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Laevinoids A and B: Two Diterpenoids with an Unprecedented Backbone from *Croton laevigatus*

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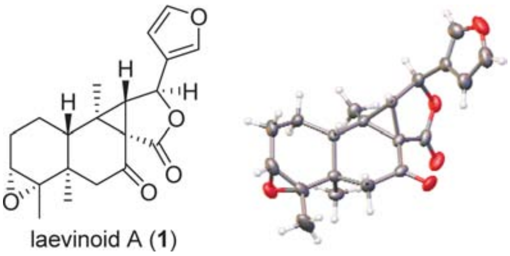
ABSTRACT



Chemical fractionation of the ethanolic extract of a Chinese herbal plant, *Croton laevigatus*, yielded laevinoids A (1) and B (2) with a new rearranged *ent*-clerodane scaffold named as laevinane. The structures of 1 and 2 were assigned on the basis of detailed spectroscopic analyses with their absolute configurations being established via single-crystal X-ray diffraction studies.

The genus *Croton* (Euphorbiaceae) consists of nearly 1300 species widely distributed in the tropical and subtropical regions all over the world.[1] Many *Croton* species have long been used as natural remedies in Africa, Asia, and South America, for the treatment of a number of ailments such as cancer and hypertension.[2] Previous chemical investigations on the *Croton* plants revealed their predominant secondary metabolites as diterpenoids including labdanes, pimaranes, and clerodanes, as well as other types of constituents such as polyphenols, alkaloids, flavonoids, etc.[2] These *Croton* metabolites exhibited a wide variety of biological properties such as antimicrobial, anti-inflammatory, and cytotoxic activities.[2]

Croton laevigatus Vahl, one of the 15 Chinese endemic species, grows as shrubs or trees mainly found in Hainan and Yunnan provinces of China.[1] It is an ethnomedicinal plant whose leaves and roots are used by the Dai people of the Xishuangbanna area to cure bone fracture, malaria, and stomach ache.[3] Recently, Zou and co-workers have reported their studies on the chemical composition of *C. laevigatus* in a series of papers, revealing the occurrence of cembrane and clerodane diterpenes, together with flavonoids and monoterpenoid glycosides.[4] Meanwhile, Li et al. have also discovered clerodane diterpenoids from this herb.[5] As part of our recent research work for bioactive agents from Chinese folk medicine,[6] we collected the branches and leaves of *C. laevigatus* from Hainan Island and performed an intensive chemical analysis. The present project revealed different chemistries in this plant of

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(1) Li, B.; Esser, H.-J. In *Flora of China*; Science Press: Beijing, 2008; Vol. 11, pp 258−264.

(2) For reviews on Croton species, see: (a) Salatino, A.; Salatino, M. L. F.; Negri, G. *J. Braz. Chem. Soc.* **2007**, *18*, 11–33. (b) Wu, X. A.; Zhao, Y. M. *Tianran Chanwu Yanjiu Yu Kaifa* **2004**, *16*, 467–472.

(3) Jiangsu New Medical College. *Dictionary of Chinese Materria Medica*; Shanghai Science & Technology Publishing House: Shanghai, 1975; pp 881−882.

(4) (a) Zou, G. A.; Ding, G.; Su, Z. H.; Yang, J. S.; Zhang, H. W.; Peng, C. Z.; Aisa, H. A.; Zou, Z. M. *J. Nat. Prod.* **2010**, *73*, 792–795. (b) Zou, G. A.; Aisa, H. A.; Zhang, H. W.; Yang, J. S.; Zou, Z. M.; Shakhidoyatov, Kh. M. *Chem. Nat. Compd.* **2012**, *48*, 687–688. (c) Zou, G. A.; Aisa, H. A.; Zhang, H. W.; Yang, J. S.; Zou, Z. M. *Chem. Nat. Compd.* **2012**, *47*, 993–994. (d) Zou, G. A.; Zhang, H. W.; Aisa, H. A.; Yang, J. S.; Peng, C. Z.; Zou, Z. M. *J. Nat. Med.* **2011**, *65*, 391–394.

(5) Li, C. S.; Zhao, J. L.; Zhang, L. X.; Gao, W. W. *Zhongchengyao* **2011**, *33*, 1740–1742.

(6) (a) Liu, H. B.; Zhang, H.; Yu, J. H.; Xu, C. H.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2012**, *75*, 722–727. (b) Zhang, H.; Zhang, C. R.; Zhu, K. K.; Gao, A. H.; Luo, C.; Li, J.; Yue, J. M. *Org. Lett.* **2013**, *15*, 120–123. (c) Han, M. L.; Shen, Y.; Wang, G. C.; Leng, Y.; Zhang, H.; Yue, J. M. *J. Nat. Prod.* **2013**, *76*, 1317–1329.

