Cussosaponins A—E, Triterpene Saponins from the Leaves of *Cussonia racemosa*, a Malagasy Endemic Plant

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Five new triterpene saponins, cussosaponins A (2), B (3), C (4), D (5), and E (6), were isolated from the dried leaves of *Cussonia racemosa* BAKER. The structures of these new compounds were deduced on the basis of chemical and spectroscopic evidence.

Key words *Cussonia racemosa*; cussosaponin; triterpene saponin; Araliaceae

Cussonia racemosa BAKER (Araliaceae), one of the 40 species of the genus, is a Malagasy endemic plant widely distributed in the center and east of Madagascar. In our earlier investigation of this plant, we isolated flavonoid glycosides, *ent*-kaurane, clerodane, and labdane diterpenoid glycosides.[1,2] Although the uses of this plant do not appear in the traditional Malagasy pharmacopoeia, some species of the genus have been used to treat acne, diarrhea, syphilis, mental diseases, malaria, and rheumatism and as antispasmodic.[3,4] The present study deals with the isolation and structure elucidation of five new triterpene saponins from the leaves of the plant.

The methanolic extract of the dried leaves of *C. racemosa* was suspended in water and partitioned successively with hexane and EtOAc. The aqueous layer, after evaporation, was repeatedly chromatographed (Diaion HP-20, silica gel, LiChroprep RP-18, and then HPLC) to afford six compounds (**1**—**6**).

Compound **1** was identified as 3-*O*-β-D-glucopyranosyl betulinic acid 28-*O*-glucopyranosyl (1→6)-β-D-glucopyranosyl ester, previously isolated from *Oplopanax nakai*,[5] based on the ^{1}H- and ^{13}C-NMR data, as well as comparison of their physical data.

The absolute configuration of the component sugars of the isolated saponins was determined by acid hydrolysis of the crude saponins (see Experimental). The monosaccharides were identified as D-glucose, D-galactose, L-rhamnose, and L-arabinose.

Cussosaponin A (**2**), obtained as a white powder, $[\alpha]_D^{30}$ −14.7° (c=0.3, pyridine). The negative high-resolution (HR)-FAB-MS exhibited a quasimolecular ion peak due to $[M-H]^-$ at m/z 911.4979 ($C_{47}H_{75}O_{17}$). Acid hydrolysis of **2** gave betulinic acid (**1a**), arabinose, and glucose. The ^{1}H-NMR spectrum showed resonances of five tertiary methyl groups (δ 0.77, 0.91, 1.03, 1.10, 1.23), one methyl group attached to a double bond (δ 1.69), an exometylene (δ 4.68 and 4.85 each br s), and three anomeric protons (δ 4.75, d, J=7.0 Hz; δ 5.00, d, J=7.8 Hz; δ 6.34, d, J=7.6 Hz) ascribable to those of α-arabinopyranosyl, β-glucopyranosyl, and β-glucopyranosyl ester, respectively. The ^{13}C-NMR spectral data of **2** were very similar to those of **1** in all respects, except for the appearance of the signals of one terminal arabinopyranosyl unit (105.3, 72.8, 74.4, 69.4, 65.4) instead of the signals due to the terminal glucopyranosyl. To assign the attachment of the terminal arabinopyranosyl unit, the chemical shift of each proton of the sugar moieties was determined by correlated spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) (see Experimental). COSY correlations were observed from H-1′ to H-6′a, and H-6′b of the glucose at the 28-position (δ 6.34). Moreover, long-range correlations were shown between the following protons and carbons: the anomeric proton at δ_H 6.34 and C-28 (δ_C 174.9); the anomeric proton at δ_H 5.00 and C-3 (δ_C 88.7); and the anomeric proton of α-arabinose (δ_H 4.75) and C-6′ (δ_C 69.4), by heteronuclear multiple bond correlation (HMBC) experiments. From the above data, the structure of **2** was concluded to be 3-*O*-β-D-glucopyranosyl betulinic acid 28-*O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl ester.

