Phytochemistry 69 (2008) 2406–2410

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Ent-3,4-*seco*-labdane and *ent*-labdane diterpenoids from *Croton stipuliformis* (Euphorbiaceae)

Freddy Ramos [a,b,*], Yoshihisa Takaishi [a], Yoshiki Kashiwada [a], Coralia Osorio [b], Carmenza Duque [b], Ricardo Acuña [c], Yoshinori Fujimoto [d]

[a] *Graduate School of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan*
[b] *Departamento de Química, Universidad Nacional de Colombia, AA 14490, Bogota, Colombia*
[c] *Centro Nacional de Investigaciones del Café, Planalto Chinchina, Colombia*
[d] *Department of Chemistry and Material Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan*

ARTICLE INFO

Article history:
Received 1 October 2007
Received in revised form 4 May 2008
Available online 27 July 2008

Keywords:
Croton stipuliformis
Euphorbiaceae
Ent-labdane
Ent-3,4-*seco*-labdanes
Ent-3,4-*seco*-15-*nor*-labdanes
Absolute configuration

ABSTRACT

From a methanolic extract of the leaves of *Croton stipuliformis*, three *ent*-3,4-*seco*-labdanes (**1**–**3**) and an *ent*-labdane (**4**) together with the known compounds 6-hydroxynidorellol (**5**), maravuic acid, and sitosterol were isolated and identified from their spectroscopic data. The absolute stereochemistry of compound **4** was determined by application of Mosher's method in the NMR tube.

1. Introduction

In Colombia, *Croton* genera is represented by 80 species (Murillo, 2004). *Croton stipuliformis,* a native species known as "guacamayo"; is a 10–20 m high tree that grows at elevations from 1300 to 1800 m in the Colombian Andes (Murillo, 2004). In that region, this plant is recognized for the production of exudates that are used to cure stomach ulcers. As a part of our studies of Colombian medicinal plants (Ramos et al., 2006), the isolation of three new *ent*-3,4-*seco*-labdane diterpenes (**1**–**3**) and an *ent*-labdane (**4**) together with 6-hydroxynidorellol (**5**) (Bohlmann and Fritz, 1978), maravuic acid (Schneider et al., 1995), and sitosterol (Sakakibara et al., 1983) is presented.

2. Results and discussion

The methanolic extract of *C. stipuliformis* leaves was partitioned to obtain the hexane, CH_2Cl_2, MeOH, BuOH and water layers. After comparison of TLC profiles, the hexane and CH_2Cl_2 layers were mixed. From this mixture, three *ent*-3,4-*seco*-labdane diterpenoids (**1**–**3**), and one *ent*-labdane (**4**) were isolated.

A molecular formula of $C_{20}H_{28}O_4$ was proposed for compound **1** based on the peak obtained by HRESIMS at *m/z* 357.2026, (calcd. for $C_{20}H_{28}O_4Na$ 357.2042). The IR data indicated a carboxylic acid absorption (1649 cm^{-1}) and the absorption of an oxygen-bearing carbon (1106 and 1016 cm^{-1}). The ^1H NMR spectrum for compound **1** (Table 1) showed signals for a trisubstituted olefin (δ_H 5.59, 1H, *dd*, *J* = 4.0, 1.4 Hz), an oxygenated methylene (δ_H 4.70, 1H, *dd*, *J* = 16, 4 Hz; δ_H 4.28, 1H, *dd*, *J* = 16.4, 1.4 Hz), an oxygen-bearing methine (δ_H 4.16, 1H, *d*, *J* = 8 Hz), two vinyl methylenes (δ_H 4.92, 4.87, 4.70 and 4.55, each 1H, *s*), two methyls bound to double bonds (δ_H 1.85, 1.74, each 3H, *s*), and a methyl bound to a quaternary carbon (δ_H 0.73, 3H, *s*). From the ^{13}C NMR (Table 1) and DEPT experiments of **1**, 20 carbons were detected and assigned by HSQC to a carboxylic acid carbon (δ_C 180.5), two vinyl methylenes (δ_C 107.0, 113.6, 147.0 and 148.2), a trisubstituted olefin (δ_C 134.7, 118.3) an oxygenated methine (δ_C 80.6), an oxygenated methylene (δ_C 69.9), two methines (δ_C 50.4, 44.4), five methylenes (δ_C 37.6, 32.1, 30.2, 27.0, 25.5), three methyls (δ_C 23.6, 19.1 and 17.7) and a quaternary carbon (δ_C 41.0). These data were similar to those for maravuic acid, a 3,4-*seco*-labdane diterpene isolated from *Croton matourensis* (Schneider et al., 1995), and isolated here as the main compound present in the extract. However, some

