# Two New 3,4-Seco-*ent*-kaurenes and Other Constituents from the Costa Rican Endemic Species *Croton megistocarpus*

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Following our phytochemical studies of Costa Rican plants, we report the isolation of two new 3,4-seco-*ent*-kaurenes from the aerial parts of *Croton megistocarpus* (Euphorbiaceae). The structures of the two compounds were elucidated as 14-[(2-methylbutanoyl)oxy]-3,4-seco-*ent*-kaura-4(19),16-dien-3-oic acid (**1**) and 14-{[(2*Z*)-2-methylbut-2-enoyl]oxy}-3,4-seco-*ent*-kaura-4(19),16-dien-3-oic acid (**2**). In addition, seven known diterpene clerodanes were also isolated and identified. The structures of the compounds were elucidated by spectroscopic methods, including HR-MS, $^1$H-NMR, $^{13}$C-NMR, COSY, HMQC, HMBC, and NOESY experiments.

**Introduction.** – The *Croton* genus comprises *ca.* 1300 species of trees, shrubs, and herbs distributed in tropical areas [1]. In Costa Rica, 41 species have been identified, some of which have been used in folk medicine, *e.g.*, *C. gossypiifolius* is used in gum disease, *C. niveus* as antispasmodic and appetite stimulant, and *C. tonduzii* to treat malarial fevers [2]. This genus is a valuable source of *ent*-kaurenes [3], clerodanes [4], flavonoids [4], alkaloids [5][6], and lignans [7][8] which exhibit a wide range of pharmacological activities. Following our chemical studies of Costa Rican plants, we investigated the endemic species *Croton megistocarpus* [9], a plant that has not yet been subjected to phytochemical or pharmacological studies. In this very first study, two new 3,4-seco-*ent*-kaurenes **1**[1]) and **2**, and seven known diterpene clerodanes **3**–**9** were isolated and identified (*Fig. 1*).

**Results and Discussion.** – Compound **1**[1]) was isolated as a white powder, and its elemental composition was determined to be $C_{25}H_{39}O_4$, as deduced from its positive-ion-mode HR-MS (*m*/*z* 403.2830 ($[M+1]^+$)). Its chemical structure was assigned by the combination of spectroscopic methods including $^1$H- and $^{13}$C-NMR, COSY, NOESY, HMBC, and HMQC. The $^1$H- and $^{13}$C-NMR data (*Table*) confirmed that the molecule contained 25 C-atoms including four Me groups, ten $CH_2$, and five CH groups, four quartenary C-atoms, and two C=O groups. The correlations ($^1$H,$^1$H-COSY) between H–C(22), Me(23), $CH_2$(24), and Me(25) (sequence $MeCH_2CH(Me)$–) and the long-distance correlation of Me(23) with C(21)=O ($\delta$(C) 175.0) provided evidence for the presence of one (2-methylbutanoyl)oxy group at

[1]) Partly arbitrary atom numbering; for systematic names, see *Exper. Part.*

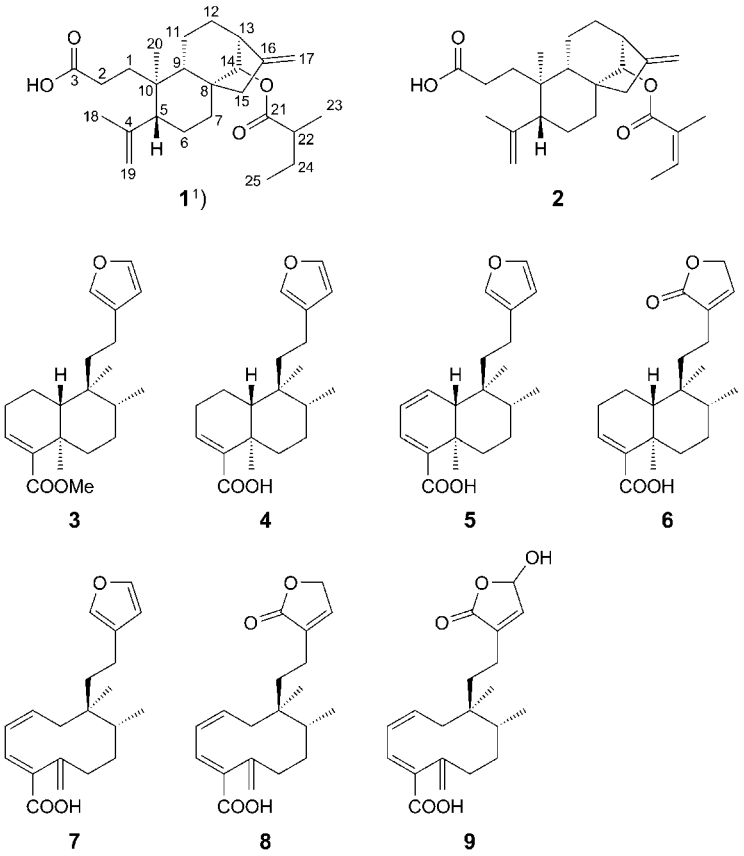


Fig. 1. *Two new 3,4-seco*-ent-*kaurenes,* **1** *and* **2***, and the known clerodanes* **3**–**9***, isolated from* C. megistocarpus

