Studies on the Constituents of *Calliandra anomala* (KUNTH) MACBR. I. Structure Elucidation of Two Acylated Triterpenoidal Saponins

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Two novel triterpenoidal saponins, called calliandra saponins A and E, were isolated from the branches of *Calliandra anomala* (KUNTH) MACBR. On the basis of the chemical and physicochemical evidence, their structures were defined as 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester (4) and 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(6′*S*)-2′-*trans*-2′,6′-dimethyl-6′-*O*-(2-*O*-(6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-xylopyranosyl-2′,7′-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester (5), respectively.

Keywords *Calliandra anomala*; Leguminosae; calliandra saponin; triterpene; bisdesmoside; monoterpenic acid

Aqueous extracts of the branches of *Calliandra anomala* (KUNTH) MACBR. (Leguminosae) are used as an antimararial and antifebrile in Mexico.[1] There is no report on the constituents making up this plant. This paper deals with the isolation and structure elucidation of saponins. The plant was extracted with chloroform, acetone, methanol and water, successively. The ether precipitate of methanol extract was separated by droplet counter-current chromatography (DCCC),[2] followed by Lobar RP-18 chromatography and repeated semi-preparative high performance liquid chromatography (HPLC) on an Asahipak ODP-50 reversed phase column. We isolated all six saponins and called them calliandra saponins (A—F). (Fig. 1).

Calliandra saponin E (**5**) revealed an $[M+Na]^+$ ion peak at *m/z* 2162 in the fast atom bombardment mass spectrum (FAB-MS) and elemental analysis data was consistent with $C_{101}H_{159}NO_{47}\cdot 6H_2O$. On acid hydrolysis with 2 N sulfuric acid, saponin E (**5**) gave echinocystic acid (**1**), which was identified with an authentic sample,[3] L-arabinose, D-glucose, L-rhamnose, D-xylose, and *N*-acetyl-D-glucosamine as the component sugars,[4] and monoterpene carboxylic acid. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum showed the signals of eleven tertiary methyl groups (δ 1.80, 1.75, 1.60, 1.33 (2×CH_3), 1.03, 0.96, 0.89, 0.82 (2×CH_3), 0.77), three trisubstituted olefinic protons (δ 6.93, 6.75, 5.43), and nine anomeric protons δ 5.79 (d, J=7.3 Hz), 5.62 (br s), 5.19 (d, J=7.4 Hz), 5.02 (d, J= 8.0 Hz), 4.91 (d, J=7.7 Hz), 4.84 (d, J=5.3 Hz), 4.83 (d, J=4.9 Hz), 4.73 (d, J=7.3 Hz), 4.66 (d, J= 7.3 Hz). The ^{13}C-NMR spectrum of **5** showed nine anomeric carbons (δ 105.08, 105.02, 104.29, 103.89, 103.76, 101.80, 101.47, 97.36, 94.22), five pairs of olefinic carbons (δ 143.81, 122.34, 142.90, 127.52, 143.47, 127.52, 141.95, 115.53, 145.41, 111.76), four carbonyl carbons (δ 175.77, 171.93, 168.01, 167.28), and one sugar-substituted methine carbon (δ 89.25) (Table I). When the ^{13}C-NMR data of **5** was compared with that of echinocystic acid, glycosylation shifts[5] were observed at C-2 (−2.23 ppm) and C-3 (+9.96 ppm). The chemical shift of the highest δ value of anomeric carbon (δ 94.22) signal showed that the sugar residue must be attached to the aglycone by an ester bond. Three reasons confirmed that the binding site of each sugar was at the C-28 of the aglycone. First, the anomeric proton signal [δ 5.79 (d, J=7.3 Hz)] shifted relatively downfield. Second, the anomeric carbon signal (δ 94.22) appeared at a higher field than usual. And third, carbon signal due to the C-28 of **5** (δ 175.77) was displaced upfield, compared with the C-28 signal of sapogenin **1** (δ 179.93). These three shifts were characteristic of ester-type-glycoside linkage. This characteristic data suggested that **5** should be a 3,28-*O*-bisdesmoside of echinocystic acid having two arabinose, two glucose, one rhamnose, three xylose, and one *N*-acetyl glucosamine and two monoterpenic acids. The proton signals of **5** were assigned by a detailed proton spin decoupling experiment. Based on