The molecular formula of cussosaponins B (**3**, $C_{59}H_{96}O_{26}$) and C (**4**, $C_{59}H_{96}O_{25}$) were determined by negative HR-FAB-MS. Acid hydrolysis of **3** gave **1a**, arabinose, glucose, galactose, and rhamnose, while **1a**, arabinose, glucose, and rham-

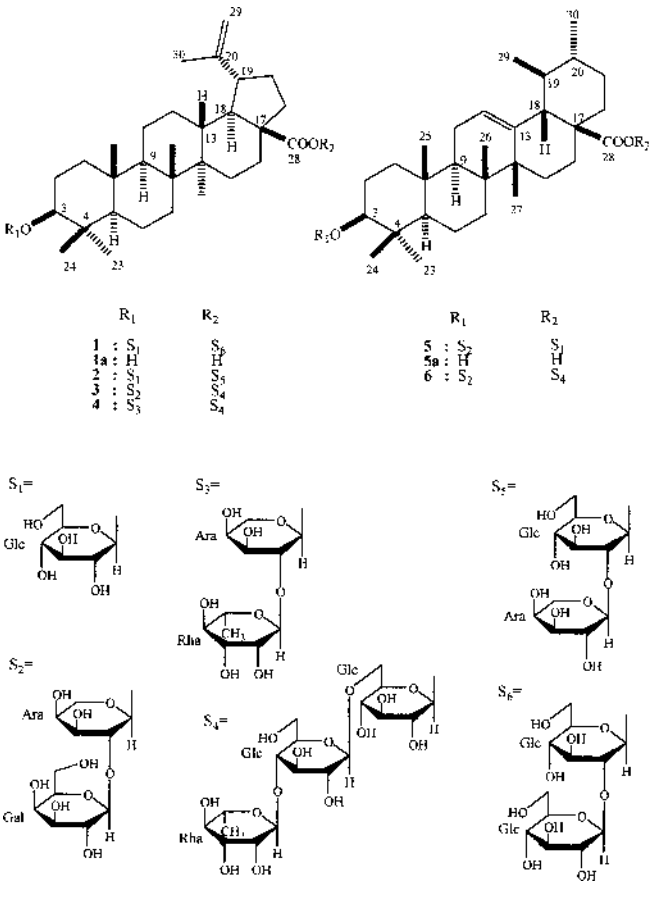

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Table 1. ^{13}C-NMR Spectral Data for the Aglycone Moieties of **2**—**6** in Pyridine-d_5 (100 MHz)

C	2	3	4	5	6
1	39.0	38.8	39.0	38.8	38.9
2	26.0	25.8	25.9	26.4	26.4
3	88.7	88.8	88.8	88.7	88.8
4	39.6	39.4	39.5	39.4	39.4
5	55.9	55.7	55.9	55.7	55.8
6	18.4	18.2	18.4	18.4	18.5
7	34.5	34.4	34.5	33.0	33.4
8	41.1	40.9	41.0	40.0	40.0
9	50.8	50.6	50.7	47.9	47.9
10	37.1	36.9	37.0	36.8	36.8
11	21.0	20.9	21.0	23.6	23.6
12	26.8	26.4	26.6	126.0	126.0
13	38.3	38.1	38.2	138.3	138.4
14	42.7	42.6	42.7	42.4	42.4
15	30.1	29.9	30.0	28.5	28.6
16	32.2	32.1	32.2	24.5	24.5
17	57.0	56.8	56.9	48.3	48.3
18	47.1	47.2	47.3	53.2	53.1
19	49.8	49.6	49.7	39.2	39.3
20	150.8	150.6	150.7	39.0	39.0
21	30.6	30.6	30.7	30.7	30.7
22	36.8	36.7	36.8	36.7	36.7
23	28.1	27.9	27.9	28.2	28.2
24	16.3	16.2	16.3	16.7	16.7
25	16.7	16.2	16.7	15.6	15.7
26	16.7	16.4	16.7	17.5	17.6
27	14.8	14.6	14.7	23.7	23.6
28	174.9	174.8	174.9	176.2	176.2
29	110.0	109.9	109.9	17.3	17.3
30	19.4	19.2	19.3	21.2	21.2