position C(14) ($\delta$(C) 68.4). In addition to this group, the presence of 20 more C-atoms suggested that compound **1** might have a diterpene skeleton. Comparison of the $^1$H- and $^{13}$C-NMR data with those of known kaurenes indicated a close structural similarity of **1** with *ent*-kaurenes isolated from *C. tonkinensis* [10–12]. To verify this observation, a detailed structural analysis was performed: The $^1$H,$^1$H-COSY plot showed correlations of an exomethylidene group, *i.e.*, $CH_2$(17) ($\delta$(H) 4.92 and 4.80 (2*s*); $\delta$(C) 103.9 and $^2J$ to C(16), $\delta$(C) 154.7), with two signals at $\delta$(H) 2.55 (*d*, 1 H of $CH_2$(15)) and 2.00 (*ddd*, 1 H of $CH_2$(15)). The HMBC spectrum inferred the presence of a CH group (C(13); $\delta$(C) 42.7; $\delta$(H) 2.45–2.47 (*m*)) allylic to the C=C bond. Long-range correlations (HMBC) were observed between C(13) and the signal of the secondary-alcohol-derived H–C(14) ($\delta$(H) 5.18 (*d*); $\delta$(C) 68.4), alcohol which is esterified with 2-methylbutanoic acid (deduced from the H–C(14) chemical shift). Moreover, other long-range correlations were observed between H–C(14) and C(8), C(9), and C(12). All these observations confirmed a similar structural fragment present in the structure of the *ent*-kaurenes. On the other hand, in the HMBC spectrum,

Table. *$^1$H- and $^{13}$C-NMR Data* (400 and 100 MHz, resp.) *of the New 3,4-Seco*-ent-*kaurenes* **1** *and* **2**, *Isolated from* C. megistocarpus.

| | **1**[a]) | | **2**[b]) | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| $CH_2$(1) | 1.79–1.82, 1.80–1.83 (2*m*) | 33.7 | 2.32–2.42, 2.47–2.55 (2*m*) | 34.6 |
| $CH_2$(2) | 2.14–2.19, 2.17–2.21 (2*m*) | 28.8 | 2.78–2.85, 2.95–3.05 (2*m*) | 29.3 |
| C(3) | | 178.8 | | 175.9 |
| C(4) | | 146.9 | | 147.7 |
| H–C(5) | 1.75–1.80 (*m*) | 50.1 | 2.08–2.10 (*m*) | 50.2 |
| $CH_2$(6) | 1.22–1.25, 1.23–1.28 (2*m*) | 39.5 | 1.70–1.78, 1.88–1.95 (2*m*) | 39.6 |
| $CH_2$(7) | 1.58–1.62, 1.87–1.92 (2*m*) | 39.7 | 2.18–2.24, 2.33–2.42 (2*m*) | 39.9 |
| C(8) | | 42.7 | | 42.9 |
| H–C(9) | 1.31–1.38 (*m*) | 52.6 | 2.47–2.55 (*m*) | 52.7 |
| C(10) | | 39.8 | | 40.1 |
| $CH_2$(11) | 1.19–1.28, 1.19–1.28 (2*m*) | 26.2 | 1.70–1.78, 2.00–2.05 (2*m*) | 26.4 |
| $CH_2$(12) | 0.88–0.98, 1.58–1.63 (2*m*) | 39.2 | 1.44–1.50, 2.18–2.21 (2*m*) | 39.3 |
| H–C(13) | 2.45–2.47 (*m*) | 42.7 | 2.95–2.99 (*m*) | 42.8 |
| H–C(14) | 5.18 (*d*, *J* = 4.8) | 68.4 | 5.85 (*d*, *J* = 5.0) | 68.9 |
| $CH_2$(15) | 2.00 (*ddd*, *J*= 16.5, 2.0, 2.0), 2.55 (br. *d*, *J* = 16.5) | 48.1 | 2.42–2.51 (*m*), 3.02–3.06 (2*m*) | 48.2 |
| C(16) | | 154.7 | | 155.2 |
| $CH_2$(17) | 4.80, 4.92 (2 br. *s*) | 103.9 | 5.17, 5.30 (2 br. *s*) | 103.8 |
| Me(18) | 1.61 (br. *s*) | 23.6 | 2.12 (br. *s*) | 23.8 |
| $CH_2$(19) | 4.67, 4.87 (2 br. *s*) | 114.4 | 5.19, 5.35 (2 br. *s*) | 114.3 |
| Me(20) | 0.64 (*s*) | 21.5 | 1.24 (*s*) | 21.8 |
| C(21) | | 175.0 | | 167.0 |
| H–C(22) or C(22) | 2.23–2.29 (*m*) | 42.0 | | 129.5 |
| Me(23) | 1.13 (*d*, *J* = 6.8) | 16.9 | 2.20 (br. *s*) | 12.1 |
| $CH_2$(24) or H–C(24) | 1.31–1.38, 1.66–1.72 (2*m*) | 26.8 | 7.31 (*ddd*, *J* = 7.0, 5.0, 2.0) | 137.0 |
| Me(25) | 0.93 (*t*, *J* = 7.5) | 12.1 | 1.96 (*dd*, *J* = 7.0, 1.0) | 14.2 |

