluted with 0.3 N HCl, and was evaporated repeatedly at 40° removing HCl to afford a colourless powder which was found to be identical with D-glucosamine hydrochloride by comparing its $[\alpha]_D^{25}$ and HP-TLC (cellulose). HP-TLC (cellulose)-Solvent: EtOAc–pyridine–AcOH–H_2O (5:5:1:3); colour reagent: Ninhydrin.

Hydrolysis of **5** *with* 0.5 N H_2SO_4. A soln of **5** (1 g) in 0.5 N-H_2SO_4 (100 ml)–EtOH (100 ml) was refluxed for 2 hr. The reaction mixture was cooled to room temp., neutralized with 5% NaOH and conc to 100 ml, then lyophylised. The MeOH-soluble fraction was chromatographed on a column of Sephadex LH-20 with MeOH to afford a prosapogenin fraction which was further separated by HPLC on an Aquasil column (30 cm × 20 mm I. D.) using $CHCl_3$–MeOH–H_2O (10:6:1) to give three colourless amorphous substances, **6** (10 mg), **7** (98 mg) and **8** (89 mg), together with sapogenin **4** and unchanged prosapogenin **5**.

Compound **6**: Mp 277–280° (dec.), $[\alpha]_D^{25}$ +7.3° (EtOH; *c* 0.4). ^{1}H NMR: δ0.88, 0.97, 1.01, 1.04, 1.16, 1.76 (21 H, *s*, 7 × Me), 2.07 (3H, *s*, Me CONH), 5.04 (1H, *d*, *J* = 7.3 Hz), 8.14 (1H, *d*, *J* = 7.3 Hz, NH). Found: C, 65.77 H, 9.15 N, 2.02. $C_{38}H_{61}NO_9 \cdot H_2O$ requires: C, 65.82 H, 9.08 N, 2.03%.

Compound **7**: Mp 236–238° (dec.), $[\alpha]_D^{25}$ +4° (EtOH; *c* 0.8). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH), 1650, 1550 (CONH). ^{1}H NMR: δ0.88, 0.95, 1.01, 1.03, 1.15, 1.16, 1.76 (21 H, *s*, 7 × Me), 2.07 (3H, *s*, Me CONH), 4.99 (1H, *d*, *J* = 7.9 Hz), 5.03 (1H, *d*, *J* = 7.9 Hz), 8.25 (1H, *d*, *J* = 7.9 Hz). Found: C, 60.16 H, 8.56 N, 1.57. $C_{44}H_{71}NO_{14} \cdot 2\frac{1}{2}H_2O$ requires: C, 59.84 H, 8.68 N, 1.58%.

Compound **8**. Mp 232–234° (dec.), $[\alpha]_D^{25}$ −6.3° (EtOH; *c* 1.05). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH), 1650, 1550 (CONH). ^{1}H NMR: δ0.87, 0.96, 1.00, 1.13, 1.16, 1.75 (21 H, *s*, 7 × Me), 2.06 (3H, *s*, Me CONH), 4.91 (1H, *d*, *J* = 6.7 Hz), 5.00 (1H, *d*, *J* = 7.8 Hz), 5.18 (1H, *d*, *J* = 7.6 Hz), 8.20 (1H, NH). Found: C, 56.70 H, 8.14 N, 1.27. $C_{49}H_{79}NO_{18} \cdot 3\frac{1}{2}H_2O$ requires: C, 56.96 H, 8.39 N, 1.36.

Hydrolysis of **6**, **7** *and* **8** *with* 2N HCl. Solns of **6**, **7** and **8** (5 mg each) in 2N HCl (2 ml) were allowed to stand at 100° for 4 hr, and the ppts formed were collected. The filtrate was evapd repeatedly at 40° until the soln indicated a neutral reaction. The residue was examined by HPTLC and GC. Compound **6** gave only glucosamine hydrochloride, **7** gave glucosamine hydrochloride and glucose, and **8** gave glucosamine hydrochloride, glucose and arabinose, respectively.

Hydrolysis of **2** *with* 1% KOH. To a soln of **2** (400 mg) in dioxane (50 ml) was added 1% KOH (50 ml), the mixture was stirred at 0° for 3 hr under N_2 gas. The reaction mixture was acidified with 10% HCl and extracted with $CHCl_3$. The $CHCl_3$ soln was washed with H_2O and evapd to dryness. The residue was chromatographed over silica gel using *n*-hexane–Me_2CO (4:1) as the solvent to afford two oily substances **12** and **13**.

Compound **12**. $[\alpha]_D^{25}$ −24.6° ($CHCl_3$; *c* 0.65). ^{1}H NMR ($CDCl_3$): δ1.32 (3H, *s*, Me), 1.82 (3H, *s*, Me), 1.57–1.73 (2H, *m*, CH_2), 2.15–2.32 (2H, *m*, CH_2), 5.10 (1H, *dd*, *J* = 11.0 and 0.9 Hz), 5.24 (1H, *dd*, *J* = 17.4 and 0.9 Hz), 5.91 (1H, *dd*, *J* = 17.4 and 11.0 Hz), 6.89 (1H, *t*, *J* = 7.3 Hz). MS (*m/z*): 166 [M − 18]$^+$, 151 [M − 18 − 15]$^+$.

