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

Takatoshi NAKAMURA, Tadahiro TAKEDA, and Yukio OGIHARA*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.
Received November 8, 1993; accepted December 28, 1993

Four new triterpenoidal saponins, called calliandra saponins B (8), C (9), D (10), and F (12), were isolated from the branches of *Calliandra anomala* (KUNTH) MACBR. The structures of these compounds were established on the basis of NMR spectra, FAB-MS, and the chemical evidence.

These saponins, interestingly, have a *N*-acetyl glucosamine at the 3 position of the genin, and one or two monoterpene glycosides at the position of the sugar chain.

Keywords *Calliandra anomala*; Leguminosae; calliandra saponin; triterpene; bisdesmoside; monoterpene carboxylic acid

In a preceding paper,[1] we reported the structures of two novel saponins, calliandra saponin A (**7**) and E (**11**), from the branches of *Calliandra anomala* (KUNTH) MACBR. (Leguminosae). Further studies have led to the isolation of four new saponins, whose structure is elucidated here: In this paper, we wish to report the structure elucidation of the four new saponins, calliandra saponin B (**8**), C (**9**), D (**10**) and F (**12**).

The ether precipitate of methanol extract was separated by droplet counter-current chromatography (DCCC), followed by Lobar RP-18 chromatography and repeated semi-preparative high performance liquid chromatography (HPLC). We isolated six main saponins (calliandra saponins A—F).

The molecular formula of calliandra saponin B (**8**) was determined as $C_{92}H_{147}NO_{45}\cdot 7H_2O$ by FAB-MS and elemental analysis data. On acid hydrolysis with 2 N sulfuric acid, saponin B (**8**) gave echinocystic acid (**1**),[2] monoterpene carboxylic acid (**2**),[3] L-arabinose, D-glucose, L-rhamnose, D-xylose, D-quinovose and *N*-acetyl-D-glucosamine as the component sugars.[4] Alkaline hydrolysis of **8** with 1 N KOH afforded a prosapogenin (**5**)[5] and monoterpene glycoside (**4**) as major products. On mild alkaline hydrolysis of **8** with 5% K_2CO_3 in ethanol, deacylated compounds **6**, **5** and **4** were obtained (Chart 1).

Compound **6** was identified 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-rhamnopyrnosyl-(1→2)-β-D-glucopyranosyl} ester, by comparing the ^{1}H-NMR and the ^{13}C-NMR spectra with those of the authentic sample.[1] Compound **4** was hydrolyzed with 2 N H_2SO_4 to afford monoterpene carboxylic acid (**2**) and quinovose. Compound **2** was identified with an authentic sample of (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid.[3] Finally, compound **4** was identified with (6*S*)-2-*trans*-2,6-dimethyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoic acid.

When the ^{1}H-NMR and the ^{13}C-NMR data of **8** were compared with those of calliandra saponin A (**7**),[1] they were similar except for the signals of the sugar moiety of the monoterpene glycoside. The assignment of a carbohydrate moiety in **8** was achieved by analysis of detailed H–H correlation spectroscopy (COSY), C–H COSY, homonuclear Hartmann-Hahn spectroscopy (HOHAHA) and heteronuclear multiple-bond correlation (HMBC) experiment. The HMBC experiments of **8** showed a correlation between H-6 (δ 4.71, 4.51 ppm) of glucose attaching to C-28 of the aglycone and C-1 (δ 168.05 ppm) of the monoterpene glycoside. Acylation shift[6] was observed at C-6 of inner glucose (Table I). Therefore, the structure of **8** 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*)-