* Corresponding author. Address: Departamento de Química, Universidad Nacional de Colombia, AA 14490, Bogota, Colombia. Tel.: +57 1 3165000x14477; fax: +57 1 3165220.
E-mail address: faramosr@unal.edu.co (F. Ramos).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.05.025

Table 1
^{1}H and ^{13}C NMR spectroscopic data in $CDCl_3$ for compounds 1 to 3 (δ_H and δ_C in ppm, J = in Hz)

	1		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	1.71, 1H, *dd*, 16, 4	32.1	1.70–1.82, 2H, *m*[c]	32.3	1.86, H, *dd*, 12.0, 4.4	32.2
1b	1.66, 2H, *m*[a]	–	1.70–1.82, 2H, *m*[c]	–	1.71, H, *m*[d]	–
2a	2.77, H, *ddd*, 16, 12.4, 5.2	27.0	2.55, 2H, *dd*, 11.6, 5.2	27.5	2.52, H, *dd*, 12.8, 5.6	27.8
2b	2.45, H, *ddd*, 16, 12.4, 4.0	–	2.55, 2H, *dd*, 11.6, 5.2	–	2.23, H, *dd*, 12.8 5.2	–
3	–	180.5	–	179.1	–	178.9
4	–	147.0	–	147.0	–	146.7
5	2.31, H, *dd*, 12.4,4.0	50.4	2.32, 2H *dd*, 12.4, 3.6	50.8	2.28, H, *dd*, 12.4, 4.4	50.7
6a	1.66, 2H, *m*[a]	30.2	1.62, 2H, *m*,	30.0	1.62, 2H, *m*	29.8
6b	1.62, H, *m*	–	1.62, 2H, *m*,	–	1.62, 2H, *m*	–
7a	2,36, H, *ddd*, 12.8, 3.6, 2.4	37.6	2.33, 2H, *dd* 12.4, 3.6	37.5	2.41, H, *dt* 12.8, 2.4	37.4
7b	2.03, H, *dt*, 12.8, 4.8	–	2.03, H, *dd*, 12.4, 4.8	–	2.02, *dd*, H, 12.8, 4.8	–
8	–	148.2		147.4		146.9
9	2.30, *t*, 10.8	44.4	2.50, H, *dd*, 9.2, 5.6	46.6	2.07, H, *dd*, 10.0, 6.0	48.6
10	-	41.0		41.1		41.2
11a	1.88, *dd*, H, 10.8, 14.4	25.5	2.73, H, *dd*, 15.6, 10.8	21.6	2.48, 2H, *m*	24.6
11b	1.77[b]	–	2.61, H, *dd*, 15.6, 2.0	–	2.48, 2H, *m*	–
12	4.16, H, *d*, 8 Hz	80.6	–	149.8	6.38, H, *t*, 6.0	154.9
13	–	134.7	–	112.8	–	139.3
14	5.59, *dd*, 4.0, 1.4	118.3	6.11, H, *d*, 1.6	113.8	9.35, H, *s*	195.2
15a	4.70, H, *dd*, 16, 4.0	69.9	7.19, H, *d*, 1.6	139.7	–	–
15b	4.28, H, *dd*, 16, 1.4	–	–	–	–	–
16	1.85, 3H, *s*	19.1	1.98, 3H, *s*	10.1	1.79, 3H, *s*	9.5
17a	4.55, H, *s*	107.0	4.59, 1H, *s*	107.7	4.44, 1H, *s*	108.9
17b	4.92, H, *s*	–	4.85, 1H, *s*	–	4.92, 1H, *s*	–
18a	4.87, H, *s*	113.6	4.89, H, *s*	113.8	4.91, H, *s*	114.7
18b	4.70, H, *s*	–	4.73, H, *s*	–	4.73, H, *s*	–
19	1.74, 3H, *s*	23.6	1.76, 3H, *s*	23.4	1.76, 3H, *s*	23.4
20	0.73, 3H, *s*	17.7	0,82, 3H, *s*	17.3	0,81, 3H, *s*	17.4

[a] May be interchangeable.
[b] Overlap with signals for methyls 16 and 19.
[c,d] Overlap with signal for methyl 19.

differences in the chemical shifts for the side chain were observed. The side-chain connectivity (Fig. 2) was deduced from the HMBC correlations shown by the C-15 oxygenated methylene protons and the correlations of the signals for the hydroxymethylene C-12 (δ_H 4.16) and the methyl C-16 (δ_H 1.85) with the olefinic carbons at δ_C 118.3, and δ_C 134.6. The absence of correlations in the HMBC between the protons and carbons at positions 12 and 15, and the molecular weight suggested the presence of the 12,15-dioxo moiety.