Table 2. ^{13}C-NMR Spectral Data for the Sugar Moieties of **2**—**6** in Pyridine-d_5 (100 MHz)

C	2	3	4	5	6
3-*O*-Glc or Ara					
1′	107.3	106.5	104.6	106.7	106.7
2′	75.1	81.0	75.9	81.2	81.3
3′	78.6	73.0	73.9	73.2	73.2
4′	71.5	67.8	68.4	68.0	68.0
5′	78.3	64.4	64.3	64.6	64.6
6′	62.6				
2′-*O*-Rha or Gal					
1″		104.4	101.6	104.8	104.8
2″		73.7	72.2	73.7	73.7
3″		74.9	72.4	75.0	75.1
4″		69.3	73.5	69.4	69.4
5″		76.5	69.8	76.7	76.7
6″		61.1	18.4	61.2	61.2
28-*O*-Glc					
1‴	95.2	95.0	95.2	95.6	95.5
2‴	74.5	73.6	73.5	73.1	73.7
3‴	78.0	78.3	78.2	78.7	78.6
4‴	70.9	70.5	70.7	71.1	70.8
5‴	78.4	77.7	77.8	79.0	77.8
6‴	69.4	69.1	69.3	62.2	69.3
6‴-*O*-Glc or Ara					
1⁗	105.3	104.7	104.6		104.6
2⁗	72.8	75.0	75.1		75.2
3⁗	74.4	76.0	76.3		76.4
4⁗	69.4	78.3	78.5		78.2
5⁗	65.4	76.8	77.0		77.0
6⁗		61.1	61.2		61.2
4⁗-*O*-Rha					
1⁗′		102.4	102.6		102.4
2⁗′		72.3	72.2		72.4
3⁗′		72.4	72.4		72.7
4⁗′		73.8	73.8		73.9
5⁗′		70.2	70.4		70.2
6⁗′		18.2	18.4		18.4

Glc: β-D-glucopyranosyl; Gal: β-D-galactopyranosyl; Ara: α-L-arabinopyranosyl; Rha: α-L-rhamnopyranosyl.

nose were obtained from **4**. Inspection of the ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) confirmed that **3** and **4** have the same aglycone **1a**. In addition, a set of signals ascribable to 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester was observed in the ^{13}C-NMR spectra of **3** and **4**, as found in cussonosides A and B previously isolated from *Cussonia barteri*.[4]

The ^{13}C-NMR spectrum of **3** exhibited 59 signals, 30 of which could be assigned as the aglycone of a bisdesmosidic betulinic acid and the remaining 29 as a 2-linked α-L-arabinopyranosyl, a 4-linked β-D-glucopyranosyl, a 6-linked β-D-glucopyranosyl ester, a terminal β-D-galactopyranosyl, and α-L-rhamnopyranosyl as observed in the ^{1}H-NMR (anomeric protons: δ 4.88, d, J=6.7 Hz; δ 4.90, d, J=7.8 Hz; δ 6.28, d, J=8.0 Hz; δ 4.98, d, J=7.6 Hz; δ 5.80, br s, respectively). The attachment of the α-arabinopyranosyl at position 3 of the aglycone and the β-galactopyranosyl unit at 2′ of the α-arabinopyranosyl were assigned by HSQC and HMBC experiments. Long-range coupling was observed between the anomeric proton signal at δ 4.88 and the carbon signal at δ 88.8 and between the anomeric proton signal at δ 4.98 and the carbon signal at δ 81.0.

The ^{13}C-NMR data of **4** were very similar to those of **3** except for the signal due to the sugars attached at C-3 of the aglycone. One set of signals due to the α-rhamnopyranosyl unit (δ 101.6, 72.2, 72.4, 73.5, 69.8, 18.4) was observed in **4** instead of that of the β-galactopyranosyl unit in **3**. This unit was assigned to attach at C-2′ of α-arabinopyranose since HMBC correlation was observed between the anomeric proton at δ 5.78 and the carbon signal at δ 75.9. Therefore the structures of **3** and **4** were concluded to be 3-*O*-α-D-galactopyranosyl(1→2)-α-L-arabinopyranosyl betulinic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl betulinic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester, respectively.

