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Clerodane and labdane diterpene glycosides from a Malagasy endemic plant, *Cussonia racemosa*

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Abstract

Four clerodane glycosides, cussosides A–D, and one labdane glycoside, cussoside E were isolated from the dried leaves of *Cussonia racemosa*, along with two known flavonol glycosides identified as rutin and kaempferol rutinoside. The structures of the compounds were deduced on the basis of their physical and spectral data.

Keywords: Araliaceae; *Cussonia racemosa*; Leaves; Cussosides A–E; Clerodane; Labdane; diterpene glycosides; Malagasy endemic plant

1. Introduction

In the continuation of our phytochemical investigation of *Cussonia racemosa* (Araliaceae), a Malagasy endemic plant, seven (**1**–**7**) compounds were obtained, these were the four new clerodane glycosides (**3**–**6**) and one new labdane glycoside (**7**) together with rutin (**1**) and kaempferol rutinoside (**2**) isolated from the leaves of the plant. The presence of clerodane diterpenes in the family Araliaceae is un-common. In our previous paper (Harinantenaina Liva et al., 2002), six new *ent*-kaurane type diterpene glycosides, named cussoracosides A–F, along with β-D-glucopyranosyl *ent*-16ß, 17-dihydroxy-kauran-19-oate (Cheng et al., 1993) and paniculoside IV (Yamasaki et al., 1977; Kaneda et al., 1978) were obtained. The present study deals with the structural elucidation of the new compounds.

2. Results and discussion

The methanol extract of the dried leaves of *Cussonia racemosa* on chromatographic separation, followed by systematic HPLC purification (see Experimental), led to the isolation of seven compounds (**1**–**7**), these were the five new diterpene glycosides (**3**–**7**) and the two flavonol glycosides rutin (**1**) and kaempferol rutinoside (**2**) (Harborne and Mabry, 1982), by comparison of physical data with literature and from spectroscopic analyses.

Compound **3**, was determined as $C_{26}H_{44}O_8$ by HR–FAB mass spectrometry. Inspection of the ^{1}H NMR spectrum (Table 1) exhibited signals ascribable to a bicyclic clerodane skeleton: three methyls at δ 0.72 (3H, *s*), δ 0.75 (3H, *d*, $J=5.7$ Hz) and δ 1.93 (3H, *s*), methylene proton resonances at δ 4.35 and δ 4.10 (each 1H, *d*, $J=9.8$ Hz), δ 4.61 (2H, *d*, $J=6.3$ Hz) and δ 4.51 (2H, *s*), two olefinic protons at δ 5.32 (1H, *br s*) and δ 5.90 (2H, *t*, $J=6.3$ Hz), and an anomeric proton at δ 4.85 (*d*, $J=7.8$ Hz). The ^{13}C NMR spectral data of **3** in CD_3OD (Table 2) indicated the signals of one β-glucopyranosyl unit together with 20 carbon signals for the aglycone moiety, which were very similar to those of jewenol A (**3a**) previously isolated from *Portulaca* cv Jewel (Ohsaki et al., 1988). However, the signal of the hydroxymethylene group at C-18 (δ_C 64.4) of **3a** was replaced by a signal of one methyl group (δ_C 20.9) at the 4-position in **3**. The other signals remained similar, except for the downfield shift of C-19 (+9.6 ppm) and the upfield shift of C-5 (−0.9 ppm) due to the glucosylation. The complete assignments were established by using HSQC, HMBC, and NOE analyses (Fig. 1). The relative configuration was confirmed as follows. In the NOESY spectrum, H-19a and H-19b were correlated with the methyl group at C-9 and the latter with the methyl group at C-8. No NOE correlation was observed between H-10 and the $C\underline{H}_2$-19. Therefore, cussoside A, (**3**) was deduced as shown.