Compound **13**. MS (*m/z*): 184 [M]$^+$, 169 [M − 15]$^+$. High MS: mol. wt. Calc. for $C_{10}H_{16}O_3$: 184.1099, obs. 184.1114. After neutralization, the aq. soln was lyophylized. The residue was chromatographed on a column of Sephadex LH-20 with MeOH–H_2O (9:1) to afford a powder, which was further purified by HPLC on an ODS column (30 cm × 20 mm I. D.) using MeOH–H_2O (7:3) to give desacyl-ES-III (**14**) (260 mg): mp 230–233° (dec.), $[\alpha]_D^{25}$ −7.5° (MeOH; *c* 0.67), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (COOR), 1640, 1550 (CONH). ^{1}H NMR: δ0.83, 0.92, 0.97, 0.98, 1.06, 1.17, 1.74 (3H, *s*, 7 × Me), 2.06 (3H, *s*, MeCONH), 8.27 (1H, NH). Found: C, 50.35 H, 7.65 N, 0.76. $C_{76}H_{123}NO_{40} \cdot 7H_2O$ requires: C, 50.24 H, 7.60 N, 0.77%.

Hydrolysis of **14** *with* 2N H_2SO_4. A soln of **14** (20 mg) in 2N H_2SO_4 (5 ml)–EtOH (5 ml) was refluxed for 3 hr. The soln was concd to 5 ml, and the ppts were collected. The aq. layer was neutralized and evaporated *in vacuo*. The residue contained glucose, arabinose, xylose and apiose (detected by HP-TLC and GC).

Hydrolysis of **2** *with* 0.025% K_2CO_3. To a soln of **2** (500 mg) in EtOH (50 ml) was added 0.025% K_2CO_3 (50 ml), and the mixture was stirred at room temp. for 10 hr under N_2. The reaction mixture was lyophylized, and the residue was chromatographed on a column of Sephadex LH-20 with MeOH–H_2O (9:1) to afford the mixture, which was further purified by HPLC on an ODS column (30 cm × 20 mm I. D.) using MeOH–H_2O (7:3) to give desacetyl-ES-III (**15**) (50 mg), together with desacyl-ES-III (**14**) and unchanged ES-III (**2**). Compound **15**: Mp. 214–217° (dec.), $[\alpha]_D^{25}$ −24.7° (MeOH; *c* 0.49). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (COOR), 1640, 1550 (CONH). ^{1}H NMR: δ0.83, 0.92, 0.98, 1.06, 1.17, 1.74 (3H, *s*, 7 × Me), 1.43 (3H, *s*, C-6-Me of **12**), 1.97 (3H, *s*, C-2-Me of **12**), 2.06 (3H, *s*, MeCONH), 8.23 (1H, NH). FAB-MS (*m/z*) (exact mass negative-ion mode): 1855 [M − 1]$^-$.

Permethylation of desacyl-ES-III (**14**). On permethylation by the Hakomori method **14** gave a syrup which was chromatographed over silica gel using *n*-hexane–Me_2CO (2:1) to afford the compound **16** (62 mg) as a powder from *n*-hexane: Mp 127–129°, $[\alpha]_{25}^{D}$ −33.3° ($CHCl_3$; *c* 2.2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil), 1745 (COOR), 1650 (CON). Found: C, 58.53 H, 8.49 N, 0.69. $C_{99}H_{169}NO_{40} \cdot H_2O$ requires: C, 58.35 H, 8.53 N, 0.66%.

Reductive cleavage of **16** *with* $LiAlH_4$. A soln of compound **16** (300 mg) in dried THF was refluxed with $LiAlH_4$ (120 mg) for 4 hr. The excess $LiAlH_4$ was decomposed with EtOAc, and the reaction mixture was poured into a large amount of H_2O. The aq. soln was extracted with Et_2O and then with $CHCl_3$. The Et_2O soln was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by HPLC on a silica gel column (8 mm × 30 cm) using $CHCl_3$–MeOH (50:1) to give the compound **18** as a powder from *n*-hexane: mp 103–105°, $[\alpha]_D^{25}$ −29.9° ($CHCl_3$; *c* 1.34), ^{1}H NMR ($CDCl_3$): 4.32 (1H, *d*, *J* = 7.6 Hz), 4.39 (2H, *d*, *J* = 7.3 Hz), 4.67 (1H, *d*, *J* = 3.1 Hz). The $CHCl_3$ soln was washed with H_2O, dried over Na_2SO_4 and conc *in vacuo*. The residue was purified by HPLC on a silica gel column (8 mm × 30 cm) using $CHCl_3$–MeOH (50:1) to give a syrup (**17**) (92 mg): $[\alpha]_D^{25}$ −45.8° ($CHCl_3$; *c* 0.2), ^{1}H NMR (100 MHz) ($CDCl_3$): 3.35–3.70 (33 H, OMe × 11), 4.30 (1H, *d*, *J* = 8.0 Hz), 4.48 (1H, *d*, *J* = 8.0 Hz), 5.44 (1H, *d*, *J* = 2.0 Hz).