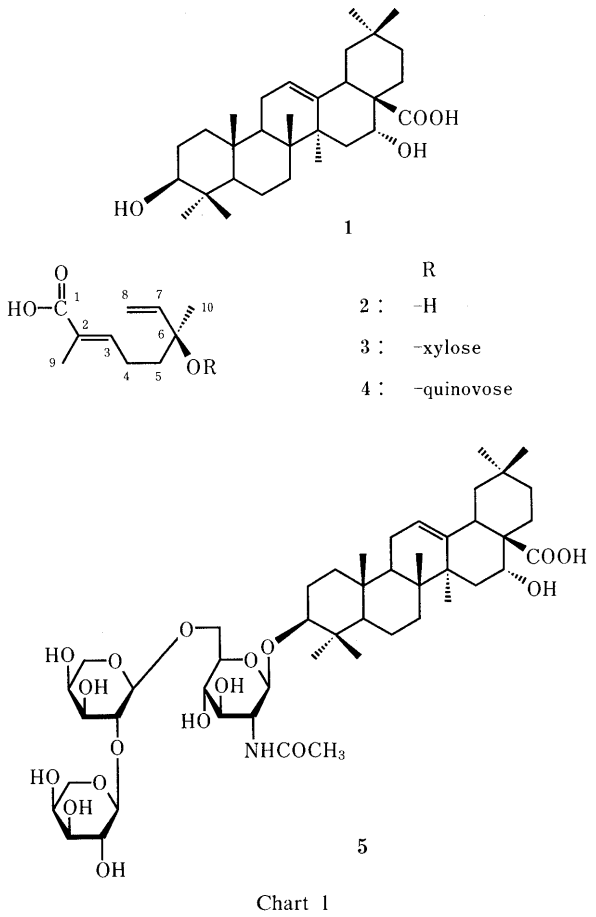

Chart 1

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

Carbon	7	8	9	10	11	12
Aglycone						
1	38.70	38.69	38.72	38.69	38.54	38.74
2	26.24	26.20	26.24	26.20	25.99	26.25
3	89.21	89.22	89.22	89.22	89.25	89.26
4	39.01	38.97	39.02	38.99	38.79	39.04
5	55.80	55.76	55.80	55.80	55.61	55.84
6	18.32	18.39	18.39	18.34	18.23	18.55
7	33.34	33.31	33.34	33.32	33.13	33.50
8	39.93	39.90	39.94	39.91	39.73	39.94
9	46.99	47.10	47.00	46.98	46.81	47.01
10	36.84	36.81	36.86	36.83	36.65	36.89
11	23.65	23.65	23.65	23.63	23.48	23.67
12	122.51	122.45	122.47	122.46	122.34	122.46
13	144.03	144.02	144.06	144.03	143.81	144.07
14	41.85	41.82	41.86	41.84	41.66	41.92
15	35.76	35.71	35.77	35.73	35.49	35.80
16	73.93	73.89	73.92	73.88	73.69	73.86
17	49.17	49.12	49.14	49.12	49.10	49.12
18	41.19	41.16	41.19	41.16	41.03	41.27
19	46.99	47.10	47.13	47.12	46.81	47.20
20	30.48	30.44	30.49	30.46	30.28	30.47
21	35.66	35.63	35.68	35.64	35.49	35.71
22	31.40	31.35	31.39	31.37	31.08	31.35
23	27.95	27.93	27.96	27.94	27.75	27.98
24	16.80	16.77	16.81	16.78	16.61	16.83
25	15.47	15.44	15.47	15.45	15.28	15.52
26	17.32	17.28	17.31	17.29	17.15	17.37
27	26.94	26.92	26.95	26.92	26.76	26.96
28	175.82	175.82	175.77	175.77	175.77	175.77
29	32.89	32.85	32.90	32.87	32.70	32.87
30	24.61	24.60	24.63	24.61	24.51	24.56
C-3 sugar						
GlcNAc						
1	104.15	104.13	104.17	104.13	103.89	104.14
2	57.41	57.31	57.38	57.32	56.99	57.42
3	75.02	74.95	74.97	74.93	74.62	75.02
4	72.16	72.04	72.16	72.07	71.55	72.19
5	75.58	75.50	75.55	75.50	75.35	75.55
6	69.10	69.10	69.07	69.02	68.75	69.07
$NH\underline{C}OCH_3$	171.32	171.45	171.34	171.44	171.93	171.31
$NHCO\underline{C}H_3$	23.19	23.15	23.19	23.16	22.96	23.21
Ara (inner)						
1	102.00	101.97	102.00	101.97	101.80	102.00
2	79.81	79.74	79.80	79.76	79.51	79.82
3	72.01	71.95	72.02	71.98	71.75	72.07
4	67.08	67.05	67.08	67.06	66.94	67.11
5	63.96	63.93	63.98	63.96	63.83	63.99
Ara (outer)						
1	105.51	105.43	105.50	105.45	105.08	105.53
2	74.71	74.72	74.76	74.73	74.50	74.79
3	77.13	77.07	77.12	77.08	76.74	77.16
4	70.35	70.29	70.34	70.30	70.03	70.35
5	66.66	66.60	66.65	66.61	66.32	66.99
C-28 sugar						
Glc (inner)						
1	94.53	94.49	94.53	94.49	94.22	94.45
2	77.97	78.07	78.02	78.04	78.05	77.86
3	77.19	77.15	77.19	77.17	76.94	76.82
4	70.98	70.94	70.98	70.96	70.72	71.05
5	75.40	75.35	75.41	75.36	75.07	75.52
6	64.19	64.15	64.20	64.19	64.05	64.18
Rham						
1	101.70	101.68	101.70	101.68	101.47	100.97
2	70.15	70.12	70.13	70.11	70.00	70.35
3	82.17	82.08	82.17	82.12	81.76	82.41
4	77.97	77.97	77.99	77.98	77.91	78.00
5	68.76	68.73	68.75	68.73	68.55	68.35
6	18.53	18.51	18.54	18.52	18.33	18.48