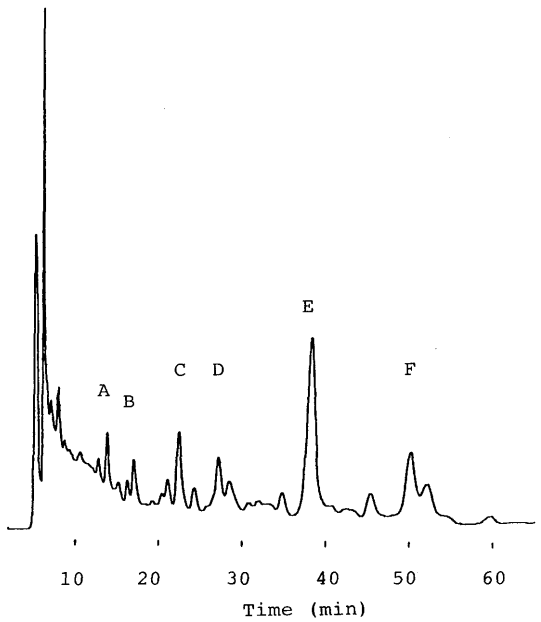


Fig. 1. Chromatogram of Calliandra Saponin A—F

HPLC conditions: column, Asahipak ODP-50 (4.6 i.d.×250 mm); flow rate, 0.5 ml/min; detection, 221 nm; eluent, CH_3CN 34%.

TABLE I. ^{13}C-NMR Spectral Data of Compounds **2**—**5** in $C_5D_5N:D_2O$ (9:1)

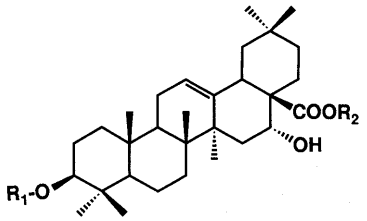
	2	3	4	5
Aglycone				
1	38.62	38.65	38.70	38.54
2	26.48	26.18	26.24	25.99
3	88.94	89.27	89.21	89.25
4	39.21	38.97	39.01	38.79
5	55.83	55.78	55.80	55.61
6	18.50	18.33	18.32	18.23
7	33.43	33.32	33.34	33.13
8	39.82	39.92	39.93	39.73
9	47.12	46.98	46.99	46.81
10	36.98	36.84	36.84	36.65
11	23.75	23.62	23.65	23.48
12	122.29	122.37	122.51	122.34
13	145.00	144.06	144.03	143.81
14	41.98	41.85	41.85	41.66
15	36.04	35.85	35.76	35.49
16	74.67	73.90	73.93	73.69
17	48.81	49.15	49.17	49.01
18	41.31	41.25	41.19	41.03
19	47.18	47.08	46.99	46.81
20	30.96	30.40	30.48	30.28
21	36.14	35.63	35.66	35.49
22	32.68	31.27	31.40	31.08
23	28.09	27.92	27.95	27.75
24	16.97	16.76	16.80	16.61
25	15.50	15.44	15.47	15.28
26	17.41	17.23	17.32	17.15
27	27.22	26.95	26.94	26.76
28	180.13	175.94	175.82	175.77
29	33.34	32.85	32.89	32.70
30	24.78	24.47	24.61	24.51
C-3 sugar				
GlcNAc				
1	104.73	104.65	104.15	103.89
2	57.69	57.36	57.41	56.99
3	75.52	75.02	75.02	74.62
4	72.78	72.10	72.16	71.55
5	77.66	75.59	75.58	75.35
6	69.35	69.04	69.10	68.75
$NHCOCH_3$	170.49	171.38	171.32	171.93
$NHCOCH_3$	23.62	23.16	23.19	22.96
Ara (inner)				
1	102.20	101.97	102.00	101.80
2	80.35	79.74	79.81	79.51
3	72.44	71.95	72.01	71.75
4	67.36	67.04	67.08	66.94
5	64.18	63.89	63.96	63.83
Ara (outer)				
1	106.12	105.45	105.51	105.08
2	75.25	74.75	74.71	74.50
3	75.98	77.11	77.13	76.74
4	70.72	70.32	70.35	70.03
5	67.07	66.64	66.66	66.32