The relative stereochemistry of **1** was proposed based on the analysis of the NOESY spectrum, which established that ring B has a chair conformation with, H_a-17, Me-20, the isopropenyl group at C-5 and the side-chain at C-9 all alpha. The relevant correlations were observed between the protons Me-20 (δ_H 0.73),

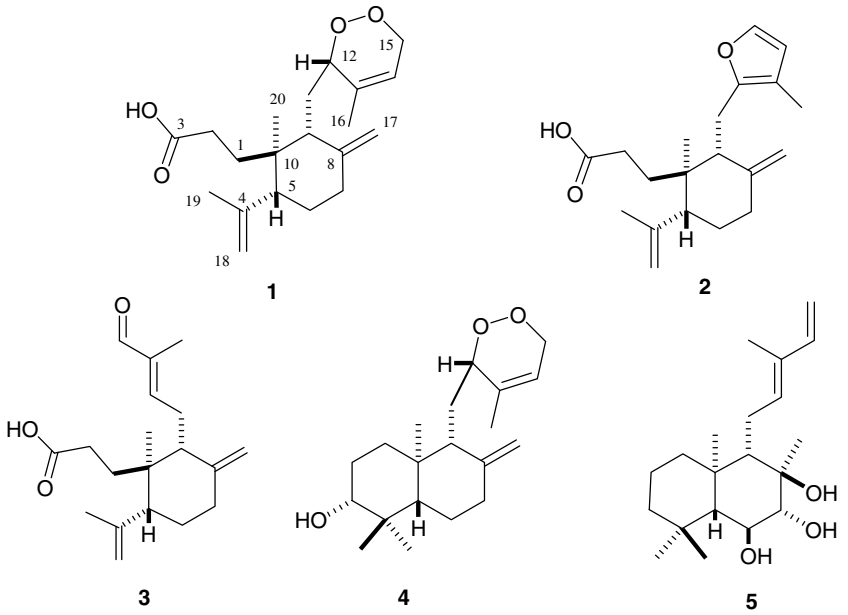

Fig. 1. Compounds isolated from *Croton stipulifomis* leaves.

H_a-17 (δ_H 4.55) and Me-19 (δ_H 1.74), and the correlations between the protons H-9 (δ_H 2.28), H-5 (δ_H 2.31), and H-12 (δ_H 4.16) (Fig. 2). From this evidence **1** was identified as *ent*-3,4-*seco*-12,15-dioxo-4,8,13-labdatrien-3-oic acid. To our knowledge there is only one report of 12,15-dioxo-labdanes in the literature, as intermediates in the synthesis of pumiloxide, and the ^{1}H-NMR data of **1** were in good agreement with those published for the dioxo moiety (Mohanraj and Herz, 1981).

Compound **2** has the molecular formula $C_{20}H_{28}O_3$ from the ion at *m/z* 339.1944 $[M+Na]^+$ obtained from the HRESIMS spectrum (calcd. for $C_{20}H_{28}O_3Na$, 339.1936). The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) showed that **2** has a 3,4-*seco*-labdane skeleton similar to that of **1**, but with differences in the side chain. The analysis of these data allowed the identification of signals for a disubstituted furan ring with resonances at δ_C 149.8 (C-12), 112.8 (C-13), δ_H 6.11, 1H, *d*, *J* = 1.6 Hz, δ_C 113.8 (C-14) and δ_H 7.19, 1H, *d*, *J* = 1.6 Hz, δ_C 139.7 (C-15), and Me-16 (δ_H 1.98, 3H, s; δ_C 10.1), in addition to the resonances for the 3,4-*seco*-labdane skeleton. The HMBC correlations of Me-16 with the carbons at C-12, C-13, C-14 led to the assignment of the furan moiety (Fig. 1) and in this way established the structure of **2**. From the NOESY spectrum, it was apparent that the ring has the same chair conformation as compound **1**, due to the correlations observed between the protons Me-20 (δ_H 0.82), H-17 (δ_H 4.59) and Me-19 (δ_H 1.76), and the correlations between the protons H-9 (δ_H 2.50), H-5 (δ_H 2.32), and H_b-18 (δ_H 4.73). This evidence allowed identification of compound **2** as *ent*-3,4-*seco*-12,15-epoxy-4,8,12,14-labdatetraen-3-oic acid.