Carbon	7	8	9	10	11	12
Glc (outer)						
1	104.67	104.60	104.66	104.61	104.29	104.51
2	74.92	74.85	74.91	74.87	74.54	74.74
3	77.63	77.56	77.63	77.58	77.22	77.46
4	71.12	71.05	71.12	71.07	70.69	71.05
5	77.63	77.56	77.63	77.58	77.22	74.79
6	62.21	62.14	62.20	62.15	61.79	64.06
$\underline{C}OCH_3$						171.09
$CO\underline{C}H_3$						20.68
Xyl (inner)						
1	104.04	104.00	104.05	104.01	103.76	104.10
2	74.13	74.07	74.11	74.07	73.82	74.11
3	87.56	87.51	87.57	87.52	87.19	87.56
4	69.10	69.08	69.11	69.09	68.92	69.07
5	65.95	65.95	65.95	65.90	65.62	66.00
Xyl (outer)						
1	105.35	105.31	105.36	105.32	105.02	105.35
2	74.77	74.67	74.71	74.67	74.43	74.67
3	77.07	76.99	77.06	77.00	76.66	77.08
4	70.24	70.19	70.24	70.20	69.94	70.24
5	66.52	66.47	66.53	66.49	66.19	66.55
Monoterpene glycoside						
1	168.03	168.05	167.98	167.98	168.01	167.96
2	127.59	127.51	127.77	127.74	127.52	127.79
3	143.15	143.26	142.86	142.86	142.90	142.83
4	23.46	23.44	23.44	23.72	23.02	23.78
5	40.15	40.11	40.15	40.12	40.54	40.73
6	79.47	79.41	79.50	79.44	79.44	79.46
7	143.45	143.55	142.35	142.30	141.95	142.35
8	114.90	114.78	115.40	115.41	115.53	115.44
9	12.19	12.22	12.21	12.19	12.04	12.22
10	23.46	23.47	23.77	23.74	23.63	23.82
Xyl						
1	99.64		97.58	97.56	97.36	97.61
2	74.53		75.00	74.95	74.69	74.98
3	77.63		75.73	75.69	77.35	75.76
4	70.59		70.81	70.77	70.48	70.82
5	66.23		66.42	66.38	66.07	66.44
Qui						
1		98.69				
2		74.88				
3		77.65				
4		76.28				
5		72.11				
6		18.39				
Monoterpene glycoside					(acid)	(acid)
1			167.24	167.24	167.28	167.24
2			127.88	127.79	127.52	127.79
3			143.10	143.24	143.47	143.39
4			23.30	23.43	23.58	23.19
5			40.31	40.27	40.93	41.26
6			79.49	79.48	72.12	72.04
7			143.35	143.46	145.41	145.96
8			114.99	114.86	111.76	111.69
9			12.36	12.39	12.16	12.36
10			23.50	23.53	27.54	27.91
Xyl						
1			99.65			
2			74.53			
3			77.79			
4			70.59			
5			66.23			
Qui						
1				98.71		
2				74.90		
3				77.68		
4				76.29		
5				72.12		
6				18.39		