The molecular formula of cussosaponin D (**5**) was found to be $C_{47}H_{78}O_{17}$ by negative HR-FAB-MS displaying a peak due to $[M-H]^-$ at m/z 913.5162. The ^{1}H-NMR spectrum showed the presence of signals of one methine at δ 2.49 (d, J=10.9 Hz), one olefininic proton 5.40 (1H, t-like), five tertiary methyl groups (δ 0.85, 0.99, 1.11, 1.16, 1.19), two secondary methyl groups (δ 0.88, d, J=6.2 Hz, δ 0.91, d, J=6.4 Hz), and three anomeric protons assignable to α-arabinopyranosyl (δ 4.88, d, J=6.7 Hz), β-galactopyranosyl (δ 4.90, d, J=7.6 Hz), and β-glucopyranosyl ester (δ 6.15, d, J=8.0 Hz).

The ^{13}C-NMR spectrum exhibited 47 signals, 30 of which were assigned to be bisdesmosidic ursolic acid aglycone[6] and the 17 remaining to 2′-linked α-arabinopyranosyl, a terminal β-galactopyranosyl, and a β-glucopyranosyl ester (Tables 1, 2). On acid hydrolysis, **5** gave arabinose, glucose, and galactose as identified by TLC and an aglycone identified as

ursolic acid (**5a**) from its ^{1}H- and ^{13}C-NMR and negative FAB-MS. To confirm the attachment of the sugar moieties, HSQC and HMBC experiments were carried out. Long-range correlations were observed between H-1′ and C-3, H-1″ and C-2′, H-1‴ and C-28. On the basis of that evidence, the structure of cussosaponin D was elucidated to be 3-*O*-*β*-D-galactopyranosyl(1→2)*α*-L-arabinopyranosyl ursolic acid 28-*O*-*β*-D-glucopyranosyl ester.

Cussosaponin E (**6**), a white powder, has the molecular formula $C_{59}H_{96}O_{26}$ as determined by HR-FAB-MS. On acid hydrolysis, **6** gave ursolic acid, arabinose, galactose, glucose, and rhamnose. The ^{13}C-NMR spectrum of the sugar part of **6** was superimposable on that of **3**, while the signals of the aglycone part were in good accordance with those of **5**. Further evidence came from the HMBC spectrum, in which long-range ^{1}H–^{13}C correlations between H-1′ and C-3, H-1″ and C-2′, H-1‴ and C-28, H-1⁗ and C-6‴, and H-1⁗ and C-4⁗ were clearly displayed. Thus the structure of cussosaponin E was concluded to be 3-*O*-*α*-D-galactopyranosyl-(1→2)-*α*-L-arabinopyranosyl ursolic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

The biological activity of the isolated compounds will be the subject of our next investigation.

Experimental

NMR spectra (^{1}H, ^{13}C, COSY, HSQC, HMBC) were recorded in pyridine-d_5 using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). MS were recorded on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150×20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector at a flow rate 6 ml/min. For column chromatography, silica gel G 60 (Merck), RP-18 (50 mm, YMC), and a highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chemical Industries) were used. The solvent systems were: (I) CH_2Cl_2–MeOH–H_2O (17 : 4 : 0.5 to 17 : 8 : 2); (II) 20—100% MeOH; (III) 45% CH_3CN, and (IV) 70% MeOH. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

Plant Material Plant material was collected in March 2000 from Ranomafana-Ifanadiana, Madagascar. The identity of the plant was confirmed by Dr. Armand Rakotozafy from the Institut Malgache de Recherches Appliquées.

Extraction and Isolation The dried leaves (1.75 kg) of *C. racemosa* were extracted with MeOH. After removal of the solvent by evaporation, the residue (362.7 g) was suspended in water and extracted with hexane and EtOAc in succession. The aqueous layer (253 g) was subjected to a column of highly porous copolymer of styrene and divinylbenzene and eluted successively with H_2O, 30% MeOH, 100% MeOH, and Me_2CO. The fraction eluted with MeOH was chromatographed on a column of silica gel (system I), affording eight fractions. Fraction 2 was subjected to chromatography on RP-18 using system II to give 18 fractions. Compound **2** (68 mg) was obtained from fraction 2—15 by precipitation in MeOH. Fraction 2—16 was subjected to preparative ODS-HPLC using system III to afford compound **5** (137.4 mg). Fraction 3 produced compound **1** (19.6 mg) by ODS MPLC using system II. Preparative ODS HPLC of fractions 5 and 6 using system IV afforded compound **4** (100 mg), and compound **3** (190 mg) and **6** (507.6 mg), respectively.

