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

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Six new triterpenoidal saponins, called calliandra saponins G(1), H(2), I(3), J(4), K(5) and L(6), 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 chemical evidence. These saponins, interestingly, have an *N*-acetyl glucosamine at the 3 position of the genin, and one or two monoterpene glycosides at the position of the sugar chain.

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

Aqueous extracts of the branches of *Calliandra anomala* (KUNTH) MACBR. (Leguminosae) are used as an antimalarial and antifebrile in Mexico.[1] In our preceding papers,[2,3] we reported the structures of six triterpenoidal saponins, called calliandra saponins A, B, C, D, E and F, from the branches of *Calliandra anomala*. Further studies have led to the isolation of six new saponins, whose structures are elucidated here: In this paper, we wish to report the struture elucidation of six new saponins, calliandra saponins G(**1**), H(**2**), I(**3**), J(**4**), K(**5**) and L(**6**). The *n*-butanol soluble fraction of methanol extract was dissolved in methanol, and ether was added to the methanol solution to give a precipitate. The ether precipitate was separated by Lobar RP-18 chromatography and subjected to repeated semi-preparative high performance liquid chromatography (HPLC) on an Asahipak ODP-50 reversed phase column. We isolated all six saponins (G—L).

Calliandra saponin G(**1**) revealed an $[M+Na]^+$ ion peak at *m/z* 2176 in the fast atom bombardment mass spectrum (FAB-MS). The molecular formula was determined to be $C_{102}H_{161}NO_{47}\cdot 10H_2O$ by elemental analysis. The ^{13}C-NMR spectrum of **1** showed nine anomeric carbons (δ 94.19, 96.27, 101.36, 101.66, 103.71, 103.82, 104.32, 105.02, 105.16), five pairs of olefinic carbons (δ 143.73, 122.13, 142.51, 127.43, 142.15, 114.96, 143.00, 127.43, 145.58, 111.36), four carbonyl carbons (δ 166.95, 167.65, 171.02, 175.43), and one sugar-substituted methine carbon (δ 88.89) (Table 1). The ^{1}H-NMR spectrum showed the signals of thirteen tertiary methyl groups, one *N*-acetyl methyl signal (δ 2.10), nine anomeric protons [δ 4.75 (d, J=7.9 Hz), 4.81 (d, J=7.3 Hz), 4.91 (d, J=8.0 Hz), 4.93 (d, J=5.5 Hz), 4.98 (d, J=8.0 Hz), 5.18 (d, J=8.0 Hz), 5.30 (d, J=7.4 Hz), 5.71 (br s), 5.88 (d, J=7.3 Hz)], and eight olefinic protons. On acid hydrolysis with 2 N sulfuric acid, calliandra saponin G(**1**) gave echinocystic acid (**7**),[4] monoterpene carboxylic acid; (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (**9**),[5] and L-arabinose, D-glucose, L-rhamnose, D-xylose, D-quinovose and *N*-acetyl-D-glucosamine as the component sugars.[6] Alkaline hydrolysis of **1** with 1 N KOH afforded monoterpene carboxylic acid (**9**), prosapogenin; 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid (**10**)[7] and monoterpene quinovoside (**12**)[3] as major products. When the ^{13}C-NMR data of **1** was compared with that of calliandra saponin B,[3] acylation shifts[8] were observed at C-1 (−2.42), C-2 (+0.03) and C-3 (−2.55) of the quinovose part of monoterpene quinovoside. The heteronuclear multiple-bound correlation spectroscopy (HMBC) experiment showed a correlation between the H-2 (δ 5.45) of quinovose and the C-1 (δ 166.95) of monoterpene carboxylic acid. The assignments of these signals were achieved by analyses of detailed H–H correlation spectroscopy (COSY), C–H COSY and homonuclear Hartmann-Hahn spectroscopy (HOHAHA) experiments. Therefore, the structure of calliandra saponin G(**1**) 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′-hydroxy-2′,7′-octadienoyl-(1→2))-β-D-quinovopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester, as shown in Chart 1.

The ^{1}H-NMR and the ^{13}C-NMR spectral data of calliandra saponin H (**2**) were very similar to those of calliandra saponin G(**1**). In comparison, the C-6 (δ 63.77) of the outer glucose in **2** appeared at a much lower field than that in **1** (δ 61.86). One carbonyl carbon (δ 170.80) and acetyl group (δ_H 2.01, δ_C 20.33) was observed. The HMBC experiment showed a cross peak between the H-6 (δ 4.69, 4.40) of the outer glucose and this carbonyl carbon (δ 170.80), confirming that the acetyl group attached to the 6 position of the outer glucose. Therefore, the structure of **2** 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-6-*O*-acetyl-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′-hydroxy-2′,7′-octadienoyl-(1→2))-β-D-quinovopyranosyl-2,7-octadienoyl-(1→6)]-β-D-