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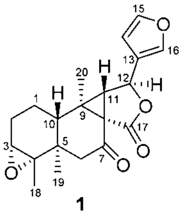
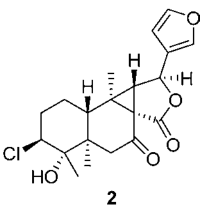
Hainan Island from those of the Xinshuangbanna area reported by Zou and Li.[4,5] We have demonstrated that the major constituents of *C. laevigatus* on Hainan Island are two diterpenoids, laevinoids A (**1**) and B (**2**), which represent a new rearranged *ent*-clerodane scaffold incorporating an unusual 3/5 bicyclic motif. The absolute structures of **1** and **2** were elucidated via MS, NMR, and X-ray crystallographic data.



1



2

The air-dried plant material of *C. laevigatus* (5 kg) was extracted three times with 95% ethanol at room temperature to afford a crude extract (230 g) which was further partitioned between EtOAc and H_2O. The EtOAc-soluble partition (110 g) was fractionated over D101 macroporous resin (EtOH/H_2O; 50%, 80%, and 95%) and MCI gel (MeOH/H_2O, from 50% to 90%) to return the subfraction containing the target molecules. Laevinoids A (**1**, 598 mg) and B (**2**, 18 mg) were purified from this subfraction via repeated column chromatography (normal-phase silica gel, reversed-phase C_{18} silica gel, and Sephadex LH-20) and semipreparative HPLC.

Laevinoid A (**1**)[7] possessed a molecular formula of $C_{20}H_{22}O_5$ with 10 degrees of unsaturation, as established by HRESI(−)MS analysis at m/z 387.1434 ($[M + HCO_2]^-$, calcd 387.1444). The IR spectrum indicated the presence of lactone (1763 cm^{-1}) and ketone (1691 cm^{-1}) functionalities. The ^1H NMR data ($CDCl_3$, Table 1) of **1** displayed diagnostic signals for three tertiary methyls (δ_H 1.17, 1.20, and 1.26) and a typical β-substituted furan ring (δ_H 6.39, 7.41, and 7.48). The ^{13}C NMR data ($CDCl_3$, Table 1) revealed 20 carbon resonances that were distinguished via DEPT and HSQC data to be three methyls, three methylenes, seven methines (including three aromatic and two oxygenated sp^3 ones), and seven quaternary carbons (including two carbonyl, one aromatic, and one oxygenated sp^3 ones). The above-mentioned analysis accounted for 5 out of 10 degrees of unsaturation, indicating five additional rings in the structure of **1** besides the furan ring.

Construction of the planar structure for **1** was accomplished by interpretation of 2D NMR data (Figure 1). Analysis of $^1H-^1H$ COSY data returned three spin systems, namely, H-10 via H_2-1 and H_2-2 to H-3, H-11 to H-12, and H-14 to H-15 of the furan ring. These structural fragments, along with other isolated groups, were connected through quaternary carbons and heteroatoms by further examination of HMBC data, especially correlations arising from the tertiary methyl protons. The presence of an A/B ring system was confirmed by the HMBC correlations of H_3-18/C-3, C-4, C-5; H_3-19/C-4, C-5, C-6, C-10; H_2-6/C-7, C-8; and H_3-20/C-8, C-9, C-10. Moreover, the 3,4-oxirane ring was supported by the chemical shifts of C-3 (δ_C 61.6) and C-4 (δ_C 61.7). The formation of a cyclopropane (C-ring) and a γ-lactone (D-ring) was implied by the HMBC cross-peaks of H_3-20/C-11; H-11/C-7, C-8, C-17; and H-12/C-8, C-9, C-17. Finally, the location of the furanyl moiety at C-12 was indicated by the HMBC correlations of H-12/C-13, C-14, C-16. The planar structure of **1** was thus established to bear a rearranged clerodane scaffold incorporating a 3-oxabicyclo[3.1.0]hexyl motif.

Table 1. 1H and ^{13}C NMR Data for laevinoids A (**1**) and B (**2**) in $CDCl_3$

	1		**2**	
no.	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C
1	α 1.58 (m) β 1.69 (m)[a]	19.4	α 1.83 (m)[a] β 1.98 (m)	26.0
2	α 2.22 (m) β 1.73 (m)[a]	26.3	α 2.23 (m) β 1.71 (m)	32.6
3	3.02 (d, 1.7)	61.6	4.31 (dd, 12.6, 4.3)	66.9
4		61.7		75.5
5		39.4		47.6
6	α 2.55 (d, 17.5) β 2.08 (br d, 17.5)	50.9	α 2.66 (d, 16.1) β 2.06 (br d, 16,1)	45.9
7		200.4		199.6
8		43.5		43.9
9		34.6		36.4
10	1.04 (dd, 12.0, 2.7)	50.3	1.81 (m)[a]	46.0
11	2.50 (br s)	49.4	2.61 (br s)	50.7
12	5.20 (br s)	70.5	5.23 (br s)	70.5
13		123.6		123.6
14	6.39 (m)	108.0	6.36 (m)	107.9
15	7.41 (t, 1.7)	144.5	7.43 (t, 1.7)	144.7
16	7.48 (m)	140.2	7.48 (m)	140.1
17		169.6		168.9
18	1.17 (s)	19.9	1.25 (s)	17.8
19	1.20 (br s)	15.8	1.17 (d, 1.0)	13.2
20	1.26 (s)	10.8	1.30 (s)	12.2

[a] Overlapping signals.