Determination of the Absolute Configuration of the Component Sugar Eighty milligrams of fractions 5 and 6 was treated with 4 ml of 1 N HCl in H_2O and refluxed for 3 h. Water was added to the reaction mixture and extracted with CH_2Cl_2 and EtOAc. The water-soluble fraction was subjected to silica gel column chromatography, using CH_2Cl_2 : MeOH : H_2O (17 : 6 : 1) as eluent, to afford D-glucose $[\alpha]_D^{30}$ +65.1°, D-galactose $[\alpha]_D^{30}$ +72.1°, L-arabinose $[\alpha]_D^{30}$ +142.4°, and L-rhamnose $[\alpha]_D^{30}$ +9.1°. TLC (CH_2Cl_2 : MeOH : H_2O (17 : 6 : 1): *Rfs*: 0.13 (glc), 0.10 (gal), 0.2 (Ara), 0.29 (Rha).

Cussosaponin A (**2**): Amorphous powder, $[\alpha]_D^{30}$ −14.7° (*c*=0.3, pyridine); ^{1}H-NMR, δ (pyridine-d_5): 0.77 (3H, s, H_3-25), 0.87 (2H, m, H-1), 0.91 (3H, s, H_3-24), 1.03 (3H, s, H_3-27), 1.10 (3H, s, H_3-26), 1.23 (3H, s, H_3-23), 1.69 (3H, s, H_3-30), 3.35 (1H, dt, *J*=10.9, 4.8 Hz, H-19), 3.86 (1H, ddd, *J*=8.7, 5.3, 2.5 Hz, H-5′), 3.97 (1H, dd, *J*=7.6, 8.7 Hz, H-2′), 4.00 (1H, dd, *J*=12.3, 1.7 Hz, H-5‴a), 4.06 (1H, ddd, *J*=8.6, 5.6, 3.0 Hz, H-5″), 4.08 (1H, dd, *J*=7.6, 9.0 Hz, H-2″), 4.13 (1H, dd, *J*=8.7, 3.9 Hz, H-3‴), 4.15 (1H, dd, *J*=8.7, 8.1 Hz, H-3′), 4.17 (1H, dd, *J*=8.2, 8.6 Hz, H-4″), 4.18 (1H, m, H-4′), 4.20 (1H, m, H-3), 4.22 (1H, dd, *J*=12.3, 3.2 Hz, H-5‴a), 4.28 (1H, dd, *J*=9.0, 8.2 Hz, H-3″), 4.29 (1H, dd, *J*=11.2, 5.3 Hz, H-6′b), 4.30 (1H, ddd, *J*=3.9, 3.2, 1.7 Hz, H-4‴), 4.32 (1H, dd, *J*=11.4, 3.0 Hz, H-6″a), 4.40 (1H, dd, *J*=7.0, 8.7 Hz, H-2‴), 4.45 (1H, dd, *J*=11.4, 5.6 Hz, H-6″b), 4.46 (1H, dd, *J*=11.2, 2.5 Hz, H-6′a), 4.68 (1H, br s, H-29a), 4.75 (1H, d, *J*=7.0 Hz, H-1‴), 4.85 (1H, br s, H-29b), 5.00 (1H, d, *J*=7.8 Hz, H-1′), 6.34 (1H, d, *J*=7.6 Hz, H-1″); ^{13}C-NMR: Tables 1 and 2; negative HR-FAB-MS, *m/z* 911.4979 $[M-H]^-$ ($C_{47}H_{75}O_{17}$ requires 911.5005).