[a]) In deuterated benzene. [b]) In deuterated pyridine.

the allylic Me(18) ($\delta$(H) 1.61 (*s*)) showed correlations with an exocyclic $CH_2$=C group ($\delta$(C) 146.9 (C(4)) and 114.4 (C(19))) and CH(5) ($\delta$(C) 50.1; $\delta$(H) 1.75–1.80 (*m*)). The Me(20) group ($\delta$(H) 0.64 (*s*)) showed correlations with C(10) ($\delta$(C) 39.8), C(1) ($\delta$(C) 33.7), C(5) ($\delta$(C) 50.1), and C(9) ($\delta$(C) 52.6). In the HSQC spectrum, C(1) ($\delta$(C) 33.7) correlated with two H-atoms at $\delta$(H) 1.79–1.82 and 1.80–1.83 (2*m*; $CH_2$(1)), which correlated in the $^1$H,$^1$H-COSY with $CH_2$(2) ($\delta$(H) 2.14–2.19 (*m*)) and in the HMBC spectrum with the C(3)OOH group ($\delta$(C) 178.8). These observations suggested that ring *A* of this *ent*-kaurene is cleaved between C(3) and C(4) revealing the structure of a 3,4-seco-*ent*-kaurene. The $^1$H,$^1$H-COSY and HMBC data enabled to complete the structure elucidation of **1**. The relative configuration of **1** was determined by NOE enhancement experiments (*Fig. 2*) which showed significant correlations of $CH_2$(17) with H–C(13) and $CH_2$(15), and correlations of H–C(14) with $CH_2$(19) and Me(20); these observations suggested a *trans* position of H–C(5) and Me(20) and a *cis*-

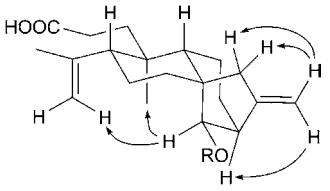


Fig. 2. *NOE Correlations of compound* **1**. R = (2-methylbutanoyl)oxy group.

decaline configuration at the fusion site of the two 6-membered rings. On the basis of the above data, the structure of compound **1** was thus established as 14-[(2-methylbutanoyl)oxy]-3,4-seco-*ent*-kaura-4(19),16-dien-3-oic acid.

Compound **2** showed NMR spectra (*Table*) similar to those of **1**; however, differences in the $^{1}$H- and $^{13}$C-NMR and NOE data suggested that instead of a (2-methylbutanoyl)oxy group in **1**, a (2*Z*)-2-methylbut-2-enoyloxy group was present in **2**. Otherwise, the $^{13}$C-NMR chemical shifts indicated the same configuration of **2** as that of **1**.

The isolation of these two new natural products from *C. megistocarpus* suggests that 3,4-seco-*ent*-kaurenes can be considered as a marker of chemotaxonomy since this diterpenoid subclass has only been reported in *Croton* species [13]. Moreover, the seven known clerodanes **3**–**9** were isolated and identified (*Fig. 1*), which established that the *Croton* genus is a valuable source of diterpenes.

Further studies to evaluate the biological activities of the new compounds are currently underway in our laboratory.

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## Experimental Part

*General.* Prep. HPLC: *Knauer* HPLC pump, type *6400*; *Econsil* $C_{18}$ column; differential refractometer detector. Column chromatography (CC): silica gel ($SiO_2$; 70–230 mesh; *Merck*). TLC: silica gel *60* $F_{254}$ (*Merck*). NMR Spectra: *Varian-Mercury-400* instrument; $\delta$ in ppm rel. to $Me_4Si$ as internal standard, *J* in Hz. HR-MS: carried out at the Mass Spectrometry Service of the Institute of Advanced Chemistry of Catalonia (IQAC-CSIC, Spain); *Acquity-UPLC* system (*Waters*) coupled to a *LCT-Premier-XE* detector (*Waters*); *Aquity-UPLC-BEH-*$C_{18}$ (*Waters*) column (1.7 μm; 2.1 × 100 mm), eluent MeCN/$H_2O$ mixtures containing 20 mM of HCOOH.