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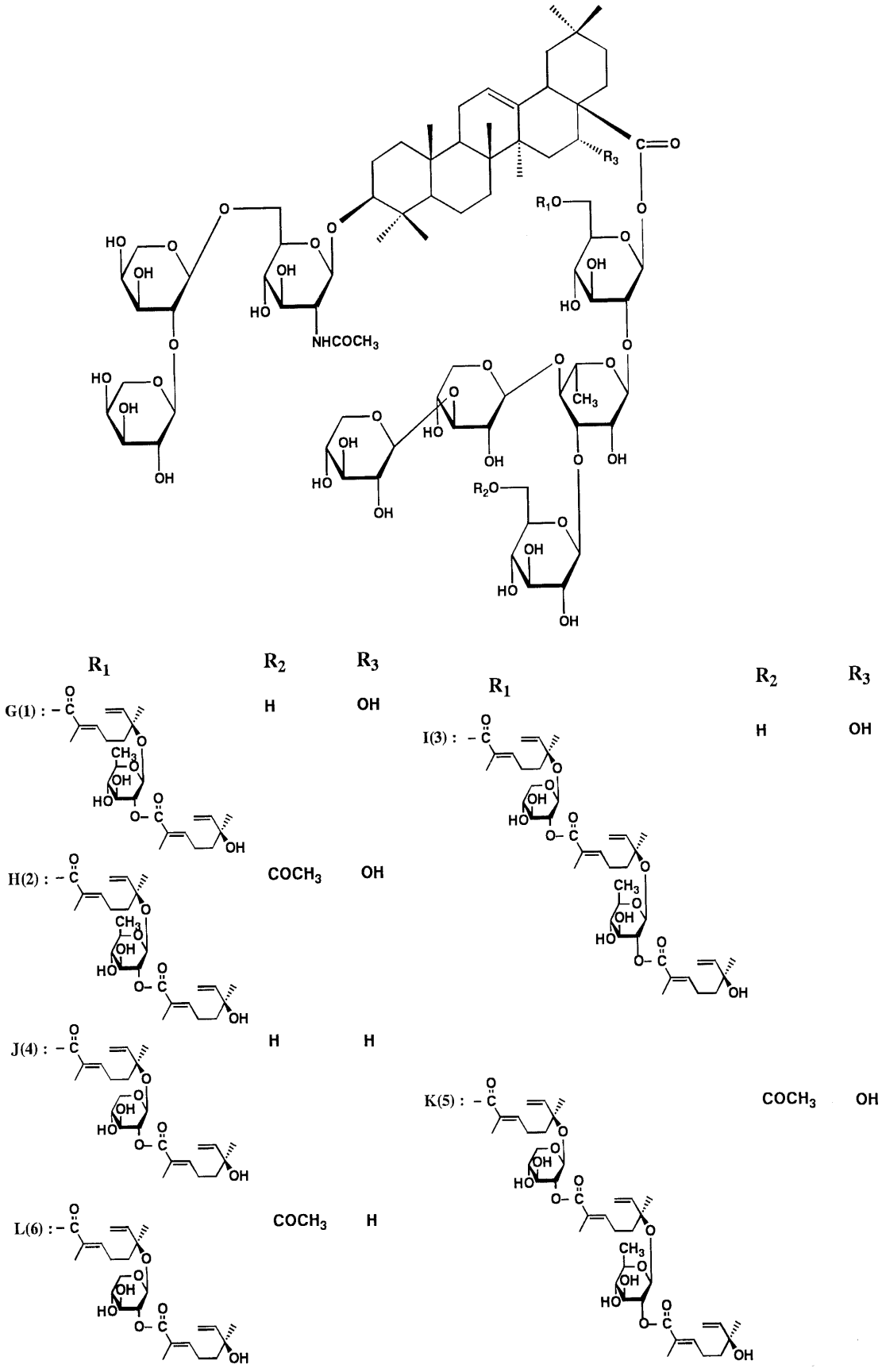

Chart 1

glucopyranosyl} ester, as shown in Chart 1.

Calliandra saponin I(**3**) revealed an $[M+Na]^+$ ion peak at *m/z* 2473 in the FAB-MS, and elemental analysis data was consistent with $C_{117}H_{183}NO_{53}\cdot 10H_2O$. The ^{1}H-NMR spectrum showed the signals of fifteen tertiary methyl groups, one acetyl methyl signal, five pairs of olefinic protons, and ten anomeric protons. When the ^{13}C-NMR data of **3** was compared with that of calliandra