Cussosaponin B (**3**): Amorphous powder, $[\alpha]_D^{30}$ −34° (*c*=0.8, pyridine); ^{1}H-NMR, δ (pyridine-d_5): 0.71 (3H, s, H_3-25), 0.86 (2H, m, H-1), 0.94 (3H, s, H_3-24), 0.97 (3H, s, H_3-27), 1.03 (3H, s, H_3-26), 1.13 (3H, s, H_3-23), 1.60 (3H, d, *J*=6.0, H_3-6⁗), 1.66 (3H, s, H_3-30), 3.53 (1H, dt, *J*=10.9, 4.8 Hz, H-19), 4.20 (1H, m, H-3), 4.66 (1H, br s, H-29a), 4.80 (1H, br s, H-29b), 4.88 (1H, d, *J*=6.7 Hz, H-1′), 4.90 (1H, d, *J*=7.8 Hz, H-1‴), 4.98 (1H, d, *J*=7.6 Hz, H-1″), 5.80 (1H, br s, H-1⁗), 6.28 (1H, d, *J*=8.0 Hz, H-1‴); ^{13}C-NMR: Tables 1 and 2; negative HR-FAB-MS, *m/z* 1219.6132 $[M-H]^-$ ($C_{59}H_{95}O_{26}$ requires 1219.6112).

Cussosaponin C (**4**): Amorphous powder $[\alpha]_D^{30}$ −6.5° (*c*=0.4, pyridine); ^{1}H-NMR, δ (pyridine-d_5): 0.68 (3H, s, H_3-25), 0.87 (2H, m, H-1), 1.00 (3H, s, H_3-24), 1.01 (3H, s, H_3-27), 1.08 (3H, s, H_3-26), 1.11 (3H, s, H_3-23), 1.58 (3H, d, *J*=6.0 Hz, H_3-6″), 1.65 (3H, d, *J*=6.2 Hz, H_3-6⁗), 1.68 (3H, s, H_3-30), 3.37 (1H, dt, *J*=10.9, 4.8 Hz, H-19), 4.18 (1H, br d, *J*=9.7 Hz, H-3), 4.62 (1H, br s, H-29a), 4.82 (1H, br s, H-29b), 4.88 (1H, d, *J*=6.0 Hz, H-1′), 4.90 (1H, d, *J*=7.8 Hz, H-1‴), 5.78 (1H, br s, H-1″), 5.99 (1H, br s, H-1⁗), 6.29 (1H, d, *J*=8.0 Hz, H-1‴); ^{13}C-NMR: Tables 1 and 2; negative HR-FAB-MS, *m/z* 1203.6169 $[M-H]^-$ ($C_{59}H_{95}O_{25}$ requires 1203.6163).

Acid Hydrolysis of 2, 3, and 4 Twenty milligrams of each sample was hydrolyzed with 5 ml of 2 N HCl in H_2O and refluxed for 4 h. The reaction mixture was neutralized with 2 N NaOH and extracted with CH_2Cl_2. The CH_2Cl_2 extract produced 5 mg (amorphous powder) of betulinic acid (**1a**) identified by its ^{13}C-NMR spectral data. The aqueous layer was examined for sugar by direct comparison on silica gel TLC with authentic arabinose, galactose, glucose, and rhamnose. *Rfs* of authentic samples (CH_2Cl_2 : MeOH : H_2O=17 : 6 : 1): 0.14 (glc), 0.10 (gal), 0.2 (ara) and 0.31 (rha); *Rfs* of produced glc: 0.13, gal: 0.10, Ara: 0.2, Rha: 0.29. **1a**: ^{13}C-NMR, δ (ppm, $CDCl_3$): 39.0 (C-1), 27.7 (C-2), 79.0 (C-3), 39.3 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.7 (C-8), 50.5 (C-9), 37.1 (C-10), 21.1 (C-11), 25.5 (C-12), 38.4 (C-13), 42.4 (C-14), 30.6 (C-15), 32.6 (C-16), 56.3 (C-17), 46.9 (C-18), 49.3 (C-19), 150.3 (C-20), 29.7 (C-21), 37.2 (C-22), 28.1 (C-23), 15.6 (C-24), 16.1 (C-25), 16.1 (C-26), 14.7 (C-27), 183.0 (C-28), 19.4 (C-29), 109.6 (C-30).