	3	4	5
C-28 sugar			
Glc (inner)			
1	94.53	94.53	94.22
2	77.72	77.97	78.05
3	77.26	77.19	76.94
4	70.91	70.98	70.72
5	78.27	75.40	75.07
6	61.82	64.19	64.05
Rham			
1	101.51	101.70	101.47
2	70.03	70.15	70.00
3	82.19	82.17	81.76
4	77.95	77.97	77.91
5	68.72	68.76	68.55
6	18.53	18.53	18.33
Glc (outer)			
1	104.65	104.67	104.29
2	74.90	74.92	74.54
3	77.61	77.63	77.22
4	71.09	71.12	70.69
5	77.59	77.63	77.22
6	62.17	62.21	61.79
Xyl (inner)			
1	104.01	104.04	103.76
2	74.11	74.13	73.82
3	87.51	87.56	87.19
4	69.09	69.10	68.92
5	65.94	65.95	65.62
Xyl (outer)			
1	105.31	105.35	105.02
2	74.68	74.77	74.43
3	77.03	77.07	76.66
4	70.22	70.24	69.94
5	66.50	66.52	66.19
Monoterpene glycoside			
1		168.03	168.01
2		127.59	127.52
3		143.15	142.90
4		23.46	23.58
5		40.15	40.54
6		79.47	79.44
7		143.45	141.95
8		114.90	115.53
9		12.19	12.04
10		23.46	23.63
Xyl			
1		99.64	97.36
2		74.53	74.69
3		77.63	75.35
4		70.59	70.48
5		66.23	66.07
Monoterpene acid			
1			167.28
2			127.52
3			143.47
4			23.02
5			40.93
6			72.12
7			145.41
8			111.76
9			12.16
10			27.54

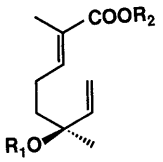
these assignments, the carbon signals were identified by carbon-13–proton correlation spectroscopy (^{13}C–^{1}H COSY). On alkaline hydrolysis of **5** with 1 N KOH, a prosapogenin (**2**), monoterpene glycoside (**6**), and monoterpene carboxylic acid (**7**) were obtained as major products. On the other hand, mild alkaline hydrolysis of **5** with 5% K_2CO_3 in ethanol afforded a deacylated compound **3**, and partially deacylated compounds **4**, **6** and **7**, respectively. Compound **7** was identified with an authentic sample of (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octa-

dienoic acid.[6] Compound **6** was hydrolyzed with 2 N H_2SO_4 to afford monoterpene carboxylic acid (**7**) and xylose. This compound showed the presence of an anomeric proton at δ 4.86 (d, $J=7.7$ Hz) and was permethylated by Hakomori's method[7] to afford the per-*O*-methyl derivative (**6a**). In conclusion, compound **6** is identical with (6*S*)-2-*trans*-6-*O*-β-D-xylopyranosyl-2,6-dimethyl-2,7-octadienoic acid. Compound **2** revealed an $[M+Na]^+$ ion peak at *m/z* 962 in FAB-MS and elemental analysis data was consistent with $C_{48}H_{77}NO_{17}\cdot 2H_2O$. On acid hydrolysis with 2 N sulfuric acid, **2** yielded echinocystic acid as well as L-arabinose, and *N*-acetyl-D-glucosamine in a ratio of 2 : 1. In the ^{1}H-NMR spectrum of **2**, three anomeric signals were observed at δ 4.98 (d, $J=7.0$ Hz), 5.03 (d, $J=8.3$ Hz) and 5.12 (d, $J=5.1$ Hz), respectively. When the ^{13}C-NMR data of **2** was compared with that of echinocystic acid, glycosylation shifts were observed at C-2 (-1.73 ppm), C-3 ($+10.65$ ppm).