A molecular formula of $C_{19}H_{28}O_3$ was assigned for compound **3** based on the ion at *m/z* 327.1915 $[M+Na]^+$ (calculated for $C_{19}H_{28}O_3Na$, 327.1936) obtained from the HRESIMS spectrum. The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) showed that **3** had a 3,4-*seco*-labdane skeleton similar to compounds **1** and **2**, but the differences in the side-chain and molecular weight suggest that compound **3** was a 3,4-*seco*-*nor*-labdane. The side-chain signals were assigned by HSQC to an aldehyde carbonyl (δ_H 9.35, 1H, *s*; δ_C 195.2), a trisubstituted olefin (δ_H 6.38, 1H, *t*, *J* = 6 Hz; δ_C 154.9 and δ_C 139.3), a methyl (δ_H 1.79, 3H, *s*; δ_C 9.5), and a methylene (δ_H 2.48, 2H, *m*; δ_C 24.6). Long-range COSY correlations between the olefinic proton at δ_H 6.38 and the methyl at δ_H 1.79, and from that methyl and the aldehyde proton at δ_H 9.35, indicated the presence of an α-methyl-α,β-unsaturated aldehyde moiety as side chain for **3**. The proposed substructure was confirmed by correlations observed in HMBC for the methyl protons C-16 (δ_H 1.79) with the carbonyl C-14 (δ_C 195.2), and the olefinic carbons C-12 and C-13 (δ_C 154.9 and δ_C 139.3, respectively), in addition to the correlation of the olefinic proton H-12 with the carbonyl at C-14 and the methine C-9 (δ_C 48.6) of the 3,4-*seco*-labdane skeleton. From the NOESY correlations of **3** the same relative stereochemistry as **1** and **2** can be inferred. Correlations between H_2-11, H_a-17 (δ_H 4.55) and H_3-16 (δ_H 1.79), H_3-20 (δ_H 0.81) established that the side-chain was equatorial. The NOESY correlation between the aldehyde proton at δ_H 9.35 and the olefinic proton H-12 (δ_H 6.38) and carbon chemical shift of Me-16 established the double bond configuration as *trans*. From the above evidence, **3** was identified as 3,4-*seco*-15-*nor*-14-oxo-4,8,12(*E*)-labdatrien-3-oic acid. The chemical shifts for protons and carbons of the side-chain were in good agreement with those published for 15-*nor*-14-oxo-8(17),12(*E*)-labdadiene-19-oic acid (Kobayashi et al., 1991). Some other 3,4-*seco*-labdanes were isolated from *Croton matourensis*, (Schneider et al., 1995), *C. geayi* (Palazzino et al., 1997), *Excoecaria agallocha* (Euphorbiaceae) (Anjaneyulu and Rao, 2003), *Stevia seleriana* (Eupatoriae) (Escamilla and Ortega, 1991), *Chilitotrichium rosmarinifolium* and *Nardophyllum lanatum* (Compositae, Astereae) (Jakupovic et al., 1986).

Compound **4** had the molecular formula $C_{20}H_{28}O_3$ obtained from the ion at *m/z* 302.2246 $[M-H_2O]^+$ by HREIMS. The ^{1}H NMR spectrum of compound **4** showed signals for an olefinic proton (δ_H 5.60, 1H, *s*), an *exo*-methylene (δ_H 4.89 and 4.53, each 1H, *s*), an oxygenated methylene (δ_H 4.69 and 4.31, each 1H, *d*, 15.2 Hz), two oxygenated methines (δ_H 4.16, 1H, *d*, *J* = 10 Hz; δ_H 3.31, 1H, *dd*, *J* = 12, 4.4 Hz), a methyl bound to an olefinic carbon (δ_H 1.81, 3H, *s*) and three quaternary methyls (δ_H 1.02, 0.80 and 0.72, each 3H, *s*). From the ^{13}C NMR and DEPT spectra, 20 resonances were detected and assigned by HSQC experiments to an *exo*-methylene (δ_C 106.4, 148.6), an olefin (δ_C 135.0 and 118.2), two oxygenated methines (δ_C 80.8 and 78.7), a methylene bound to a double-bond and an oxygen (δ_C 69.9), two methines (δ_C 54.3 and 51.6), four methyls (δ_C 28.3, 19.1, 15.4 and 14.6), five methylenes and two quaternary carbons. These signals showed that **4** was a labdane diterpenoid with a side-chain containing a dioxo group similar to that of compound **1**. However, the presence of the hydroxyl group and some differences in the ring A NMR spectroscopic data were observed. HMBC correlations for methyl protons at δ_H 1.02 (H_3-18) with C-4, C-5 and C-19 confirmed the presence of geminal methyls. Additionally, HMBC correlations between these geminal methyls and the hydroxymethine at δ_C 78.7 allowed the assignment of the hydroxyl to C-3, and led to structure of **4** (without stereochemistry) as in Fig. 1.