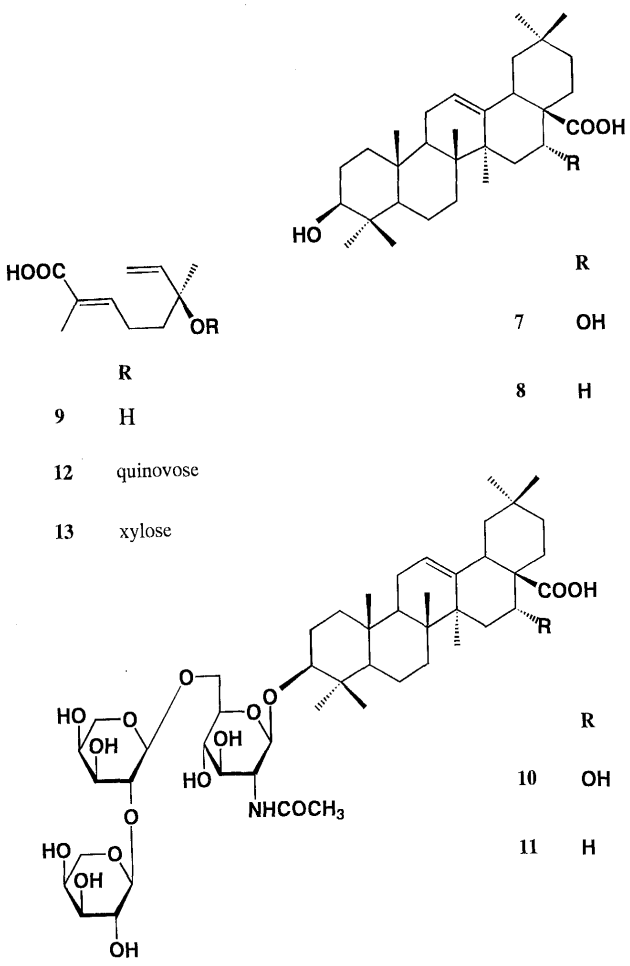

Chart 2

saponin D,[3] acylation shifts were observed at C-1 (−2.43), C-2 (+0.01) and C-3 (−2.62) of monoterpene quinovoside. The HMBC experiment showed a correlation between the H-2 (δ 5.45) of quinovose and the C-1 (δ 166.99) of monoterpene carboxylic acid. The assignments of these signals were achieved by the analyses of detailed H–H COSY, C–H COSY and HOHAHA experiments. Therefore, the structure of calliandra saponin I(**3**) 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-(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′-(6″*S*)-2″-*trans*-2″,6″-dimethyl-6″-hydroxy-2″,7″-octadienoyl-(1→2))-β-D-quinovopyranosyl-2′,7′-octadienoyl-(1→2)-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester, as shown in Chart 1.

Calliandra saponin K(**5**) revealed an $[M+Na]^+$ ion peak at *m/z* 2515 in the FAB-MS, and elemental analysis data was consistent with $C_{119}H_{185}NO_{54}\cdot 10H_2O$. The ^{1}H-NMR and the ^{13}C-NMR spectral data of calliandra saponin K(**5**) were very similar to those of calliandra saponin I(**3**). In comparison, the C-6 (δ 63.71) of the outer glucose in **5** appeared at a much lower field than that in **3** (δ 61.85). One carbonyl carbon (δ 170.80) and acetyl group (δ_H 2.01, δ_C 21.81) was observed. Therefore, the structure of **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-6-*O*-acetyl-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′-(6″*S*)-2″-*trans*-2″,6″-dimethyl-6″-hydroxy-2″,7″-octadienoyl-(1→2))-β-D-quinovopyranosyl-2′,7′-octadienoyl-(1→2)-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester, as shown in Chart 1.

The molecular formula of calliandra saponin J(4) was determined to be $C_{101}H_{159}NO_{46}\cdot 10H_2O$ by the FAB-MS and elemental analysis data. On acid hydrolysis with 2 N sulfuric acid, saponin J(**4**) gave oleanolic acid (**8**),[9] monoterpene carboxylic acid (**9**), L-arabinose, D-glucose, L-rhamnose, D-xylose, and *N*-acetyl-D-glucosamine as the component sugars. Alkaline hydrolysis of **4** with 1 N KOH afforded a prosapogenin; 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl oleanolic acid (**11**) and monoterpene xyloside (**13**) as major products. When the ^{1}H-NMR and the ^{13}C-NMR data of **4** were compared with those of calliandra saponin E,[3] they were similar except for the signals of the aglycone moiety. The ^{13}C-NMR chemical shifts of the aglycone moiety in calliandra saponin J were coincident with those in prosapogenin **11**, composed of oleanolic acid as an aglycone. The HMBC experiment of **4** showed a correlation between the H-1 (δ 5.92) of inner glucose attaching to the C-28 of the aglycone and the C-28 (δ 175.89) of oleanolic acid, and between the H-6 (δ 4.61, 4.82) of inner glucose and the C-1 (δ 167.64) of the first monoterpene xyloside, and between the H-2 (δ 5.46) of xylose of the first one and C-1 (δ 166.91) of the monoterpene carboxylic acid. Therefore, the structure of calliandra saponin J (**4**), having oleanolic acid as an aglycone, is 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl oleanolic 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′-hydroxy-2′,7′-octadienoyl-(1→2)-β-D-xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester, as shown in Chart 1.

Calliandra saponin L(**6**) revealed an $[M+Na]^+$ ion peak at *m/z* 2186 in the FAB-MS, and elemental analysis data was consistent with $C_{103}H_{161}NO_{47}\cdot 11H_2O$. The ^{1}H-NMR and the ^{13}C-NMR spectral data of calliandra saponin L (**6**) were very similar to those of calliandra saponin J (**4**). In comparison, the C-6 (δ 63.74) of the outer glucose in **6** appeared at a much lower field than **4** (δ 61.89). One carbonyl carbon (δ 170.77) and an acetyl group (δ_H 2.02, δ_C 20.37) was observed. Therefore, the structure of **6** was characterized as 3-*O*-α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl oleanolic acid 28-*O*-{β-D-6-*O*-acetyl-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′-hydroxy-2′,7′-octadienoyl-(1→2)-β-D-

Table 1. ^{13}C-NMR Spectral Data of Compounds **1**—**6** and **11**[a)]