Compound **2** was identified as 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid, because the NMR data (in pyridine-d_5 : $D_2O=9:1$) were consistent with the reference data reported by Carpani *et al.*[8] They had already isolated from *Albizzia anthelmintica* (Brongn.), which is a bush, commonly occurring from Abyssinia and Somaliland to South West Africa. The partially deacylated prosapogenin, compound **4**, was obtained as an amorphous powder and its FAB-MS gave an $[M+Na]^+$ in a peak at *m/z* 1996. Acid hydrolysis of **4** gave glucose, rhamnose, xylose, arabinose, and *N*-acetyl glucosamine. The assignment of a carbohydrate moiety in **4** was achieved by analysis of a detailed heteronuclear multiple-bond correlation (HMBC),[9] homonuclear Hartmann–Hahn spectroscopy (HOHAHA), and CH-HOHAHA experiment. The HMBC experiment showed a correlation between H-1 (5.93 ppm) of inner glucose and C-28 of the aglycone (175.82 ppm), confirming that a 2,6-disubstituted glucose was attached to C-28 of the aglycone. A cross peak between C-3 (89.21 ppm) of the aglycone and H-1 of *N*-acetyl glucosamine (4.95 ppm) was observed, demonstrating that this sugar is linked to C-3 position of the aglycone. We also observed correlations between 6-substituted *N*-acetyl glucosamine C-6 (69.10 ppm) and 2-substituted inner arabinose H-1 (4.97 ppm), between this arabinose C-2 (79.81 ppm) and terminal arabinose H-1 (4.85 ppm), between rhamnose H-1 (5.75 ppm) and 2,6-disubstituted inner glucose C-2 (77.97 ppm), between outer glucose H-1 (5.16 ppm) and 3,4-disubstituted rhamnose C-3 (82.17 ppm), between inner xylose H-1 (5.34 ppm) and 3,4-disubstituted rhamnose C-4 (77.97 ppm), between 3-substituted inner xylose C-3 (87.56 ppm) and terminal xylose H-1 (5.02 ppm), between xylose H-1 (4.72 ppm) of xylopyranosyl monoterpenic acid part (**6**) and monoterpene carboxylic acid C-6 (79.47 ppm) and between this monoterpene carboxylic acid C-1 (168.03 ppm) and inner glucose H-6 (4.80, 4.45 ppm). The anomeric configuration of six sugars in prosapogenin which belong to the C-28 ester sugars, xylose, glucose, rhamnose were determined to be β,β, and α, respectively,



	R_1	R_2
1 :	H	H
2 :	—GlcNAc^6 —^{1}Ara^2 —^{1}Ara	H
3 :	—GlcNAc^6 —^{1}Ara^2 —^{1}Ara	—Glc^2 —^{1}Rham$^3_4$ (^{1}Glc, ^{1}Xyl^3 — ^{1}Xyl)
4 :	—GlcNAc^6 —^{1}Ara^2 —^{1}Ara	—Glc$^2_6$ (—C(=O)—O-^{1}Xyl; ^{1}Rham$^3_4$ (^{1}Glc, ^{1}Xyl^3 — ^{1}Xyl))
5 :	—GlcNAc^6 —^{1}Ara^2 —^{1}Ara	—Glc$^2_6$ (—C(=O)—O-^{1}Xyl^2 —C(=O)—OH; ^{1}Rham$^3_4$ (^{1}Glc, ^{1}Xyl^3 — ^{1}Xyl))



	R_1	R_2
6 :	Xyl	H
6a :	Xyl(Me)$_3$	Me
7 :	H	H

Chart 1

from the J values of those anomeric proton signals. Acylation shift[10] was observed at C-6 of glucose attaching to C-28 of the aglycone. Therefore, the structure of **4** was characterized as shown in Chart 1. The peaks at m/z 1996 $[M+Na]^+$, 1055 (M − prosapogenin **2** + Na]$^+$, 962 [prosapogenin **2** + Na]$^+$ and 467 [M − prosapogenin **2** − Rham − 2Xyl − Glc + Na]$^+$ in the positive FAB-MS spectrum of **4** were also in good agreement with the above deduced structure. The deacylated prosapogenin **3** was obtained as an amorphous powder. Compound **3** revealed an $[M+Na]^+$ ion peak at m/z 1696 in FAB-MS and elemental analysis data was consistent with $C_{76}H_{123}NO_{39}\cdot H_2O$. The ^{1}H- and ^{13}C-NMR spectra (C–H COSY, H–H COSY, HMBC, HOHAHA) of **3** resembled those of compound **4**. On hydrolysis with 2 N sulfuric acid, **3** yielded echinocystic acid and monosaccharides, arabinose, glucose, rhamnose, xylose, and *N*-acetyl glucosamine. It was evident, therefore, that **3** is missing one monoterpene carboxylic acid and one xylose unit with respect to compound **4**. The structure of prosapogenin **3** was established as 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl} ester as shown in Chart 1.

Comparing the ^{1}H-NMR spectra of **4** and **5**, the H-2 (δ 5.29 ppm) of xylopyranose in **5** appeared as a field so much lower than **4** that we thought the monoterpene carboxylic acid group (C-1 167.28 ppm) was attached to the H-2 position of xyl. The ^{1}H- and ^{13}C-NMR spectra (C–H COSY, H–H COSY, HMBC, HOHAHA) also led us to determine the structure of **5**. Therefore, the structure of calliandra saponin E (**5**) was characterized as 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2- acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(6′*S*)-2′-*trans*-2′,6′-dimethyl-6′-*O*-(2-*O*-(6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-xylopyranosyl-2′,7′-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester as shown in Chart 2. The peaks at m/z 2162 $[M+Na]^+$, 1221 [M − prosapogenin **2** + Na]$^+$, 962 [prosapogenin **2** + Na]$^+$ and 633 [M − prosapogenin **2** − Rham − 2Xyl − Glc + Na]$^+$ in Chart 2 in the positive FAB-MS spectrum of **5** were also in good agreement with the above deduced structure.