Cussosaponin D (**5**): Amorphous powder, $[\alpha]_D^{30}$ −50° (*c*=1.2, pyridine); ^{1}H-NMR, δ (pyridine-d_5): 0.85 (3H, s, H_3-25), 0.88 (3H, d, *J*=6.2 Hz, H_3-30), 0.91 (3H, d, *J*=6.4 Hz, H_3-29), 0.99 (3H, s, H_3-24), 1.11 (3H, s, H_3-26), 1.16 (3H, s, H_3-27), 1.19 (3H, s, H_3-23), 2.49 (1H, d, *J*=10.9 Hz, H-18), 3.20 (1H, dd, *J*=11.8, 4.3 Hz, H-3), 4.88 (1H, d, *J*=6.7 Hz, H-1′), 4.90 (1H, d, *J*=7.6 Hz, H-1″), 5.40 (1H, t-like H-12), 6.15 (1H, d, *J*=8.0 Hz, H-1‴); ^{13}C-NMR: Tables 1 and 2; negative HR-FAB-MS, *m/z*: 913.5162 $[M-H]^-$ ($C_{47}H_{77}O_{17}$ requires 913.5152).

Cussosaponin E (**6**): $[\alpha]_D^{30}$ −14.8° (*c*=1.6, pyridine); ^{1}H-NMR, δ (pyridine-d_5): 0.86 (3H, d, *J*=6.2 Hz, H_3-30), 0.87 (3H, s, H_3-25), 0.90 (3H, d, *J*=6.4 Hz, H_3-29), 1.01 (3H, s, H_3-24), 1.10 (3H, s, H_3-26), 1.17 (3H, s, H_3-27), 1.21 (3H, s, H_3-23), 1.65 (3H, d, *J*=6.1 Hz, H_3-6⁗), 2.47 (1H, d, *J*=10.9 Hz, H-18), 3.20 (1H, dd, *J*=11.8, 4.3 Hz, H-3), 4.87 (1H, d, *J*=6.7 Hz, H-1′), 4.90 (1H, d, *J*=7.8 Hz, H-1‴), 4.92 (1H, d, *J*=7.6 Hz, H-1″), 5.40 (1H, t-like H-12), 5.80 (1H, br s, H-1⁗), 6.20 (1H, d, *J*=8.0 Hz, H-1‴); ^{13}C-NMR: Tables 1 and 2; negative HR-FAB-MS, *m/z*: 1219.6124 $[M-H]^-$ ($C_{59}H_{95}O_{26}$ requires 1219.6112).

Acid Hydrolysis of 5 and 6 Twenty milligrams of each compound was treated with acid in the same manner as above to give **5a** (amorphous powder, identified as ursolic acid from its ^{1}H- and ^{13}C-NMR spectral data), arabinose, glucose, and galactose from **5** and **5a**, and arabinose, glucose, galactose, and rhamnose from **6**.

Acknowledgments Liva Harinantenaina is grateful to the Japanese Ministry of Education, Science, Culture and Sport for the award of a scholarship to study for a Ph.D. at the Institute of Pharmaceutical Sciences, Hiroshima University, Japan. We also thank the Research Center for Molecular Medicine, Hiroshima University School of Medicine, Japan, for the use of

its facilities.

References

1) Harinantenaina L., Kasai R., Yamasaki K., *Chem. Pharm. Bull.*, **50**, 268—271 (2002).
2) Harinantenaina L., Kasai R., Yamasaki K., *Phytochemistry*, **60**, 339—343 (2002).
3) Samyn J.-M., "Plantes Utiles des Hautes Terres de Madagascar," Graphoprint, Madagascar, 1999, p. 16.
4) Dubois M. A., Ilyas M., Wagner H., *Planta Med.*, **2**, 80—83 (1986).
5) Wang G., Chen X., Xu J., Murayama T., Shoji J., *Yaoxue Xuebau*, **31**, 940—944 (1996).
6) Miyase T., Shiokawa K., Zhang D. M., Ueno A., *Phytochemistry*, **41**, 1411—1418 (1996).