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Table 1
^{1}H NMR spectral data for compounds **3**–**6** (400 MHz in pyridine-d_5)

H	**3**	**4**	**5**	6
3	5.32 *br s*	5.31 *br s*	5.38 *br s*	5.27 *br s*
6	1.45–1.65[a]	1.49–1.62[a]	1.42–1.60[a]	1.46–1.65[a]
7a	1.31 *m*	1.35 *m*	1.29 *m*	1.29 *m*
7b	1.45–1.65[a]	1.49–1.62[a]	1.42–1.60[a]	1.46–1.65[a]
8	1.45–1.65[a]	1.49–1.62[a]	1.42–1.60[a]	1.46–1.65[a]
10	1.45–1.65[a]	1.49–1.62[a]	1.42–1.60[a]	1.46–1.65[a]
11a	1.01–1.04[a]	0.95–1.07[a]	1.10–1.16[a]	1.02–1.05[a]
11b	1.01–1.04[a]	0.95–1.07[a]	1.10–1.16[a]	1.02–1.05[a]
14	5.90 *t* (6.3)	5.70 *t* (6.3)	5.71 *t* (6.3)	5.80 *t*(6.3)
15a,b	4.61 *d* (6.3)	4.60 *d* (6.3)	4.60 *d* (6.3)	4.61 *d*(6.3)
16a,b	4.51 *s*	4.45 *s*	4.48 *s*	4.49 *s*
17	0.75 *d* (5.7)	0.74 *d* (5.8)	0.76 *d* (5.7)	0.75 *d* (5.8)
18	1.93 *s*	1.92 *s*	1.87 *s*	1.91 *s*
19a	4.10 *d* (9.8)	4.08 *d* (9.8)	4.09 *d* (10.5)	4.07 *d*(10.0)
19b	4.35 *d* (9.8)	4.32 *d* (9.8)	4.22 *d* (10.5)	4.17 *d*(10.0)
20	0.72 *s*	0.71 *s*	0.80 *s*	0.79 *s*
19-*O* -Glc				
1′	4.85 *d* (7.8)	4.93 *d* (7.8)	4.90 *d* (7.8)	4.77 *d* (7.6)
		15-*O*-Glc	15-*O*-Api	Api
1″		4.82 *d* (7.8)	5.49 *d* (1.8)	5.68 *d* (1.6)

[a] Overlapped signals.

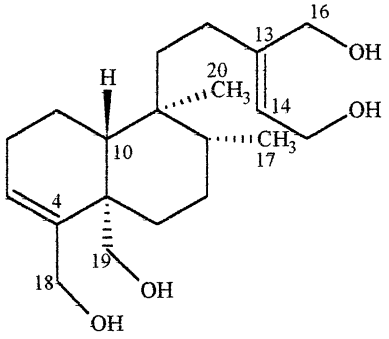
Compounds **4** and **5** (cussosides B, and C) have the molecular formula $C_{32}H_{54}O_{13}$ and $C_{31}H_{52}O_{12}$, respectively, as determined by HR–FAB-mass spectrometry.

The ^{1}H and ^{13}C NMR spectral data of **4** and **5** were correlated with those of **3**, except for the presence of additional signals due to a β-glucopyranosyl unit (δ 4.82, *d*, J=7.8 Hz) in **4** and a β-apiofuranosyl unit (δ 5.49, *d*, J=1.8 Hz) in **5**. The ^{13}C NMR spectra of **4** and **5** showed the signal of the hydroxymethylene at C-15 shifted downfield (δ 65.3 and 64.4, respectively) while that of C-14 was shifted upfield at δ 122.5, 122.9, respectively, and compared to those of **3**. This suggested that the additional glucopyranosyl or apiofuranosyl unit must be attached at C-15. HSQC, HMBC, and NOESY experiments were carried out in order to confirm the connectivities and the relative stereochemistry. Thus, the structures of **4** and **5** were determined as shown.

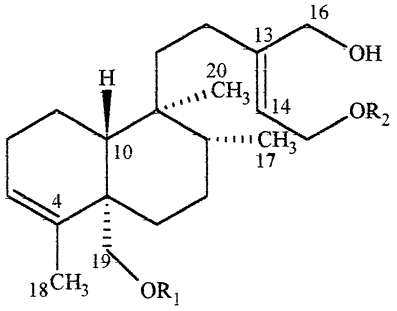
Compound **6**, named cussoside D, has the same molecular formula as **5**. The ^{1}H and ^{13}C NMR spectroscopic data of **6**, which are very similar to **3**, suggested that **6** is a clerodane diterpene glycoside with a terminal β-apiofuranose and a 6-linked β-glucopyranose. The ^{13}C NMR spectral data of the aglycone of **6** are superimposable with those of **3**. However, differences were observed in the signals due to the sugar moiety. On going from **3** to **6**, the carbon signal due to C-6 of the glucopyranosyl unit was shifted by +6.1 ppm and that of C-5 by 1.0 ppm. The biose was confirmed to be at C-19 by the observation of a HMBC correlation between the anomeric proton signal of β-glucopyranose at δ 4.77 (*d*, J =7.6 Hz) and C-19 carbon signal at δ 74.9. Based on these results, **6** could be formulated as shown.