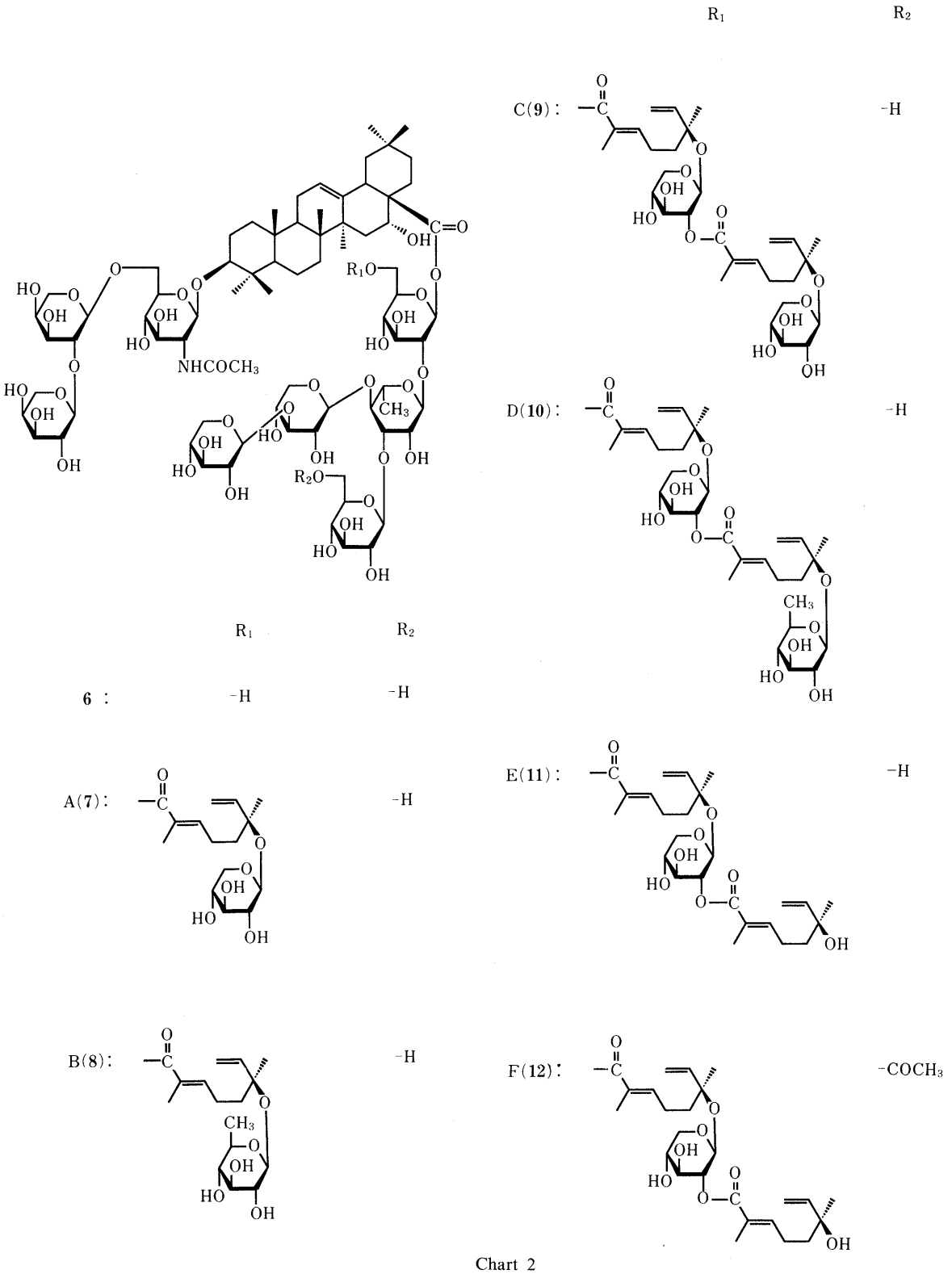

Chart 2

2-*trans*-2,6-dimethyl-6-*O*-*β*-D-quinovopyranosyl-2,7-octadienoyl-(1→6)]-*β*-D-glucopyranosyl} ester (Chart 2).