Calliandra saponin A (**4**) was obtained as an amorphous powder. Elemental analysis data was consistent with $C_{91}H_{145}NO_{45}\cdot 6H_2O$. On acid hydrolysis, it gave echinocystic acid, arabinose, glucose, rhamnose, xylose, *N*-acetyl glucosamine and monoterpene carboxylic acid. The NMR spectra indicated that calliandra saponin A is also an echinocystic acid 3,28-*O*-bisdesmoside. Calliandra saponin A (**4**) showed NMR signals similar to those of **5** except that the signals of one monoterpene carboxylic acid are missing. Calliandra saponin A (**4**) showed the same behavior on TLC and the same ^{1}H- and ^{13}C-NMR spectra of a partially deacylated compound **4**. Plant origin bisdesmoside saponin containing both *N*-acetyl-D-glucosamine and monoterpene carboxylic acid was very unique.[11]

Experimental

General Procedures Melting points were determined with a Yanagimoto microapparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were reduced on a JEOL GSX-400 and/or JNM A500 FT-NMR, and chemical shifts were given in ppm with tetramethylsilane as an internal standard.

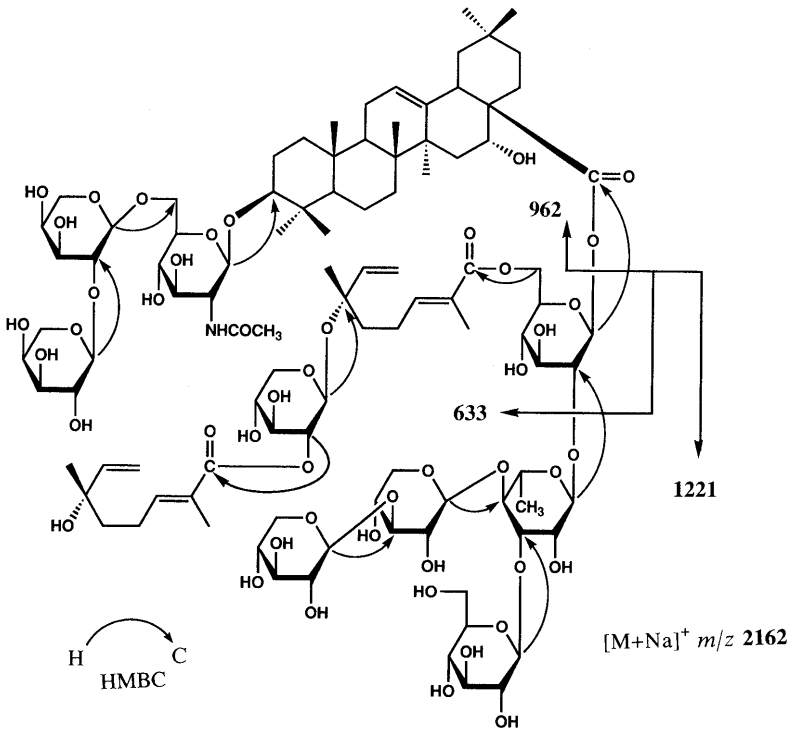


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Chart 2

FAB-MS was recorded on a JEOL JMS-HX 110 mass spectrometer. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Gas chromatography (GLC) was run on a Shimadzu GC-6A Gas chromatograph. The Lobar column used was LiChroprep RP-18 (Merck). Semi-preparative HPLC was carried out on a column of Asahipak ODP-50 (10 mm × 250 mm). TLC was conducted on precoated silica gel plates (Merck 60F-254). Column chromatography was carried out on silica gel (Merck Kiesel gel 60).

Materials Branches of *Calliandra anomala* were collected in Morelos, Mexico in 1987 and voucher specimens were deposited in the Jardin de Etno-botanico, Instituto Nacional de Antropologia e Historia. The material was identified as *C. anomala* (KUNTH) MACBR. by Dr. Guillermo Suarez Ortega (Botanical Garden Director at Jardin de Etnobotanica, Morelos, Mexico).