Compound **4** was shown to be an *ent*-labdane by application of the Mosher's Method. NOESY correlations established its relative configuration, through the correlations between the protons H-17b, H_2-11, H_3-20 and H_3-19, and the correlations between the protons H-3, H_3-18, H-5, and H-9. From these correlations, rings A and B were shown to have chair–chair conformations. Furthermore, correlations between H-12 and H-9, and the correlation between H-17b and the methyl at H_3-16 suggest the orientation of ring C with respect to the decalin moiety as shown in the Fig. 3a. In order to establish the absolute stereochemistry for **4**, 0.4 mg of this compound were derivatized with Mosher's reagents, (*R*) and (*S*) methoxytrifluoromethylphenylacetic chloride in the NMR tube using pyridine-d_5 (Ohtani et al., 1991; Su et al., 2002). After the data analysis, the values of $\Delta\delta_{SR}$ unambiguously indicates the absolute configuration of C-3 as *R* and identified **4** as an *ent*-labdane and allows assigning the absolute configuration for C-5*S*,

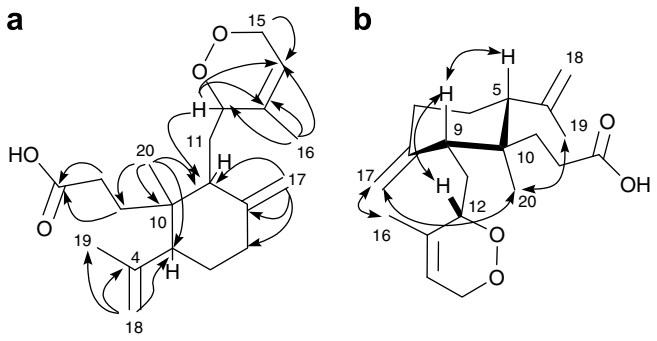

Fig. 2. Key correlations for 1. (a) HMBC and (b) NOESY.

Fig. 3. Stereochemical analyses for 4. (a) Noe correlations and (b) values of $\Delta\delta_{SR} = \delta_S - \delta_R$ measured in d_5-pyridine.

C-9*R*, C-10*S* and C-12*S*. From the above mentioned evidence, **4** was proposed to be *ent*-12,15-dioxo-8,13-labdadien-3α-ol.

The mono- and bidimensional NMR data for compound **5** as well as the negative sign of the optical rotation were in good agreement with those reported for 6-hydroxynidorellol with 6*R*, 7*S*, 8*S* stereochemistry (Bohlmann and Fritz, 1978), an *ent*-labdane related to the normal labdane austroinulin with 6*S*, 7*R*, 8*R* stereochemistry (Ohtsuki et al., 2004), but with positive sign in its optical rotation. Additionally, the ^{13}C NMR spectroscopic data for 6-hydroxynidorellol are presented here for the first time.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a JASCO DIP-370 digital polarimeter (Tokyo, Japan). IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/IR-420) (Tokyo Japan). NMR (400 MHz for ^1H, 100 MHz for ^{13}C, both use TMS as internal standard) was measured on a Bruker AVANCE 400 spectrometer (Karlsruhe, Germany). HREIMS spectra were obtained on a JEOL JMSDX-303 instrument (Tokyo, Japan). HRESIMS was obtained on Waters LCT Premier instrument. For HPLC a JASCO equipment was used with detectors UV (at 256 nm) and RI. The solid supports for column chromatography and gel permeation chromatography (GPC) were: silica gel 60 N (Spherical Neutrial, Kanto Kagaku, Tokyo, Japan), YMC GEL ODS-A (12 nm S50um), Sephadex LH-20 (Pharmacia), Toyopearl HW-40 (Tosoh Bioscience). The packed columns used were: Shodex H-2001, 2002 for GPC ($CHCl_3$); Shodex Asahipack GS310-2G for GPC (MeOH); Mightysil Si60 (250 × 20 mm; Merck) for HPLC normal phase and Mightysil RP-18 (250 × 20 mm; Merck) for HPLC-ODS.

3.2. Plant material

The leaves of *C. stipuliformis* (Euphorbiaceae) were collected in October 2002 at Chinchiná village, near the city of Manizales. A voucher specimen was identified by Dr. J. Murillo at the Instituto de Ciencias Naturales at Universidad Nacional de Colombia and registered as COL-512797.

3.3. Extraction and isolation

The dried leaves (1.3 kg) of *C. stipuliformis* were crushed and extracted three times with 3 L of MeOH at 60 °C for 3 h each time. Then, the methanolic extract was concentrated *in vacuo* to give a residue (254 g).