Calliandra saponin C (**9**) revealed an $[M+Na]^+$ ion peak at *m/z* 2293 in FAB-MS and elemental analysis data was consistent with $C_{106}H_{167}NO_{51}\cdot 8H_2O$. On mild alkaline hydrolysis, **9** afforded compounds **6** and **3** as major products. Compound **3** was identified with an authentic sample of (6*S*)-2-*trans*-2,6-dimethyl-6-*O*-*β*-D-xylopyranosyl-2,7-octadienoic acid.[1] Comparing the ^{1}H-NMR spectra of **6**, **7** and **9**, the H-6 (δ 4.72, 4.52 ppm) of inner glucose and the H-2 (δ 5.37 ppm) of xylose of the first monoterpene glycoside appeared at lower field than usual (**6**; H-6 of inner glucose, δ 4.23, 4.13 ppm, **7**; H-2 of xylose of monoterpene glycoside, δ 3.82 ppm); we therefore thought two monoterpene glycosides were attached to these positions. The HMBC experiment showed a cor-

relation between H-6 of glucose and C-1 of first monoterpene glycoside, and between H-2 (δ 5.37 ppm) of xylose of the first one and C-1 (δ 167.24 ppm) of the second one. The assignment of these signals was achieved by analysis of a detailed H–H COSY, C–H COSY and HOHAHA experiment. Therefore, the structure of calliandra saponin C (**9**) is 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*-((6′*S*)-2′-*trans*-2′,6′-dimethyl-6′-*O*-β-D-xylopyranosyl-2′,7′-octadienoyl-(1→2))-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester as shown in Chart 2.

Calliandra saponin D (**10**) gave compounds **3**, **4** and **6** on mild alkaline hydrolysis. In the HMBC experiment, cross peaks were observed between H-6 (δ 4.72, 4.50 ppm) of inner glucose and C-1 (δ 167.98 ppm) of xylopyranosyl monoterpenic acid part, and between H-2 (δ 5.36 ppm) of xylose of that and C-1 (δ 167.24 ppm) of monoterpene quinovoside. Acylation shift was observed at C-6 of inner glucose and C-2 of xylose (Table I). Thus, **10** 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)-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-((6′*S*)-2′-*trans*-2′,6′-dimethyl-6′-*O*-β-D-quinovopyranosyl-2′,7′-octadienoyl-(1→2))-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester as shown in Chart 2.

The ^{1}H-NMR and the ^{13}C-NMR spectral data of calliandra saponin F (**12**) were very similar to those of calliandra saponin E (**11**). In comparison, H-6 (δ 4.69, 4.40 ppm) of outer glucose in **12** appeared at much lower field than **11** (δ 4.19, 3.95 ppm). One carbonyl carbon (δ 171.09 ppm) and acetyl group (^{1}H-NMR: δ 1.97 ppm, ^{13}C-NMR: δ 20.68 ppm) was observed for the first time. The HMBC experiment showed a cross peak between H-6 (δ 4.69, 4.40 ppm) of outer glucose and this carbonyl carbon (δ 171.09 ppm), confirming that the acetyl group attached to 6 position of the outer glucose. Therefore, the structure of **12** was characterized as shown in Chart 2.

Experimental

General Procedures Melting points were determined with a Yanagimoto microapparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM EX-270 and/or A-500 FT-NMR, and chemical shifts were given in ppm with tetramethylsilane as an internal standard. FAB-MS was recorded on a JEOL JMS-SX 102 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) with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase) as a developing solvent. 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 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 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 with chloroform–methanol–water (35 : 65 : 40) as solvent system in the ascending mode, 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) with 35% acetonitrile solution yielded saponin A (**7**) (322.7 mg), B (**8**) (362.1 mg), C (**9**) (200.8 mg), D (**10**) (257.0 mg), respectively. Fraction 2 (1.2 g) was chromatographed on Lobar RP-18 with 35% acetonitrile solution to give a saponin E (**11**) rich fraction, which was rechromatographed with HPLC on an Asahipak ODP-50 column (10 mm × 250 mm) with the same solution and gave **11** (803.0 mg). Saponin F (**12**) (173.2 mg) was obtained from fraction 3.