Compound **7**, was determined as $C_{31}H_{52}O_{11}$ by HR–FAB mass spectrometry. The ^{1}H NMR (Table 1) spectrum exhibited signals due to one vinyl group, two tertiary methyl groups (δ 0.75 and 1.14), an exocyclic methylene (δ 4.84 and 4.54, each *brs*), a hydroxymethylene group attached to a quaternary carbon, two anomeric protons of β-glucopyranosyl and β-apiofuranosyl [at δ 4.75 (*d*, J =7.8 Hz) and δ 5.72 (*d*, J =1.7 Hz), respectively]. The ^{13}C NMR spectral data indicated the presence of eleven signals ascribable to one terminal β-apiofuranosyl and one 6-linked β-glucopyranosyl units as observed in **6**, together with twenty signals for the aglycone, which suggested being a labdane diterpene. On comparison of the ^{13}C NMR spectral data of **7** and *ent*-labda-8(17),13-diene-15,18-diol (**8**) (Tanaka et al., 1984), differences were recognized in the chemical shifts of C-5, C-18, C-19, except the signals due to the sugars. This data assumed that **7** and **8** are C-4 epimer. Moreover the observation of NOE correlation between the two methyls at δ 0.75 and 1.14 allowed us to conclude that the orientation of the hydroxymethylene in **7** was equatorial, hence α. The allocation of the primary hydroxyl group to be at C-14 and the exocyclic methylene at C-8(17) was deduced by HSQC, HMBC, and NOE experiments. Based on the above data, the structure of cussoside E(7) was established as shown.

The absolute configurations of all new isolated compounds were not determined because of the small amount of samples. Interestingly, the *ent*-kaurane diterpene glycosides previously isolated from *C. racemosa* could be biogenetically synthesized from labdane, as proposed in the literature (Railton et al., 1984), since compound **7** has been found in this species.



3a : Jewenol A

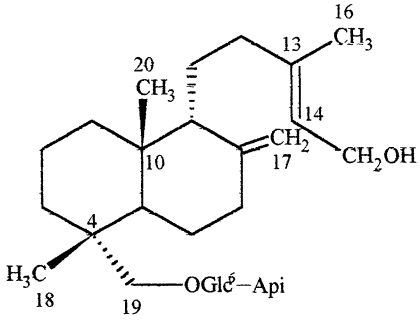


3 : R_1= Glc R_2= H

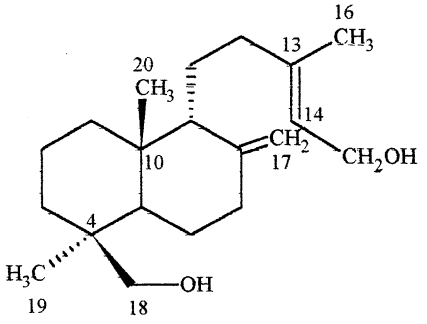
4 : R_1= Glc R_2= Glc

5 : R_1= Glc R_2= Api

6 : R_1= Glc^6–Api R_2= H



7



8

Glc: β-D-glucopyranosyl

Api: β-D-apiofuranosyl

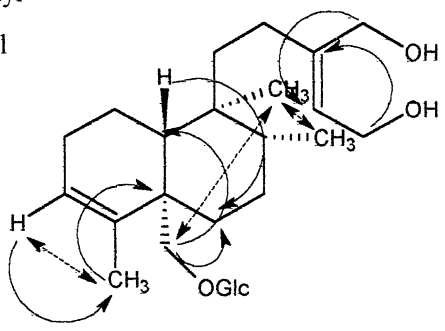


Fig. 1. Important HMBC (↷) and NOE (↷) correlations of 3.

3. Experimental

3.1. General

NMR spectra (^{1}H, ^{13}C, 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 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, flow rate 6 ml/min. For CC, silica gel G 60 (Merck), RP-18 (50 mm, YMC) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) 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) 30% CH_3CN, (IV) 45% CH_3CN, (V) 50% MeOH, (VI) 70% MeOH. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. 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 Institut Malgache de Recherches Appliquées. A voucher specimen (CUSSRAC-LI01) has been deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University Faculty of Medicine.