The methanolic extract of the *C. stipuliformis* leaves was partitioned in CH_2Cl_2 and H_2O. The organic layer was evaporated under vacuum, dissolved in a mixture of MeOH–H_2O (9:1, v/v) and partitioned with *n*-hexane to obtain the *n*-hexane layer (10.7 g). The methanolic layer was adjusted to MeOH–H_2O (1:1, v/v) and partitioned with CH_2Cl_2 to obtain the CH_2Cl_2 layer (104 g) and the MeOH layer (7.6 g).

After comparison by TLC, the *n*-hexane and CH_2Cl_2 layers (114 g) were combined and further purified by silica gel cc with a discontinuous gradient of *n*-hexane-EtOAc-MeOH (100:0:0 to 0:0:100). From this, 114 fractions were collected and pooled according to their TLC profiles in 16 fractions F1 to F16. Fraction F6 was further separated by silica gel cc using a gradient of $CHCl_3$–MeOH (100:0 to 90:10, v/v) to collect 51 fractions finally grouped in nine fractions (F6.1 to F6.9) by their TLC profiles. From these, fractions F6.3 and F6.4 (320 mg) were mixed and then applied to a Toyopearl HW-40 open column eluted with $CHCl_3$–MeOH (1:1) to yield three fractions F6.3.1 to F6.3.3. Compound **5** (6 mg) was isolated by means of silica gel cc from F6.3.3 using a gradient of CH_2Cl_2-acetone (100:0 to 98:2, v/v). Fraction F6.5 (3.6 g) was fractionated by Toyopearl HW-40 eluted with $CHCl_3$–MeOH (1:1, v/v) to yield six fractions F6.5.1 to F6.5.6; from these, β-sitosterol (63 mg) was isolated by HPLC in silica gel with $CHCl_3$–MeOH (99:1, v/v) as solvent from the fraction F6.5.2 and maravuic acid (4 g) was isolated by HPLC-GPC in $CHCl_3$ from F6.5.5. The fractions F6.6 and F6.7 (3.6 g) were mixed and fractionated by ODS open column with a discontinuous gradient of MeOH–H_2O (50:50 to 100:0, v/v) to obtain 13 fractions F6.6.1 to F6.6.13. Compounds **1** (23 mg) and **2** (3 mg) were obtained by HPLC in silica gel with CH_2Cl_2–Me_2CO (93:7, v/v) as solvent, from the fraction F6.6.13.6. Fraction F6.6.13.3 yields **3** (3 mg) isolated by HPLC-GPC with MeOH as solvent. Fraction F12 and F13 (16.3 g) were mixed and separated on a silica gel with a gradient of $CHCl_3$–MeOH (100:0 to 90:10 90:1, v/v) 42 fractions were collected and pooled in 6 fractions (F12.1 to F12.6), according with their TLC profile. F12.4 (1.9 g) was fractionated by Toyopearl HW-40 with $CHCl_3$–MeOH (1:1, v/v) to obtain 20 fractions (F12.4.1 to F12.4.20). Compound **4** (14 mg) was obtained from F12.4.9 after HPLC on a silica gel column with $CHCl_3$–MeOH (95:5, v/v) as eluent.

3.4. ent-12,15-dioxo-3,4-seco-4,8,13-labdatrien-3-oic acid (*1*)

Colorless crystals, $[\alpha]_D^{25}$ −9.2 (*c* 0.13, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3208 (O—H), 1649 (C=O), 1106(C—O), 1016(C—O); For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1. HRESIMS *m/z* 357.2026 [M+Na], calculated for $C_{20}H_{28}O_4Na$ 357.2042.

3.5. ent-12,15-epoxy-3,4-seco-4,8,12,14-labdatetraen-3-oic acid (*2*)

Obtained as a pale yellow oil, $[\alpha]_D^{25}$ + 20.3 (*c* 0.51, $CHCl_3$); IR, ν_{max}^{KBr} cm^{-1}: 3079 (O—H), 1643 (C=O), 1178(C—O), 1036 (C—O) cm^{-1}; For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data see Table 1. HR-ESIMS: *m/z* 339.1944 $[M+Na]^+$, calculated for $C_{20}H_{28}O_3Na$, 339.1936.

3.6. ent-15-nor-14-oxo-3,4-seco-4,8,12(E)-labdatrien-3-oic acid (*3*)

Obtained as a colorless oil, $[\alpha]_D^{25}$ − 2.0 (*c* 0.51, $CHCl_3$); IR, ν_{max}^{KBr} cm^{-1}: 3077 (O—H), 1643 (C=O), 1116(C—O), 1078(C—O) cm^{-1}; For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data see Table 1. HR-ESIMS: *m/z* 327.1915 $[M+Na]^+$, calculated for $C_{19}H_{28}O_3Na$, 327.1936.