Extraction and Isolation The cut branches of *Calliandra anomala* (2.03 kg) were extracted successively with chloroform, acetone, methanol and water (5.5 l × 2, 6 h in each) under reflux. The methanol extract was concentrated under reduced pressure and the residue (80.3 g) was suspended in water. The suspension was extracted with *n*-butanol and then the *n*-butanol soluble fraction was concentrated *in vacuo* to give a residue (33.4 g). This residue was dissolved in methanol (20 ml) and ether (1 l) was added to the methanol solution to give a precipitate (10.7 g). The precipitate (5.0 g) was submitted to DCCC using chloroform–methanol–water (35 : 65 : 40) as ascending solvent to give four fractions (frs. 1—4). Fraction 1 (1.6 g) was further chromatographed on Lobar RP-18 with 35% acetonitrile solution to give four fractions, frs. A—D. Repeated semi-preparative HPLC of fractions A—D separately on an Asahipak ODP-50 column (10 mm × 250 mm) yielded saponin A (**4**) (322.7 mg), B (362.1 mg), C (200.8 mg), D (257.0 mg), respectively. Fraction 2 (1.2 g) was chromatographed on Lobar RP-18 to give a compound E rich fraction, which was rechromatographed with HPLC on Asahipak ODP-50 column (10 mm × 250 mm) and gave compound **5** (803.0 mg). Compound F (173.2 mg) was obtained from fraction 3.

Calliandra Saponin E (5) Amorphous powder, mp 193—197 °C (dec.), $[\alpha]_D^{20}$ +4.4° (*c* = 0.3, MeOH). ^{1}H-NMR (pyridine-d_5 : D_2O = 9 : 1) δ: 0.77, 0.82 (2 × CH_3), 0.89, 0.96, 1.03, 1.33 (2 × CH_3), 1.60, 1.75, 1.80 (tertiary methyl groups), 1.09 (Rham-CH_3), 5.43, 6.75, 6.93 (trisubstituted olefinic protons), 4.66 (d, *J* = 7.3 Hz), 4.73 (d, *J* = 7.3 Hz), 4.83 (d, *J* = 4.9 Hz), 4.84 (d, *J* = 5.3 Hz), 4.91 (d, *J* = 7.7 Hz), 5.02 (d, *J* = 8.0 Hz), 5.19 (d, *J* = 7.4 Hz), 5.62 (br s), 5.79 (d, *J* = 7.3 Hz) (anomeric protons). ^{13}C-NMR (pyridine-d_5 : D_2O = 9 : 1): Table I. FAB-MS *m/z*: 2162.1 $[M+Na]^+$, *Anal.* Calcd for $C_{101}H_{159}NO_{47}\cdot 6H_2O$: C, 53.98; H, 7.67; N, 0.62. Found: C, 53.95; H, 7.30; N, 0.45.

Calliandra Saponin A (4) Amorphous powder, mp 204—210 °C (dec.), $[\alpha]_D^{20}$ −22.1° (*c* = 0.7, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O = 9 : 1) δ: 4.72 (d, *J* = 7.7 Hz), 4.85 (d, *J* = 7.5 Hz), 4.95 (d, *J* = 7.5 Hz), 4.97 (d, *J* = 4.9 Hz), 5.02 (d, *J* = 7.7 Hz), 5.16 (d, *J* = 7.0 Hz), 5.34 (d, *J* = 7.7 Hz), 5.75 (br s), 5.93 (d, *J* = 7.7 Hz) (anomeric protons). ^{13}C-NMR (pyridine-d_5 : D_2O = 9 : 1): Table I. FAB-MS *m/z*: 1995.7 $[M+Na]^+$, *Anal.* Calcd for $C_{91}H_{145}NO_{45}\cdot 6H_2O$; C, 52.52; H, 7.60; N, 0.67. Found: C, 52.30; H, 7.48; N, 0.39.

Acid Hydrolysis of Compounds 2—5 a) Determination of Sugar Species: Compound **5** (20 mg) was heated at 100 °C with 2 ml of 2 N-H_2SO_4 for 6 h. The reaction mixture was diluted with H_2O and extracted with diethyl ether. The organic layer was concentrated *in vacuo*. The residue was recrystallized from methanol to give echinocystic acid. The aqueous solution was passed through an Amberlite IRA-410 column. The eluate was concentrated to give a residue, which was reduced with $NaBH_4$ (4 mg) in water (0.5 ml) for 6 h at room temperature and passed through an Amberlite IRA-120 column. The eluate was concentrated to dryness under reduced pressure and then the reaction mixture was acetylated with 0.2 ml of acetic anhydride and pyridine for 1 h. The acetylated mixture was subjected to GLC, which revealed 5 peaks for the derivatives of arabinose, xylose, glucose, rhamnose and *N*-acetyl glucosamine 2 : 3 : 2 : 1 : 1, respectively. Acid hydrolysis of the other compounds (**2**—**4**) was performed by the same method used for compound **5**.