3.3. Extraction and isolation

The dried leaves (1.75 kg) of *C. racemosa* were extracted with hot MeOH. After removal of the solvent by evaporation, the residue (200 g) was suspended in water and extracted with hexane and EtOAc successively. The aqueous layer (45.0 g) was subjected to a column of highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, 30% MeOH, MeOH and Me_2CO, successively. The fraction eluted with MeOH was applied to a column of silica gel (system I), affording eight fractions. Fraction 1 was subjected to a CC on RP-18 and ODS-HPLC using systems II and III, respectively to afford compounds **3** (13.4 mg), **5** (7.4 mg) and **7** (12 mg). Fraction 2 was further separated on column of RP-18 using system II to give 18 fractions. Fractions 2-5 and 2-8 were similarly purified by ODS–HPLC (system V and IV, respectively) to afford compounds **2** (7 mg) and **4** (13.7 mg). Fractions 2–11 were purified by ODS–HPLC (system VI) to afford compound **6** (16.2 mg). Fraction 8 was subjected to a CC on RP-18 using system II to afford compound **1** (35.1 mg).

3.4. Cussoside A (*3*)

Amorphous powder. $[\alpha]_D^{29} = -60.7^\circ$ (MeOH; *c* 0.8); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; Negative HR–FABMS, *m/z*: $[M-H]^-$ 483.2932 ($C_{26}H_{43}O_8$ requires 483.2957).

3.5. Cussoside B (*4*)

Amorphous powder. $[\alpha]_D^{29} = -91.8^\circ$ (MeOH; *c* 0.7); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; Negative H–R-FABMS, *m/z*: $[M-H]^-$ 645.3448 ($C_{32}H_{53}O_{13}$ requires 645.3485).

3.6. Cussoside C (*5*)

Amorphous powder. $[\alpha]_D^{29} = -66.6^\circ$ (MeOH; *c* 0.2); ^{1}H NMR: Table 1 and ^{13}C NMR: Table 2; Negative H–R–FABMS, *m/z*: $[M-H]^-$ 615.3411 ($C_{31}H_{51}O_{12}$ requires 615.3380).

Table 2
^{13}C NMR spectral data for compounds **3**–**8** (100 MHz in pyridine-*d5*)

C	**3a**[a]	**3**[a]	**3**	**4**	**5**	**6**	**7**	**8**
1	18.2	18.6	18.0	18.0	18.0	18.0	39.1	38.9
2	27.2	27.5	26.8	26.8	26.9	26.7	19.3	19.3
3	128.8	123.3	122.4	122.4	122.3	122.6	36.3	36.0
4	146.3	143.7	142.9	142.9	142.9	142.6	38.5	38.5
5	43.9	43.0	42.2	42.2	42.5	42.1	56.6	48.5
6	32.1	32.9	32.6	32.6	32.9	32.8	24.7	24.5
7	28.0	28.5	27.9	27.9	27.9	27.9	38.8	38.9
8	37.5	37.7	36.6	36.6	36.7	36.6	148.6	149.0
9	39.8	39.8	38.9	38.9	38.9	38.9	56.3	56.7
10	47.6	47.9	46.8	46.8	46.7	46.8	39.8	39.8
11	38.3	38.4	37.7	37.5	37.5	37.7	22.3	22.4
12	29.1	29.1	28.5	28.3	28.4	28.4	38.7	38.5
13	143.4	143.7	143.0	145.6	145.6	142.6	137.4	137.5
14	127.2	127.3	127.2	122.5	122.9	127.2	125.9	126.0
15	58.6	58.7	58.4	65.3	64.4	58.4	58.9	59.0
16	60.0	60.2	60.0	60.0	60.0	60.0	16.4	16.5
17	16.1	16.3	16.1	16.1	16.1	16.1	106.5	106.5
18	64.4	20.9	21.1	21.2	20.7	21.1	28.1	71.4
19	65.6	75.2	74.4	74.4	74.8	74.9	72.2	18.2
20	19.3	19.2	18.9	18.9	18.8	18.9	15.6	15.2
19-*O* -Glc								
1′		105.5	105.9	106.0	105.3	105.4	104.9	
2′		75.4	75.5	75.5	75.0	75.3	75.2	
3′		77.9	78.4	78.4	78.4	78.7	78.6	
4′		71.6	71.6	71.6	71.5	71.5	71.8	
5′		78.2	78.7	78.7	78.5	77.7	77.0	
6′		62.7	62.7	62.8	62.6	68.8	68.8	
15-*O*- or 6′-*O*-				Glc	Api	Api	Api	
1″				103.5	109.3	111.0	110.9	
2″				75.2	78.3	77.7	77.8	
3″				78.4	80.0	80.4	80.4	
4″				71.9	75.0	75.1	75.0	
5″				78.5	65.7	65.7	65.7	
6″				62.8				