Carbon	1	2	3	4	5	6	11
Aglycone							
1	38.39	38.41	38.41	38.31	38.40	38.34	38.6
2	25.90	25.61	25.89	25.92	25.86	25.94	26.6
3	88.89	88.90	88.89	88.74	88.90	88.82	88.9
4	38.68	38.70	38.68	38.70	38.69	38.72	39.4
5	55.47	55.60	55.47	55.36	55.49	55.40	55.8
6	18.08	18.08	18.07	18.08	18.11	18.10	18.6
7	33.00	33.17	32.99	32.67	33.17	32.67	33.5
8	39.60	39.61	39.59	39.46	39.59	39.47	39.8
9	46.66	46.68	46.66	47.54	46.66	47.55	48.1
10	36.52	36.55	36.51	36.50	36.51	36.51	37.1
11	23.34	23.34	23.25	23.30	23.35	23.30	23.9
12	122.13	122.02	122.11	122.33	122.09	122.35	122.8
13	143.73	144.58	143.73	143.46	143.73	143.52	145.0
14	41.53	41.59	41.52	41.66	41.57	41.67	42.2
15	35.45	35.45	35.44	27.56	35.47	27.56	28.5
16	73.58	73.60	73.58	22.22	73.50	22.22	23.9
17	48.80	48.87	48.83	46.66	48.83	46.66	46.9
18	40.91	40.92	40.85	41.23	40.87	41.36	42.2
19	46.82	46.80	46.80	45.89	46.85	45.88	46.7
20	30.15	30.13	30.14	30.15	30.12	30.15	31.1
21	35.35	35.36	35.35	33.52	35.41	33.52	34.5
22	31.42	31.42	31.41	31.42	31.41	31.42	33.3
23	27.62	27.65	27.61	27.63	27.64	27.65	28.2
24	16.47	16.49	16.46	16.44	16.48	16.48	17.0
25	15.13	15.18	15.13	15.01	15.17	15.09	15.5
26	16.98	17.04	16.96	16.97	17.02	16.99	17.6
27	26.61	26.63	26.60	25.47	26.62	25.47	26.3
28	175.43	174.89	175.41	175.89	175.43	175.94	180.0
29	32.56	32.53	32.56	32.56	32.52	32.56	33.5
30	24.29	24.26	24.28	23.34	24.25	23.33	23.9
C-3 sugar							
GlcNAc							
1	103.82	103.80	103.94	103.84	103.85	103.90	104.9
2	57.04	57.09	57.02	57.04	57.04	57.09	57.9
3	74.66	74.67	74.59	74.62	74.60	74.64	75.9
4	71.81	71.90	71.74	72.15	71.74	72.10	73.1
5	75.20	75.20	75.17	75.30	75.17	75.30	75.5
6	68.72	68.70	68.75	68.72	68.72	68.74	69.5
$NH\underline{C}OCH_3$	171.02	171.01	170.80	171.07	171.05	171.08	170.2
$NHCO\underline{C}H_3$	22.85	22.69	22.86	22.86	22.85	22.87	23.7
Ara (inner)							
1	101.66	101.92	101.65	101.73	101.65	101.73	102.4
2	79.46	79.48	79.44	79.55	79.47	79.58	80.6
3	71.68	71.74	71.68	72.01	71.68	72.01	72.6
4	66.75	66.86	66.75	66.80	66.76	66.81	67.5
5	63.64	63.70	63.64	63.73	63.64	63.60	64.2
Ara (outer)							
1	105.16	105.19	105.65	105.21	105.21	105.25	106.4
2	74.43	74.39	74.42	74.47	74.43	74.49	74.3
3	76.79	76.81	76.78	76.77	76.78	76.79	76.3
4	70.00	70.01	69.98	70.00	69.98	70.02	70.9
5	66.31	66.32	66.31	66.31	66.30	66.32	67.3
C-28 sugar							
Glc (inner)							
1	94.19	94.20	94.18	94.21	94.14	94.22	
2	77.66	77.72	77.66	77.76	77.68	77.65	
3	76.88	76.84	76.86	76.89	76.80	76.89	
4	70.67	70.70	70.66	70.67	70.67	70.73	
5	75.07	75.01	75.06	75.05	75.07	75.08	
6	63.86	63.85	63.81	63.89	63.81	63.80	
Rham							
1	101.36	101.40	101.35	101.30	101.35	101.31	
2	69.80	69.80	69.75	69.74	69.75	69.80	
3	81.83	81.91	82.82	81.80	81.80	81.81	
4	77.66	77.55	77.66	77.52	77.49	77.65	
5	68.40	68.48	68.41	68.37	68.40	68.21	
6	18.20	18.13	18.20	18.19	18.20	18.21	
Glc (outer)							
1	104.32	104.40	104.30	104.31	104.31	104.33	
2	74.57	74.45	74.57	74.56	74.57	74.60	
3	77.29	77.30	77.28	77.31	77.27	77.30	
4	70.78	70.70	70.77	70.82	70.68	70.73	
5	77.29	74.45	77.28	77.31	74.43	74.49	
6	61.86	63.77	61.85	61.89	63.71	63.74	
$\underline{C}OCH_3$		170.80			170.80	170.77	
$CO\underline{C}H_3$		20.33			21.81	20.37	

Table 1. (continued)