Compound **4**: Arabinose, xylose, glucose, rhamnose, *N*-acetyl glucosamine = 2 : 3 : 2 : 1 : 1

Compound **3**: 2 : 2 : 2 : 1 : 1

Compound **2**: Arabinose, *N*-acetyl glucosamine = 2 : 1

GLC Conditions: Column, 3% ECNSS-M (0.3 mm × 2 m); column temperature 190 °C; injection temperature 210 °C; retention times (min); rhamnose 8.6, arabinose 14.4, *N*-acetyl glucosamine 19.2, xylose 19.3, glucose 49.2.

b) Determination of Their Absolute Configurations: Determination of the absolute configuration was performed according to the method reported by Hara *et al.*[4] Compound **5** (2 mg) was hydrolyzed in 0.2 ml of 10% H_2SO_4–1,4-dioxane mixture (1 : 1) at 100 °C for 2 h. After usual work-up, the residue was dissolved in pyridine (0.2 ml). After addition of pyridine solution (0.4 ml) of L-cysteine methyl ester hydrochloride (0.06 mol/l), the mixture was warmed at 60 °C for 1 h. The residue was trimethylsilylated and checked by GLC.

GLC Conditions: Column, OV-17 (0.3 mm × 50 m); column temperature 200 °C; injection temperature 270 °C; retention times (min); D-xylose 11.08, L-arabinose 10.92, L-rhamnose 12.19, D-glucose 17.92.

Alkaline Hydrolysis of Saponin E (5) Compound **5** (100 mg) was hydrolyzed with 1 N KOH (2.5 ml) at room temperature for 24 h. The reaction mixture was acidified with dil. HCl and extracted with BuOH. The BuOH extract was evaporated to dryness. The residue was chromatographed on silica gel and afforded prosapogenin (**2**) (38 mg), xylosyl monoterpenic acid (**6**) (10 mg) and monoterpenic acid (**7**) (7 mg).

Mild Alkaline Hydrolysis of Saponin E (5) A solution of compound **5** (150 mg) and 5% K_2CO_3 (15 ml) in EtOH (15 ml) was refluxed for 1 h. The reaction mixture was neutralized with Dowex 50 WX8 and concentrated to half the initial volume. The BuOH extract of the concentrated solution was evaporated to dryness, and the residue was purified on a silica gel column to yield four major compounds, compound **3** (33 mg), **4** (55 mg), **6** (18 mg) and **7** (5 mg).

(6*S*)-2-*trans*-2,6-Dimethyl-6-hydroxy-2,7-octadienoic Acid (7) $[\alpha]_D^{20}$ +15.2° (*c* = 0.5, $CHCl_3$), TLC *Rf*: 0.84 ($CHCl_3$: MeOH : H_2O = 8 : 3 : 1), ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, C_6-CH_3), 1.60—1.73 (2H, m, H-5), 1.83 (3H, s, C_2-CH_3), 2.19—2.31 (2H, m, H-4), 5.02 (1H, dd, *J* = 10.4, 1.3 Hz, H-8), 5.24 (1H, dd, *J* = 17.4, 1.3 Hz, H-8), 5.91 (1H, dd, *J* = 17.4, 10.4 Hz, H-7), 6.90 (1H, td, *J* = 6.3, 1.3 Hz, H-3). ^{13}C-NMR (pyridine-d_5) δ: 170.6 (C-1), 128.9 (C-2), 142.6 (C-3), 24.2 (C-4), 41.8 (C-5), 72.2 (C-6), 146.7 (C-7), 111.6 (C-8), 12.8 (C-9), 28.5 (C-10).