Calliandra Saponin B (8) Amorphous powder, mp 220—226 °C (dec.), $[\alpha]_D^{20}$ −9.8° (c=1.3, MeOH). ^{1}H-NMR (pyridine-d_5 : D_2O=9 : 1) aglycone moiety: δ 0.79 (3H, s, H_3-25), 0.83 (3H, s, H_3-29), 0.85 (3H, s, H_3-24), 0.93 (3H, s, H_3-26), 0.97 (3H, s, H_3-30), 1.06 (3H, s, H_3-23), 1.64 (3H, s, H_3-27), 5.45 (1H, br s, H-12); sugar moiety: δ 1.51 (3H, d, J=7.3 Hz, Rham, Me-6), 2.06 (3H, s, NHCOCH_3), 4.76 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.87 (1H, d, J=8.4 Hz, GlcNAc, H-1), 4.89 (1H, d, J=4.9 Hz, inner Ara, H-1), 4.94 (1H, d, J=7.4 Hz, outer Xyl, H-1), 5.07 (1H, d, J=8.0 Hz, outer Glc, H-1), 5.26 (1H, d, J=7.9 Hz, inner Xyl, H-1), 5.67 (1H, br s, Rham, H-1), 5.85 (1H, d, J=8.0 Hz, inner Glc, H-1); monoterpene glycoside moiety: δ 1.41 (6H, s, H_3-10, Qui, Me-6), 1.74 (3H, s, H_3-9), 4.66 (1H, d, J=7.9 Hz, Qui, H-1), 5.14 (1H, d, J=11.0 Hz, H_a-8), 5.29 (1H, d, J=17.7 Hz, H_b-8), 6.05 (1H, dd, J=11.0, 17.7 Hz, H-7), 6.83 (1H, t, H-3). ^{13}C-NMR (pyridine-d_5: D_2O=9 : 1): Table I. FAB-MS m/z: 2009 $[M+Na]^+$, 1987 $[M+H]^+$. *Anal.* Calcd for $C_{92}H_{147}NO_{45}\cdot 7H_2O$: C, 52.29; H, 7.68; N, 0.66. Found: C, 52.14; H, 7.70; N, 0.60.

Calliandra Saponin C (9) Amorphous powder, mp 192—195 °C (dec.),$[\alpha]_D^{20}$ −10.2° (c=0.25, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9 : 1) sugar moiety: δ 4.77 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.88 (1H, d, J=8.5 Hz, GlcNAc, H-1), 4.90 (1H, d, J=4.8 Hz, inner Ara, H-1), 4.95 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.08 (1H, d, J=8.0 Hz, outer Glc, H-1), 5.26 (1H, d, J=7.9 Hz, inner Xyl, H-1), 5.67 (1H, br s, Rham, H-1), 5.85 (1H, d, J=7.3 Hz, inner Glc, H-1); monoterpene glycoside moiety: δ 1.35 (3H, s, H_3-10), 1.40 (3H, s, H_3-10′), 1.78 (3H, s, H_3-9), 1.81 (3H, s, H_3-9′), 4.66 (1H, d, J=7.9 Hz, Xyl′, H-1), 4.72 (1H, d, J=8.0 Hz, Xyl, H-1), 5.13 (1H, d, J=11.0 Hz, H_a-8′), 5.17 (1H, d, J=11.0 Hz, H_a-8), 5.22 (1H, d, J=17.7 Hz, H_b-8), 5.27 (1H, d, J=17.5 Hz, H_b-8′), 5.81 (1H, dd, J=11.0, 17.7 Hz, H-7), 6.06 (1H, dd, J=11.0, 17.5 Hz, H-7′), 6.78 (1H, t, H-3), 6.91 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O=9 : 1): Table I. FAB-MS m/z: 2293 $[M+Na]^+$, 2271 $[M+H]^+$. *Anal.* Calcd for $C_{106}H_{167}NO_{51}\cdot 8H_2O$: C, 52.71; H, 7.64; N, 0.58. Found: C, 52.45; H, 7.31; N, 0.46.