Carbon	1	2	3	4	5	6
Xyl (inner)						
1	103.71	103.76	103.70	103.79	103.75	103.83
2	73.78	73.78	73.75	77.82	73.75	73.87
3	87.23	87.20	87.22	87.31	87.22	87.32
4	68.77	68.73	68.77	68.78	68.73	68.76
5	65.61	65.63	65.59	65.60	65.62	65.62
Xyl (outer)						
1	105.02	105.01	105.01	105.02	104.98	105.02
2	74.37	74.34	74.36	74.38	74.37	74.40
3	76.72	76.72	76.70	76.73	76.70	76.76
4	69.90	69.89	69.89	69.92	69.88	69.92
5	66.19	66.20	66.18	66.19	66.19	66.21
Monoterpene glycoside						
1	167.65	167.65	167.63	167.64	167.63	167.64
2	127.43	127.40	127.41	127.42	127.41	127.45
3	142.51	142.51	142.50	142.52	142.50	142.51
4	23.46	23.46	23.40	23.45	23.39	23.44
5	39.81	39.80	40.36	40.36	40.37	40.39
6	79.07	79.07	79.19	79.15	79.13	79.15
7	142.15	142.15	142.03	142.01	142.02	142.01
8	114.96	114.98	114.99	115.08	115.07	115.09
9	11.87	11.94	11.86	11.86	11.86	11.87
10	23.48	23.48	23.48	23.48	23.48	23.48
Xyl						
1			97.21	97.26	97.22	97.28
2			74.66	74.66	74.67	74.69
3			75.35	75.40	75.35	75.42
4			70.43	70.47	70.43	70.48
5			66.08	66.08	66.07	66.10
Qui						
1	96.27	96.28				
2	74.91	74.92				
3	75.10	75.11				
4	76.20	76.20				
5	72.09	72.10				
6	17.98	17.98				
Monoterpene glycoside						
1	166.95	166.96	166.81	166.91	166.81	166.91
2	127.43	127.47	127.69	127.42	127.69	127.49
3	143.00	143.00	143.02	143.05	142.80	143.05
4	23.12	23.12	23.18	23.11	23.20	23.11
5	40.89	40.90	40.55	40.91	40.56	40.91
6	71.74	71.74	79.11	71.74	79.11	71.71
7	145.58	145.59	143.15	145.58	143.02	145.60
8	111.36	111.36	114.52	111.36	114.99	111.36
9	12.01	12.02	11.99	12.00	11.99	12.01
10	27.53	27.55	23.30	23.30	27.53	27.55
Qui						
1			96.28		96.29	
2			74.91		74.92	
3			75.06		75.07	
4			76.19		76.19	
5			72.09		72.09	
6			17.97		17.97	
Monoterpene carboxylic acid						
1			166.99		166.99	
2			127.46		127.46	
3			143.55		143.46	
4			22.92		22.93	
5			40.89		40.90	
6			71.78		71.79	
7			145.56		145.56	
8			111.36		111.35	
9			12.06		12.06	
10			27.53		27.53	

a) The spectra of compounds **1**—**6** were measured in $C_5D_5N:D_2O$ (9:1) and that of compound **11** in C_5D_5N.

xylopyranosyl-2,7-octadienoyl-(1→6)]-β-D-glucopyranosyl} ester, as shown in Chart 1.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.[3]

Extraction and Isolation This was performed as described in a previous report.[3] The material was 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). 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 ether precipitate (1.8 g) of the methanol extract was chromatographed on Lobar RP-18 with 35% acetonitrile solution to give a crude saponin fraction. The repeated semi-preparative HPLC of crude saponin fraction separately on an Asahipak ODP-50 column (10 mm × 250 mm) with 35% acetonitrile solution yielded calliandra saponins G(**1**) (51.1 mg), H(**2**) (7.5 mg), I(**3**) (25.6 mg), J(**4**) (41.5 mg), K(**5**) (12.3 mg), and L(**6**) (13.0 mg).

Calliandra Saponin G(1) An amorphous powder, $[\alpha]_D^{27}$ −15.6° (c=3.5, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.84 (3H, s, H_3-25), 0.89 (6H, s, H_3-24, H_3-29), 0.98 (3H, s, H_3-26), 1.02 (3H, s, H_3-30), 1.11 (3H, s, H_3-23), 1.69 (3H, s, H_3-27), 5.50 (1H, br s, H-12); sugar moiety: 1.56 (3H, d, J=6.1 Hz, Rham, Me-6), 2.10 (3H, s, NHCOC$\underline{H}_3$), 4.81 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.91 (1H, d, J=8.0 Hz, GlcNAc, H-1), 4.93 (1H, d, J=5.5 Hz, inner Ara, H-1), 4.98 (1H, d, J=8.0 Hz, outer Xyl, H-1), 5.18 (1H, d, J=8.0 Hz, outer Glc, H-1), 5.30 (1H, d, J=7.4 Hz, inner Xyl, H-1), 5.71 (1H, br s, Rham, H-1), 5.88 (1H, d, J=7.3 Hz, inner Glc, H-1); monoterpene glycoside moiety: 1.40 (3H, s, H_3-10′), 1.41 (3H, s, H_3-10), 1.47 (3H, d, J=5.5 Hz, Qui, Me-6), 1.82 (3H, s, H_3-9), 1.87 (3H, s, H_3-9′), 4.75 (1H, d, J=7.9 Hz, Qui, H-1), 5.10 (1H, d, J=10.6 Hz, H_a-8′), 5.21 (1H, d, J=11.0 Hz, H_a-8), 5.30 (1H, d, J=17.7 Hz, H_b-8), 5.37 (1H, d, J=17.7 Hz, H_b-8′), 5.45 (1H, t, J=7.9 Hz, Qui, H-2), 5.89 (1H, dd, J=10.6, 17.3 Hz, H-7), 6.05 (1H, dd, J=10.5, 17.3 Hz, H-7′), 6.82 (1H, t, H-3), 7.01 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O=9:1): Table 1. FAB-MS m/z: 2176 $[M+Na]^+$. *Anal.* Calcd for $C_{102}H_{161}NO_{47}\cdot 10H_2O$: C, 52.50; H, 7.82; N, 0.60. Found: C,52.39; H,7.78; N, 0.92.