[a] Measured in CD_3OD.

3.7. Cussoside D (*6*)

Amorphous powder. $[\alpha]_D^{29} = -83.8^\circ$ (MeOH; *c* 1.0); ^{1}H NMR: Table 1 and ^{13}C NMR: Table 2; Negative HR–FABMS, *m/z*: $[M-H]^-$ 615.3411 ($C_{31}H_{51}O_{12}$ requires 615.3380).

3.8. Cussoside E (*7*)

Amorphous powder. $[\alpha]_D^{29} = -30.5^\circ$ (MeOH; *c* 1.6); ^{1}H NMR spectral data (pyridine-d_5): δ 5.75 (1H, *t*, $J = 6.3$ Hz, H-14), 5.72 (1H, *d*, $J = 1.7$ Hz, H-1″), 4.84 (1H, *brs*, H-17a), 4.75 (1H, *d*, $J = 7.8$ Hz, H-1′), 4.54 (1H, *brs*, H-17b), 4.47 (2H, *d*, $J = 6.3$ Hz, H-15), 4.37 (1H, *d*, $J = 9.5$ Hz, H-19a), 3.45 (1H, *d*, $J = 9.5$ Hz, H-19b), 1.67 (3H, *s*, 16-CH_3), 1.58 (1H, *brd*, $J = 10.25$ Hz, H-5), 1.16 (1H, *m*, H-9), 1.14 (3H, *s*, 18-CH_3), 0.75 (3H, *s*, 20-CH_3). ^{13}C

NMR: (Table 2). Negative HR–FABMS, m/z: $[M-H]^-$ 599.3456 ($C_{31}H_{51}O_{11}$ requires 599.3431).

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References

Cheng, D.-L., Cao, X.-P., Wei, H.-X., He, L., 1993. Kaurane diterpenoids from *Aster ageratoides*. Phytochemistry 33, 1181–1183.

Harborne, J.B., Mabry, T.J., 1982. The Flavonoids: Advances in Research. Chapman & Hall, London.

Harinantenaina Liva, R.R., Kasai, R., Yamasaki, K., 2002. Ent-kaurane diterpenoid glycosides from the leaves of *Cussonia racemosa*, a Malagasy endemic plant. Chemical and Pharmaceutical Bulletin 50, 268–271.

Kaneda, N., Kohda, H., Yamasaki, K., Tanaka, O., Nishi, K., 1978. Paniculosides-IV, diterpene-glucosides from *Stevia ovata* Lag. Chemical and Pharmaceutical Bulletin 26, 2266–2267.

Ohsaki, A., Ohno, N., Shibata, K., Tokoroyama, T., Kubota, T., Hirotsu, K., Higuchi, T., 1988. Minor diterpenoids from *Portulaca* cv Jewel. Phytochemistry 27, 2171–2173.

Railton, I.D., Fellows, B., West, C.A., 1984. *Ent*-kaurene synthesis in chloroplasts from higher plants. Phytochemistry 23, 1261–1267.

Tanaka, T., Kawamura, K., Kitahara, T., Kohda, H., Tanaka, O., 1984. *Ent*-labdane-type diterpene glucosides from leaves of *Rubus chingii*. Phytochemistry 23, 615–621.

Yamasaki, K., Kohda, H., Kobayashi, T., Kaneda, N., Kasai, R., Tanaka, O., Nishi, K., 1977. Application of ^{13}C NMR spectroscopy to chemistry of glycosides: Structures of Paniculosides-I, -II, -III, -IV, and -V, diterpene glucosides of *Stevia paniculata* Lac. Chemical and Pharmaceutical Bulletin 25, 2895–2899.