(6*S*)-2-*trans*-6-*O*-β-D-Xylopyranosyl-2,6-dimethyl-2,7-octadienoic Acid (6) ^{1}H-NMR (pyridine-d_5) δ: 1.55 (3H, s, C_6-CH_3), 1.79—1.83 (2H, m, H-5), 2.02 (3H, s, C_2-CH_3), 2.45—2.51 (2H, m, H-4), 3.65 (1H, dd, *J* = 11.0, 9.9 Hz, H-5′), 3.99 (1H, dd, *J* = 8.8, 7.7 Hz, H-2′), 4.13 (1H, dd, *J* = 8.8 Hz, H-3′), 4.22 (1H, dt, *J* = 9.9, 8.8, 4.8 Hz, H-4′), 4.28 (1H, dd, *J* = 11.0, 4.8 Hz, H-5′), 4.86 (1H, d, *J* = 7.7 Hz, H-1′), 5.23 (1H, dd, *J* = 11.0, 1.3 Hz, H-8), 5.42 (1H, dd, *J* = 17.8, 1.3 Hz, H-8), 6.24 (1H, dd, *J* = 17.8, 11.0 Hz, H-7), 7.15 (1H, dd, *J* = 7.5, 1.3 Hz, H-3). ^{13}C-NMR (pyridine-d_5) δ: monoterpenic acid unit: 170.7 (C-1), 129.1 (C-2), 144.2 (C-3), 23.8 (C-4), 40.6 (C-5), 79.7 (C-6), 142.1 (C-7), 115.0 (C-8), 12.8 (C-9), 23.8 (C-10); xylose unit; 100.3 (C-1), 75.2 (C-2), 78.6 (C-3), 71.1 (C-4), 66.9 (C-5).

Compound **6** (50 mg) was methylated by Hakomori's method.[7] Compound **6a** was obtained (41 mg) as a syrup. $[\alpha]_D^{20}$ −33.1° (*c* = 0.7, $CHCl_3$). ^{13}C-NMR (pyridine-d_5) δ: 168.3 (C-1), 127.8 (C-2), 143.2 (C-3), 23.7 (C-4), 40.6 (C-5), 79.9 (C-6), 142.9 (C-7), 115.3 (C-8), 12.5 (C-9), 23.7 (C-10); xylose unit 99.2 (C-1), 84.5 (C-2), 86.2 (C-3), 80.1 (C-4), 63.5 (C-5), 51.6 ($COOCH_3$) 58.4, 60.6, 60.7 (OCH_3).

3-*O*-α-L-Arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl Echinocystic Acid (2) Amorphous powder, mp 209—215 °C (dec.), $[\alpha]_D^{20}$ +8.8° (*c* = 0.3, MeOH). ^{1}H-NMR (pyridine-d_5 : D_2O = 9 : 1) δ: 0.87, 0.98, 1.02, 1.05, 1.18, 1.19, 1.86 (each s, CH_3), 2.14 (s, –$NHCOCH_3$), 4.98 (d, *J* = 7.0 Hz), 5.03 (d, *J* = 8.3 Hz), 5.12 (d, *J* = 5.1 Hz), 8.77 (d, *J* = 9.0 Hz, –$NHCOCH_3$). ^{13}C-NMR (pyridine-d_5 : D_2O = 9 : 1): Table I. FAB-MS *m/z*: 962 $[M+Na]^+$, 808 $[M+H-132]^+$, 676 $[M+H-2\times132]^+$, 454 $[M+H-2\times132-222]^+$ (matrix: glycerol 6 kV, xenone), *Anal.* Calcd for $C_{48}H_{77}NO_{17}\cdot 2H_2O$: C, 59.06; H, 8.36; N, 1.43. Found: C, 58.96; H, 8.34; N, 1.20.

3-*O*-α-L-Arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl Echinocystic Acid 28-*O*-{β-D-Glucopyranosyl-(1→3)-{β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl} Ester (3) Amorphous powder, mp 227—235 °C (dec.) $[\alpha]_D^{17}$ −14.7° (*c* = 0.4, pyridine). ^{1}H-NMR (pyridine-d_5 : D_2O = 9 : 1) δ: 4.94 (d, *J* = 7.3 Hz), 5.04 (d, *J* = 8.4 Hz), 5.11 (d, *J* = 5.0 Hz), 5.15 (d, *J* = 7.3 Hz), 5.33 (d, *J* = 7.0 Hz), 5.52 (d, *J* = 7.9 Hz), 5.86 (br s) 6.05 (d, *J* = 7.9 Hz). ^{13}C-NMR (pyridine-d_5 : D_2O = 9 : 1): Table I. FAB-MS *m/z*: 1696 $[M+Na]^+$, *Anal.* Calcd for $C_{76}H_{123}NO_{39}\cdot H_2O$: C, 53.92; H, 7.44; N, 0.67. Found: C, 53.62; H, 7.41; N, 0.68.

Acknowledgements The authors thank Miss S. Kato for recording the NMR spectra and Miss T. Naito for performing the microanalysis. They are grateful to Dr. K. Masuda of Meijo University for measurements of FAB-MS.

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