*Plant Material.* The leaves of *C. megistocarpus* were collected in Sarapiquí, Heredia Province, Costa Rica, in March, 2003. A voucher specimen has been deposited with the Herbarium Juvenal Valerio, Heredia, Costa Rica, under reference No. 12090.

*Extraction and Isolation.* Air-dried and powdered leaves (0.79 kg) were extracted with *tert*-butylmethylether ($^{t}$BuOMe)/MeOH 9:1 at r.t. for 24 h. After evaporation, the residue (14.6 g) was resuspended in $CHCl_3$. The $CHCl_3$ extract was applied to CC ($SiO_2$, hexane/$^{t}$BuOMe 85:15 → 0:100, then $^{t}$BuOMe/MeOH 90:10 and 80:20): *Frs.* $F_a$–$F_g$. *Fr.* $F_b$ was subjected to flash CC (hexane/$^{t}$BuOMe 85:15 → 0:100): *Frs.* $F_{b1}$–$F_{b20}$. *Fr.* $F_{b3}$ was separated by prep. TLC (hexane/$^{t}$BuOMe 98:2): *Frs.* $F_{b3.1}$–$F_{b3.3}$. *Fr.* $F_{b3.3}$ was repurified by prep. TLC (hexane/$^{t}$BuOMe 95:5): *hardwickiic acid methyl ester* (**3**; 21 mg) [14]. *Fr.* $F_c$ was applied to flash CC (hexane/$^{t}$BuOMe 85:15 → 0:100): *Frs.* $F_{c1}$–$F_{c20}$. *Fr.* $F_{c3}$ was fractionated by prep. TLC (hexane/$^{t}$BuOMe 97:3): to give $F_{c3.1}$ which was repurified by prep. TLC (hexane/$CH_2Cl_2$/$^{t}$BuOMe 50:45:5): *hardwickiic acid* (**4**; 56 mg) [15]. *Fr.* $F_{c4}$ was subjected to reversed-phase HPLC (MeOH/$H_2O$ 9:1): *10-β-nidoresedic acid* (**5**; 39 mg) [16] in pure form. Futhermore, in the

HPLC separation, a mixture containing *16-oxo-15,16*H-*hardwickiic acid* (**6**; 12 mg) [16], which was repurified by TLC, was eluted with hexane/$^t$BuOMe 97:3. *Fr.* $F_{c5}$ was fractionated by prep. TLC (hexane/$CH_2Cl_2$/$^t$BuOMe 50:45:5): $F_{c5.1}$ and $F_{c5.2}$. *Fr.* $F_{c5.1}$ corresponded to *strictic acid* (**7**; 81 mg) [17], and $F_{c5.2}$ was subjected to TLC (benzene/$CH_2Cl_2$/$^t$BuOMe 50:45:5): *16-oxo-15,16*H-*strictic acid* (**8**; 3 mg) [18] and *15-hydroxy-16-oxo-15,16*H-*strictic acid* (**9**; 6 mg) [18]. *Fr.* $F_d$ was applied to flash CC (hexane/$^t$BuOMe (85:15 → 0:100): *Frs.* $F_{d1} - F_{d20}$. *Fr.* $F_{d4}$ was fractionated by TLC (hexane/$^t$BuOMe 7:3): $F_{d4.1}$ and $F_{d4.2}$. *Fr.* $F_{d4.2}$ was separated by TLC (benzene/$CH_2Cl_2$/$^t$BuOMe 50:45:5): **1** (38 mg). Finally, *Fr.* $F_f$ was subjected to flash CC (hexane/$^t$BuOMe (85:15 → 0:100): *Frs.* $F_{f1} - F_{f20}$. *Fr.* $F_{f1}$ was separated by TLC (hexane/$^t$BuOMe 6:4): **2** (320 mg).

The structure determination of the isolated compounds was based on spectroscopic methods (HR-MS, 1D- and 2D-NMR), and by comparison with those reported in the literature.

*14-[(2-Methylbutanoyl)oxy]-3,4-seco*-ent-*kaura-4(19),16-dien-3-oic Acid* (**1**): White powder. $^1$H- and $^{13}$C-NMR: *Table.* HR-MS: 403.2830 ($[M+1]^+$, $C_{25}H_{39}O_4^+$; calc. 403.2849).

*14-{[(2Z)-2-Methylbut-2-enoyl]oxy}-3,4-seco*-ent-*kaura-4(19),16-dien-3-oic Acid* (**2**): White powder. $^1$H- and $^{13}$C-NMR: *Table.* HR-MS: 401.2710 ($[M+1]^+$, $C_{25}H_{37}O_4^+$; calc. 401.2692).

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