Calliandra Saponin H(2) An amorphous powder, $[\alpha]_D^{27}$ −12.8° (c=0.1, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.88 (3H, s, H_3-25), 0.92 (6H, s, H_3-24, H_3-29), 0.96 (3H, s, H_3-26), 1.02 (3H, s, H_3-30), 1.13 (3H, s, H_3-23), 1.71 (3H, s, H_3-27), 5.52 (1H, br s, H-12); sugar moiety: 1.59 (3H, d, J=6.1 Hz, Rham, Me-6), 2.01 (3H, s, outer Glc COC$\underline{H}_3$), 2.09 (3H, s, NHCOC$\underline{H}_3$), 4.69, 4.40 (each 1H, m, outer Glc H-6), 4.82 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.91 (1H, d, J=7.3 Hz, GlcNAc, H-1), 4.95 (1H, d, J=4.9 Hz, inner Ara, H-1), 4.97 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.18 (1H, d, J=8.6 Hz, outer Glc, H-1), 5.27 (1H, d, J=7.3 Hz, inner Xyl, H-1), 5.72 (1H, br s, Rham, H-1), 5.89 (1H, d, J=6.1 Hz, inner Glc, H-1); monoterpene glycoside moiety: 1.40 (3H, s, H_3-10′), 1.42 (3H, s, H_3-10), 1.48 (3H, s, J=6.1 Hz, Qui, Me-6), 1.82 (3H, s, H_3-9), 1.88 (3H, s, H_3-9′), 4.75 (1H, d, J=7.9 Hz, Qui, H-1), 5.07 (1H, d, J=11.6 Hz, H_a-8′), 5.23 (1H, d, J=11.0 Hz, H_a-8), 5.32 (1H, d, J=17.1 Hz, H_b-8), 5.44 (1H, d, J=17.7 Hz, H_b-8′), 5.89 (1H, dd, J=11.0, 17.1 Hz, H-7), 6.05 (1H, dd, J=11.0, 17.1 Hz, H-7′), 6.83 (1H, t, H-3), 7.02 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O=9:1): Table 1. FAB-MS m/z: 2218 $[M+H]^+$. *Anal.* Calcd for $C_{104}H_{163}NO_{48}\cdot 11H_2O$: C, 52.18; H, 7.79; N, 0.59. Found: C, 52.13; H, 7.65; N, 0.89.

Calliandra Saponin I(3) An amorphous powder, $[\alpha]_D^{20}$ +3.1° (c=0.6, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.84 (3H, s, H_3-25), 0.90 (6H, s, H_3-24, H_3-29), 0.98 (3H, s, H_3-26), 1.01 (3H, s, H_3-30), 1.11 (3H, s, H_3-23), 1.67 (3H, s, H_3-27), 5.50 (1H, br s, H-12); sugar moiety: 1.56 (3H, d, J=6.1 Hz, Rham, Me-6), 2.10 (3H, s, NHCOC$\underline{H}_3$), 4.81 (1H, d, J=6.7 Hz, outer Ara, H-1), 4.91 (1H, d, J=7.4 Hz, GlcNAc, H-1), 4.93 (1H, d, J=5.5 Hz, inner Ara, H-1), 4.99 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.18 (1H, d, J=7.3 Hz, outer Glc, H-1), 5.28 (1H, d, J=7.3 Hz, inner Xyl, H-1), 5.71 (1H, br s, Rham, H-1), 5.84 (1H, d, J=7.3 Hz, inner Glc, H-1); monoterpene glycoside moiety: 1.40 (6H, s, H_3-10, H_3-10″), 1.42 (3H, s, H_3-10′), 1.47 (3H, s, J=6.1 Hz, Qui, Me-6), 1.82 (3H, s, H_3-9), 1.87 (3H, s, H_3-9″), 1.88 (3H, s, H_3-9′), 4.76 (1H, d, J=8.6 Hz, Xyl, H-1), 4.78 (1H, d, J=8.0 Hz, Qui, H-1), 5.09 (1H, d, J=11.0 Hz, H_a-8″), 5.22 (1H, d, J=11.0 Hz, H_a-8), 5.24 (1H, d, J=11.0 Hz, H_a-8′), 5.27 (1H, d, J=17.7 Hz, H_b-8), 5.29 (1H, d, J=17.7 Hz, H_b-8′), 5.43 (1H, d, J=17.7 Hz, H_b-8″), 5.45 (1H, t, J=8.0 Hz, Qui, H-2), 5.82—5.89 (2H, m, H-7, H-7′), 6.05 (1H, dd, J=10.7, 17.7 Hz, H-7″), 6.82 (1H, t, H-3), 6.90 (1H, t, H-3′), 7.02 (1H, t, H-3″). ^{13}C-NMR (pyridine-d_5: D_2O=9:1): Table 1. FAB-MS m/z: 2473 $[M+Na]^+$. *Anal.* Calcd for $C_{117}H_{183}NO_{53}\cdot 10H_2O$: C, 53.39; H, 7.77; N, 0.53. Found: C, 53.29; H, 7.87; N, 0.99.