3.7. ent-12,15-dioxo-8,13-labdadien-3α-ol (*4*)

Amorphous powder, $[\alpha]_D^{25}$ − 3.5 (*c* 0.45, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3348 (O—H), 1463 (C=C), 1035 (C—O) cm^{-1}; ^1H NMR (in $CDCl_3$) δ_H 5.60 (1H, *brs*, H-14), 4.89 (1H, *s*, H-17a), 4.69 (1H, *d*, *J* = 15.2 Hz, H-15a), 4.53 (1H, *s*, H-17b), 4.31 (1H, *d*, *J* = 15.2 Hz, H-15b), 4.16 (1H, *d*, *J* = 10 Hz, H-12), 3.31 (1H, *dd*, *J* = 12.0, 4.4 Hz, H-3), 2.43 (1H, *brd*, *J* = 16.0 Hz, H-7a), 2.09 (1H, *d*, *J* = 11.6 Hz, H-9), 2.04 (1H, *brd*, *J* = 12.5 Hz, H-7b), 1.93 (1H, *dd*, *J* = 15.0, 11.6 Hz, H-11a), 1.81 (3H, *s*, H_3-16), 1.77 (2H, *m*, H-1a, H-6a), 1.73(2H, *m*, H-2a, H-11b), 1.60 (1H, *brd*, 12.0 Hz, H-2b), 1.43 (1H, *dd*, 12.8, 3.8 Hz, H-6b), 1.37(1H, *m*, H-1b), 1.22 (1H, *dd*, 12.8, 2.8, H-5), 1.02 (3H, *s*, H_3-18), 0.80 (3H, *s*, H_3-19), 0.72, (3H, *s*, H_3-20). ^{13}C NMR (in $CDCl_3$) δ_C 180.5 (C-3), 148.6 (C-8), 147.0 (C-4), 135.0 (C-13), 118.2 (C-14), 106.4 (C-17) 80.6 (C-12), 78.7 (C-3) 69.9 (C-15), 54.3 (C-9), 51.6 (C-5), 49.0 (C-10), 38.0 (C-7), 36.7 (C-1), 23.9 (C-6), 27.8 (C-2), 25.3 (C-11), 28.3 (C-19), 15.4 (C-16), 14.6 (C-20). HRESIMS: *m/z* 302.2246 $[M-H_2O]^+$ (calculated for $C_{20}H_{30}O_2$, 302.2220).

3.8. Preparation of (S)-MTPA **4a** and (R)-MTPA esters **4b**

The sample (0.4 mg) was transferred to a NMR tube, kept in vacuum for 2 h and then dissolved in pyridine-d_5 400 μL under a N_2 stream. *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*R*-MTPA and *S*-MTPA chlorides, respectively, 5 μl) were added into each NMR tube and then mixed. The sample was next kept overnight at room temperature, and monitored at the mixing time, and after 12 and 24 h. The assignment was made on the basis of COSY correlations (Ohtani et al., 1991; Su et al., 2002).

3.9. (S)-MTPA ester (**4a**)

^{1}H NMR (in pyridine-d_5) δ_H 5.57 (1H, *brs*, H-14), 4.93 (1H, *s*, H-17a), 4.85 (1H, *dd*, *J* = 12.0, 4.4 Hz, H-3), 4.77 (1H, *d*, *J* = 15.2 Hz, H-15a), 4.62 (1H, *s*, H-17b), 4.41 (1H, *d*, *J* = 15.2 Hz, H-15b), 4.28 (1H, *d*, *J* = 15,2 Hz, H-12), 2.43 (1H, *brd*, *J* = 16.0 Hz, H-7a), 2.19 (1H, *brd*, *J* = 11.6 Hz, H-9), 1.73 (3H, *s*, H_3-16), 1.70 (1H, *brd*, 12.0 Hz, H-2b), 2.19 (1H, *m*, H-1b), 0.81 (3H, *s*, H_3-18), 0.76 (3H, *s*, H_3-19), 0.66, (3H, *s*, H_3-20).

3.10. (R)-MTPA ester (**4b**)

^{1}H NMR (in pyridine-d_5) δ_H 5.57 (1H, *brs*, H-14), 4.93 (1H, *s*, H-17a), 4.85 (1H, *dd*, *J* = 12.0, 4.4 Hz, H-3), 4.75 (1H, *brd*, *J* = 15.2 Hz, H-15a), 4.61 (1H, *s*, H-17b), 4.40 (1H, *d*, *J* = 15.2 Hz, H-15b), 4.27 (1H, *d*, *J* = 15,2 Hz, H-12), 1.88 (1H, *brd*, *J* = 11.6 Hz, H-9), 1.72 (3H, *s*, H_3-16), 1.55 (1H, *brd*, 12.0 Hz, H-2b), 1.88 (1H, *m*, H-1b), 0.93 (3H, *s*, H_3-18), 0.79 (3H, *s*, H_3-19), 0.62, (3H, *s*, H_3-20).