Calliandra Saponin D (10) Amorphous powder, mp 194—196 °C (dec.), $[\alpha]_D^{20}$ −14.6° (c=0.55, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9 : 1) sugar moiety: δ 4.77 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.88 (1H, d, J=8.6 Hz, GlcNAc, H-1), 4.89 (1H, d, J=4.9 Hz, inner Ara, H-1), 4.94 (1H, d, J=7.4 Hz, outer Xyl, H-1), 5.07 (1H, d, J=8.0 Hz, outer Glc, H-1), 5.26 (1H, d, J=7.9 Hz, inner Xyl, H-1), 5.67 (1H, br s, Rham, H-1), 5.84 (1H, d, J=7.4 Hz, inner Glc, H-1); monoterpene glycoside moiety: δ 1.35 (3H, s, H_3-10), 1.41 (3H, s, H_3-10′), 1.76 (3H, s, H_3-9), 1.80 (3H, s, H_3-9′), 4.67 (1H, d, J=7.3 Hz, Qui, H-1), 4.71 (1H, d, J=7.9 Hz, Xyl, H-1), 5.12 (1H, d, J=11.0 Hz, H_a-8′), 5.17 (1H, d, J=11.0 Hz, H_a-8), 5.23 (1H, d, J=17.7 Hz, H_b-8), 5.29 (1H, d, J=17.7 Hz, H_b-8′), 5.81 (1H, dd, J=11.0, 17.7 Hz, H-7), 6.06 (1H, dd, J=11.0, 17.7 Hz, H-7′), 6.77 (1H, t, H-3), 6.91 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O=9 : 1): Table I. FAB-MS m/z: 2307 $[M+Na]^+$. *Anal.* Calcd for $C_{107}H_{169}NO_{51}\cdot 7H_2O$: C, 53.29; H, 7.65; N, 0.58. Found: C, 53.08; H, 7.37; N, 0.48.

Calliandra Saponin F (12) Amorphous powder, mp 186—189 °C (dec.), $[\alpha]_D^{20}$ −3.6° (c=0.1, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9 : 1) sugar moiety: δ 4.77 (1H, d, J=7.4 Hz, outer Ara, H-1), 4.87 (1H, d,

$J=8.4$ Hz, GlcNAc, H-1), 4.89 (1H, d, $J=5.5$ Hz, inner Ara, H-1), 4.92 (1H, d, $J=7.3$ Hz, outer Xyl, H-1), 5.01 (1H, d, $J=8.0$ Hz, outer Glc, H-1), 5.22 (1H, d, $J=8.6$ Hz, inner Xyl, H-1), 5.89 (1H, d, $J=7.5$ Hz, inner Glc, H-1), 5.92 (1H, br s, Rham, H-1); monoterpene glycoside moiety: δ 1.35 (6H, s, H_3-10, H_3-10'), 1.78 (3H, s, H_3-9), 1.83 (3H, s, H_3-9'), 4.71 (1H, d, $J=7.9$ Hz, Xyl, H-1), 5.04 (1H, d, $J=11.0$ Hz, H_a-8'), 5.17 (1H, d, $J=11.0$ Hz, H_a-8), 5.23 (1H, d, $J=17.0$ Hz, H_b-8), 5.38 (1H, d, $J=17.0$ Hz, H_b-8'), 5.83 (1H, dd, $J=11.0$, 17.0 Hz, H-7), 6.01 (1H, dd, $J=11.0$, 17.0 Hz, H-7'), 6.77 (1H, t, H-3), 6.97 (1H, t, H-3'). ^{13}C-NMR (pyridine-d_5: $D_2O=9:1$): Table I. FAB-MS *m/z*: 2181 $[M+H]^+$. *Anal.* Calcd for $C_{103}H_{161}NO_{48}\cdot 10H_2O$: C, 52.39; H, 7.73; N, 0.59. Found: C, 52.37; H, 7.63; N, 0.59.