Calliandra Saponin J(4) An amorphous powder, $[\alpha]_D^{27}$ −11.1° (c=0.3, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.77 (3H, s, H_3-29), 0.79 (3H, s, H_3-25), 0.86 (3H, s, H_3-30), 0.88 (3H, s, H_3-24), 0.94 (3H, s, H_3-26), 1.13 (3H, s, H_3-23), 1.21 (3H, s, H_3-27), 5.51 (1H, br s, H-12); sugar moiety: 1.59 (3H, d, J=6.7 Hz, Rham, Me-6), 2.12 (3H, s, NHCOC$\underline{H}_3$), 4.81 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.92 (1H, d, J=7.9 Hz, GlcNAc, H-1), 4.93 (1H, d, J=5.5 Hz, inner Ara, H-1), 5.04 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.17 (1H, d, J=8.5 Hz, outer Glc, H-1), 5.29 (1H, d, J=6.7 Hz, inner Xyl, H-1), 5.72 (1H, br s, Rham, H-1), 5.92 (1H, d, J=7.3 Hz, inner Glc, H-1), 4.61, 4.82 (each 1H, m, inner Glc, H-6); monoterpene glycoside moiety: 1.40 (3H, s, H_3-10′), 1.42 (3H, s, H_3-10), 1.81 (3H, s, H_3-9), 1.85 (3H, s, H_3-9′), 4.75 (1H, d, J=7.3 Hz, Xyl, H-1), 5.09 (1H, d, J=11.0 Hz, H_a-8′), 5.22 (1H, d, J=11.0 Hz, H_a-8), 5.27 (1H, d, J=17.7 Hz, H_b-8), 5.43 (1H, d, J=16.5 Hz, H_b-8′), 5.46 (1H, t, J=7.3 Hz, Xyl, H-2), 5.86 (1H, dd, J=10.4, 17.7 Hz, H-7), 6.05 (1H, dd, J=10.4, 17.4 Hz, H-7′), 6.80 (1H, t, H-3), 7.01 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O =9:1): Table 1. FAB-MS m/z: 2144 $[M+Na]^+$. *Anal.* Calcd for $C_{101}H_{159}NO_{46}\cdot 10H_2O$: C, 52.66; H, 7.82; N, 0.61. Found: C, 52.62; H, 7.85; N, 1.03.

Calliandra Saponin K(5) An amorphous powder, $[\alpha]_D^{20}$ +7.7° (c= 1.2, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.88 (3H, s, H_3-25), 0.92 (6H, s, H_3-24, H_3-29), 0.98 (3H, s, H_3-26), 1.01 (3H, s, H_3-30), 1.13 (3H, s, H_3-23), 1.71 (3H, s, H_3-27), 5.52 (1H, br s, H-12); sugar moiety: 1.56 (3H, d, J=6.1 Hz, Rham, Me-6), 2.01 (3H, s, outer Glc COC$\underline{H}_3$), 2.09 (3H, s, NHCOC$\underline{H}_3$), 4.84 (1H, d, J=7.3 Hz, outer Ara, H-1), 4.91 (1H, d, J=7.4 Hz, GlcNAc, H-1), 4.94 (1H, d, J=4.9 Hz, inner Ara, H-1), 4.99 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.13 (1H, d, J=7.9 Hz, outer Glc, H-1), 5.28 (1H, d, J=7.3 Hz, inner Xyl, H-1), 5.71 (1H, br s, Rham, H-1), 5.90 (1H, d, J=7.3 Hz, inner Glc, H-1); monoterpene glycoside moiety: 1.40 (6H, s, H_3-10, H_3-10″), 1.42 (3H, s, H_3-10′), 1.47 (3H, s, J=5.5 Hz, Qui, Me-6), 1.82 (3H, s, H_3-9), 1.87 (3H, s, H_3-9″), 1.88 (3H, s, H_3-9′), 4.76 (1H, d, J=7.9 Hz, Xyl, H-1), 4.81 (1H, d, J=7.9 Hz, Qui, H-1), 5.10 (1H, d, J=10.4 Hz, H_a-8″), 5.21 (1H, d, J=10.4 Hz, H_a-8), 5.25 (1H, d, J=10.4 Hz, H_a-8′), 5.27 (1H, d, J=17.7 Hz, H_b-8), 5.29 (1H, d, J=17.7 Hz, H_b-8′), 5.43 (1H, d, J=17.1 Hz, H_b-8″), 5.82—5.94 (2H, m, H-7, H-7′), 6.05 (1H, dd, J=11.0, 17.1 Hz, H-7″), 6.81 (1H, t, H-3), 6.90 (1H, t, H-3′), 7.02 (1H, t, H-3″). ^{13}C-NMR (pyridine-d_5: D_2O=9:1): Table 1. FAB-MS m/z: 2515 $[M+Na]^+$. *Anal.* Calcd for $C_{119}H_{185}NO_{54}\cdot 6H_2O$: C, 54.93; H, 7.63; N, 0.53. Found: C, 55.02; H, 7.82; N, 1.31.