Methanolysis of **17** *with* 5% HCl–MeOH. A soln of **17** (5 mg) in methanolic 5% HCl (1 ml) was refluxed for 2 hr in a sealed tube. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated *in vacuo*. The *O*-methylated sugar was

identified with an authentic sample.

GC/MS analysis of **17**. To a soln of **17** (10 mg) in dioxane (2 ml) was added 2 N H_2SO_4 (2 ml), and the mixture was refluxed for 4 hr. The soln was neutralized, and the filtrate was evapd. To a solution of the residue in H_2O (5 ml) was added $NaBH_4$ (10 mg) and the mixture allowed to stand overnight. The soln was neutralized with Amberlite IR 120B, and was repeatedly evaporated with MeOH to dryness. After acetylation with Ac_2O–pyridine, the mixture of partially methylated alditol acetates was dissolved in $CHCl_3$, and was injected analysed by GC/MS. GC column: 3% ECNSS-M Gaschrom Q, 100–120 mesh, 2 m × 2 mm, N_2 gas at 0.7 kg/cm², column temp. 170°, MS: inlet temp. 200°, ionizing potential 70 eV, ionizing current 300 μamp., temp. of the ion source: 230°.

Partial methanolysis of **17** *with methanolic* 0.2% HCl. Compound **17** (80 mg) was stirred with methanolic 0.2% HCl (6 ml) for 24 hr, then the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was separated by HPLC on a silica gel column (8 mm × 30 cm) using $CHCl_3$–MeOH (50:1) to give methyl 2,3,4-tri-*O*-methyl-apiofuranoside and compound **19** (48 mg): $[\alpha]_D^{25}$ −18.7° ($CHCl_3$; *c* 0.11), ^{1}H NMR ($CDCl_3$): 3.38–3.63 (24 H, OMe × 8), 4.27 (1H, *d*, *J* = 7.9 Hz), 4.52 (1H, *d*, *J* = 7.3 Hz).

Partial methanolysis of **17** *with methanolic* 0.5% HCl. Compound **17** (70 mg) was refluxed with methanolic 0.5% HCl (6 ml) for 12 hr at 50°, then the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was separated by HPLC on a silica gel column (8 mm × 30 cm) using $CHCl_3$–MeOH (50:1) to give methyl 2,3,5-tri-*O*-methyl-apiofuranoside, 2,4-di-*O*-methyl-xylopyranoside and compound **20** (41 mg): $[\alpha]_D^{25}$ −5.6° ($CHCl_3$; *c* 0.18), ^{1}H NMR ($CDCl_3$): 3.39–3.62 (18 H, OMe × 6), 4.28 (1H, *d*, *J* = 7.6 Hz).

Methylation of **20**. Compound **20** (18 mg) was methylated by the Hakomori method and the product was chromatographed over silica gel using *n*-hexane–Me_2CO (4:1) to afford compound **21** (12 mg): $[\alpha]_D^{25}$ −6.6° ($CHCl_3$; *c* 0.3), ^{1}H NMR ($CDCl_3$): δ3.35, 3.38 × 2, 3.42, 3.48, 3.52, 3.558, 3.564, 3.62 (27 H, *s*, OMe × 9), 4.39 (1H, *d*, *J* = 7.9 Hz).

Synthesis of **22** *from* D-(+)-*cellobiose*. An aq. soln (30 ml) of D-(+)-cellobiose (1.0 g) was allowed to stand for 12 hr with $NaBH_4$ (300 mg). The soln was neutralized with Amberlite IR 120B, and evaporated repeatedly with MeOH to dryness. The residue was methylated by the Hakomori method. The product was chromatographed over silica gel using *n*-hexane–Me_2CO (4:1) to afford the compound **22** (618 mg): $[\alpha]_D^{25}$ −6.3° ($CHCl_3$; *c* 0.8), ^{1}H NMR ($CDCl_3$): δ3.35, 3.38 × 2, 3.42, 3.48, 3.52, 3.560, 3.564, 3.62 (27 H, *s*, OMe × 9), 4.39 (1H, *d*, *J* = 7.9 Hz).

Methylation of **2** *by the Kuhn method*. Treatment of **2** by the Kuhn method gave a syrup. The residue was purified by CC on a silica gel column using *n*-hexane–Me_2CO (2:1) to afford compound **23** (81 mg) as a powder from *n*-hexane, mp 124–127°, $[\alpha]_D^{25}$ −36.2° ($CHCl_3$; *c* 0.38).

GC/MS analysis of **23**. A soln of **23** (70 mg) in dried THF (30 ml) was refluxed with $LiAlH_4$ (30 mg) for 3 hr. The excess $LiAlH_4$ was decomposed with EtOAc, and the reaction mixture was poured into a large amount of water. The aq. soln was extracted with Et_2O and then with $CHCl_3$. The organic layers were washed with H_2O, dried over Na_2SO_4, evaporated to dryness *in vacuo*, and the product chromatographed over silica gel using *n*-hexane–Me_2CO (3:2). Each residue was subjected to GC/MS analysis for partially methylated alditol acetates.

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