Acid Hydrolysis of Compounds 8—10 Compound **8** (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 eluent 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 6 peaks for the derivatives of arabinose, xylose, glucose, quinovose, rhamnose, and *N*-acetyl glucosamine 2:2:2:1:1:1, respectively. Acid hydrolysis of the other compounds (**9**, **10**) was performed by the same method used for compound **8**.

Compound **9**: Arabinose, xylose, glucose, rhamnose, *N*-acetyl glucosamine = 2:4:2:1:1. Compound **10**: Arabniose, xylose, glucose, quinovose, rhamnose, *N*-acetyl glucosamine 2:3:2:1:1:1. 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, quinovose 11.7, arabinose 14.4, *N*-acetyl glucosamine 19.2, xylose 19.3, glucose 49.2.

Alkaline Hydrolysis of Saponin B (8) Compound **8** (50 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 (**5**) (17 mg) and quinovosyl monoterpenic acid (**4**) (6 mg).

Mild Alkaline Hydrolysis of Saponin B (8) A solution of compound **8** (50 mg) and 5% K_2CO_3 (5 ml) in EtOH (5 ml) was refluxed for 1 h. The reaction mixture was neutralized with Dowex 50Wx8 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 three major compounds, compounds **4** (6 mg), **5** (8 mg) and **6** (16 mg). Mild alkaline hydrolysis of the other compounds (**9**, **10**, each 10 mg) was performed by the same method used for compound **8**. Compound **9** gave compounds **3** (ref. 1) and **6**, and similarly, compound **10** gave **3**, **4** and **6**. Each reaction product was confirmed by TLC.

(6*S*)-2-*trans*-2,6-Dimethyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoic Acid (4) ^{1}H-NMR (pyridine-d_5) δ: 1.57 (3H, s, H_3-10), 1.62 (3H, d, $J=5.7$ Hz, Qui, Me-6), 1.80—1.85 (2H, m, H-5), 2.03 (3H, s, H_3-9), 2.48—2.51 (2H, m, H-4), 3.71—3.74 (2H, m, H-4'. H-5'), 4.02 (1H, dd, $J=7.7$, 9.0 Hz, H-2'), 4.13 (1H, dd, $J=8.6$, 9.0 Hz, H-3'), 4.91 (1H, d, $J=7.7$ Hz, H-1'), 5.24 (1H, dd, $J=1.3$, 10.8 Hz, H_a-8), 5.45 (1H, dd, $J=1.3$, 17.6 Hz, H_b-8), 6.25 (1H, dd, $J=10.8$, 17.6 Hz, H-7), 7.17 (1H, td, $J=1.3$, 7.5 Hz, H-3). ^{13}C-NMR (pyridine-d_5) δ: monoterpene acid unit; 170.6 (C-1), 129.0 (C-2), 144.2 (C-3), 23.8 (C-4), 40.7 (C-5), 79.6 (C-6), 142.3 (C-7), 114.8 (C-8), 12.8 (C-9), 23.8 (C-10); quinovose unit; 96.8 (C-1), 75.6 (C-2), 76.6 (C-3), 76.1 (C-4), 73.0 (C-5), 18.0 (C-6).

Acknowledgments The authors thank Miss. S. Kato for recording the NMR spectra, Miss T. Naito for performing the microanalysis and Miss K. Takahashi for measurements of FAB-MS.

References

1) T. Takeda, T. Nakamura, S. Takashima, O. Yano, Y. Ogihara, *Chem. Pharm. Bull.*, **41**, 2132 (1993).
2) T. Konoshima, H. Fukushima, H. Inui, K. Sato, T. Sawada, *Phytochemistry*, **20**, 139 (1981).
3) Y. Okada, K. Koyama, K. Takahashi, T. Okuyama, S. Shibata, *Planta Medica*, **40**, 185 (1980).
4) S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.*, **35**, 501 (1987).
5) G. Carpani, F. Orsini, M. Sisti, L. Verotta, *Phytochemistry*, **28**, 863 (1989).
6) H. Ishii, K. Tori, T. Tojo, Y. Yoshimura, *Chem. Pharm. Bull.*, **26**, 674 (1978).