Calliandra Saponin L(6) An amorphous powder, $[\alpha]_D^{27}$ −17.8° (c=1.1, MeOH). ^{1}H-NMR (pyridine-d_5: D_2O=9:1) δ: aglycone moiety: 0.82 (6H, s, H_3-25, H_3-29), 0.90 (6H, s, H_3-24, H_3-30), 1.00 (3H, s, H_3-26), 1.18 (3H, s, H_3-23), 1.26 (3H, s, H_3-27), 5.50 (1H, br s, H-12); sugar moiety: 1.61 (3H, d, J=6.1 Hz, Rham, Me-6), 2.02 (3H, s, outer Glc COC$\underline{H}_3$), 2.09 (3H, s, NHCOC$\underline{H}_3$), 4.81 (1H, d, J=6.7 Hz, outer Ara, H-1), 4.91 (1H, d, J=7.9 Hz, GlcNAc, H-1), 4.94 (1H, d, J=5.5 Hz, inner Ara, H-1), 5.01 (1H, d, J=7.3 Hz, outer Xyl, H-1), 5.18 (1H, d, J=8.5 Hz, outer Glc, H-1), 5.28 (1H, d, J=7.9 Hz, inner Xyl, H-1), 5.72 (1H, br s, Rham, H-1), 5.92 (1H, d, J=7.9 Hz, inner Glc, H-1); monoterpene glycoside moiety: 1.40 (6H, s, H_3-10, H_3-10′), 1.82 (3H, s, H_3-9), 1.88 (3H, s, H_3-9′), 4.77 (1H, d, J=7.9 Hz, Xyl, H-1), 5.10 (1H, d, J=10.4 Hz, H_a-8′), 5.22 (1H, d, J=10.4 Hz, H_a-8), 5.26 (1H, d, J=17.7 Hz, H_b-8), 5.41 (1H, d, J=17.1 Hz, H_b-8′), 5.88 (1H, dd, J=10.4, 17.7 Hz, H-7), 6.05 (1H, dd, J=10.7, 17.4 Hz, H-7′), 6.82 (1H, t, H-3), 7.02 (1H, t, H-3′). ^{13}C-NMR (pyridine-d_5: D_2O=9:1): Table 1. FAB-MS m/z: 2186 $[M+Na]^+$. *Anal.* Calcd for $C_{103}H_{161}NO_{47}\cdot 11H_2O$: C, 51.55; H, 7.85; N, 0.58. Found: C, 51.44; H, 7.79; N, 0.71.

Acid Hydrolysis of Compounds 1, 3 and 4 Compound **1** (10 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 (1.5 mg). 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*-acetylglucosamine at 2:2:2:1:1:1, respectively.

Acid hydrolyses of compounds (**3**, **4**) were performed by the same method used for compound **1**. Compound **3**: Arabinose, xylose, glucose, quinovose, rhamnose, *N*-acetylglucosamine = 2:3:2:1:1:1. Compound **4**: Residue of organic layer gave oleanolic acid (1.5 mg). Arabinose, xylose, glucose, rhamnose, *N*-acetylglucosamine = 2:3:2:1:1. 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, quionovose 11.7, arabinose 14.4, *N*-acetylglucosamine 19.2, xylose 19.3, glucose 49.2. Determination of their absolute configuration was performed according to the method reported by Hara *et al.*[6]

Alkaline Hydrolyses of Calliandra Saponins G(1) and J(4) Compound **1** (25 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 monoterpene carboxylic acid (**9**) [2 mg, $[\alpha]_D^{20}$ +15.2° (c=0.5, $CHCl_3$), TLC *Rf*: 0.84 ($CHCl_3$: MeOH : H_2O = 8:3:1)], prosapogenin (**10**) [9 mg, mp 209—215 °C (dec.) $[\alpha]_D^{20}$ +8.8° (c=0.3, MeOH)] and monoterpene quinovoside (**12**) (3 mg).[3] Compound **4** (25 mg) was hydrolyzed by the same procedure as compound **1**, and then monoterpene carboxylic acid (**9**) (2 mg), prosapogenin (**11**) (10 mg) and monoterpene xyloside (**13**) (3 mg) were obtained.

3-*O*-α-L-Arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl Oleanolic Acid (11) An amorphous powder, $[\alpha]_D^{16}$ +8.0° (c=0.2, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 0.84, 0.94, 0.98, 1.02, 1.21, 1.30, 1.32 (3H, each s, CH_3), 2.14 (3H, s, $NHCOC\underline{H}_3$), 4.98 (1H, d, J=7.0 Hz, outer Ara, H-1), 5.04 (1H, d, J=8.4 Hz, GlcNAc, H-1), 5.13 (1H, d, J=5.1 Hz, inner Ara, H-1), 8.76 (1H, d, J=9.0 Hz, $N\underline{H}COCH_3$). ^{13}C-NMR (pyridine-d_5): Table 1. *Anal.* Calcd for $C_{48}H_{77}NO_{16}\cdot 2.5H_2O$: C, 59.49; H, 8.50; N, 1.45. Found: C, 59.34; H, 8.00; N, 1.33.

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