3.11. 6-hydroxynidorellol (**5**)

The ^{1}H NMR spectroscopic data where in good agreement with those published previously by Bohlmann and Fritz (1978). ^{13}C NMR (in $CDCl_3$) δ_C141.5 (C-14), 135.7 (C-12), 132.6 (C-13), 110.5 (C-15), 85.0 (C-7), 76.9 (C-8), 71.7 (C-6), 59.5 (C-9), 57.2 (C-5), 43.3 (C-3), 39.8 (C-1), 39.2 (C-10), 36.2 (C-18), 33.7 (C-4), 23.7 (C-11), 22.1 (C-19), 19.3 (C-20), 18.2 (C-2), 16.9 (C-17), 11.9 (C-16). HR-ESIMS: *m/z* 345.2426 $[M+Na]^+$, calcd. for $C_{20}H_{34}O_3Na$, 345.2406.

Acknowledgments

This study was supported by a Grant-in-Aid from the Ministry of Education, Science Sports, Culture and Technology of Japan, from the Ministry of Environment Housing and Territorial Development, and the National Coffee Growers in Colombia (Contract No 21/2002).

References

Anjaneyulu, A.S.R., Rao, V.L., 2003. *Seco* diterpenoids from *Excoecaria agallocha* L.. Phytochemistry 62, 585–589.
Bohlmann, F., Fritz, U., 1978. Neue Diterpene und Acetylenverbindungen aus *Nidorella*-Arten. Phytochemistry 17, 1769–1772.
Escamilla, E., Ortega, A., 1991. Labdane diterpenoids from *Stevia seleriana*. Phytochemistry 30, 599–602.
Jakupovic, J., Banerjee, S., Bohlmann, F., King, R.M., Robinson, H., 1986. New diterpenes from *Chiliotrichium rosmarinifolium* and *Nardophyllum lanthanum*. Tetrahedron 42, 1305–1313.
Kobayashi, M., Ishida, K., Terabayashi, S., Mitsuhasi, H., 1991. 10-Hydroxypheophytins and new norlabdane diterpene from the leaves of *Cupressus funebris* ENDL. Chemical and Pharmaceutical Bulletin 39, 3348–3349.
Mohanraj, S., Herz, W., 1981. Photosensitized oxygenation of labda-8(17),12-diene, labda-8(17),13-diene, and the biformenes. Synthesis of pumiloxide. Journal of Organic Chemistry 46, 1362–1366.
Murillo, J.A., 2004. Las Euphorbiaceae de Colombia. Biota Colombiana 5, 183–200.
Ohtani, I., Kusumi, T., Kashman, Y., Kakisawa, H., 1991. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. Journal of the American Chemical Society 113, 4092–4096.
Ohtsuki, T., Koyano, T., Kowithayakorn, T., Yamaguchi, N., Ishibayashi, M., 2004. Isolation of austroinulin possessing cell cycle inhibition activity from *Blumea glomerata* and revision of its absolute stereochemistry. Planta Medica 70, 1170–1173.
Palazzino, G., Federici, E., Rasoanaivo, P., Galeffi, C., 1997. 3,4-*seco* diterpenes from *Croton geayi*. Gazzetta Chimica Italiana 127, 311–314.
Ramos, F., Takaishi, Y., Kawazoe, K., Osorio, C., Duque, C., Acuña, R., Fujimoto, Y., Sato, M., Okamoto, M., Oshikawa, T., Ahmed, S.U., 2006. Immunosuppressive diacetylenes, ceramides and cerebrosides from Hydrocotyle leucocephala. Phytochemistry 67, 1143–1150.
Sakakibara, J., Kaiya, T., Fukuda, H., Ohki, T., 1983. 6β-Hydroxyursolic acid and other triterpenoids of *Enkianthus cernus*. Phytochemistry 22, 2553–2555.
Schneider, C., Breitmaier, E., Bayma, J.C., de França, L.F., Kneifel, H., Krebs, H.C., 1995. Maravuic acid, a new seco-labdane diterpene from Croton matourensis. Liebigs Annals 1995, 709–710.
Su, B.N., Park, E.J., Mbwambo, Z.H., Santarsiero, B.D., Mesecar, A., Fong H.H.S., Pezzuto, J.M., Kinghorn A.D., 2002. New chemical constituents of *Euphorbia quinquecostata* and absolute configuration assignment by a convenient Mosher ester procedure carried out in NMR tubes. Journal of Natural Products, 65, 1278–1282.