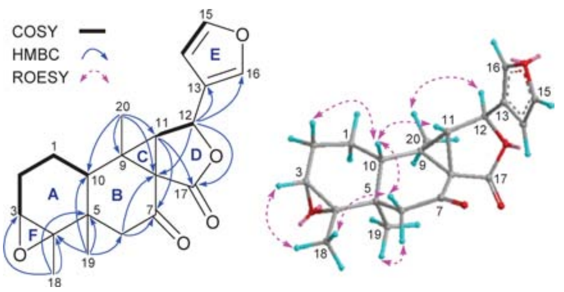


Figure 1. Key 2D NMR correlations for laevinoid A (**1**).

The relative configuration of **1** was established via the ROESY data (Figure 1). In particular, the strong ROESY

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[7] Laevinoid A (**1**): colorless crystals; mp 216−218 °C $[\alpha]^{23}_D$ 5.9 (c 0.187, MeOH); IR (KBr) ν_{max} 3136, 3059, 2968, 1763, 1691, 1506, 1329, 1020, 874 cm^{-1}; CD (MeOH) λ (Δ_ε) 195 (+12.3), 220 (−3.8) nm; ^1H and ^{13}C NMR data, see Table 1; ESI(±)MS m/z 343.2 $[M + H]^+$, 707.3 $[2 M + Na]^+$, 387.1 $[M + HCO_2]^-$; HRESI(−)MS m/z 387.1434 $[M + HCO_2]^-$ (calcd for $C_{21}H_{23}O_7$, 387.1444).

correlations of H-10 with H-6 (δ_H 2.08) and H-2 (δ_H1.73) suggested that they were axially bonded and were assigned a β-orientation. Consequently, the cross-peaks of H-10/H-11, H-6/H_3-18, and H_3-18/H-3 supported that H-3, H-11, and Me-18 were also β-oriented and the 3,4-epoxy ring was thus α-oriented. Then the observation of ROESY correlations of H-6α/H_3-19 and H_3-20/H-12 confirmed the α-orientation of Me-19, Me-20, and H-12.

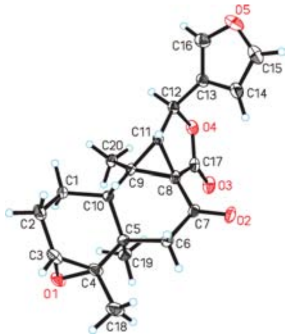

Figure 2. Structure of **1** from X-ray crystallographic analysis.

As *ent*-clerodane diterpenoids also widely exist in nature, it has proven challenging via conventional spectroscopic methods to assign which antipodal series laevinoid A (**1**) belongs to. We thus resorted to an X-ray diffraction experiment using Cu Kα radiation (λ = 1.54178 Å), as performed on a high-quality single crystal of **1** that was obtained from petroleum ether/acetone. The X-ray crystallographic data (Figure 2) corroborated the planar structure and relative configuration of **1** elucidated via NMR data and further allowed the assignment of its absolute configuration as 3*R*, 4*S*, 5*R*, 8*S*, 9*S*, 10*R*, 11*S*, 12*S* [absolute structure parameter: −0.02 (14)],[8] being a member of the *ent*-clerodane series.

Laevinoid B (**2**)[9] showed a cluster of protonated ions at *m*/*z* 379.1/381.1 with a ratio of 3:1 in the ESI(+)MS analysis, indicative of a monochlorinated compound. A molecular formula of $C_{20}H_{23}O_5Cl$ was assigned to **2** based on the HRESI(+)MS ion at *m*/*z* 379.1311 ($[M + H]^+$, calcd 379.1312), which suggested a hydrochloride adduct of **1**. Analysis of the NMR data (Table 1) confirmed this hypothesis with diagnostic signals for C-2 to C-5 being markedly shifted to the downfield ($\Delta\delta_C$ 6.3, 5.3, 13.8, and 8.2 ppm, respectively) owing to the absence of the shielding effect from the oxirane ring. This supported that compound **2** was the 3,4-epoxy ring-opening derivative of **1** by addition of a molecule of hydrogen chloride. Further examination of the 2D NMR data [Figure S19 in the Supporting Information (SI)] confirmed the structure and relative configuration of **2** as shown, bearing 3β-Cl and 4α-OH functionalities. The absolute configuration of **2** was established as 3*S*, 4*S*, 5*R*, 8*S*, 9*S*, 10*R*, 11*S*, 12*S* on the basis of the similar CD spectrum (Figure 3) to that of **1** and was secured by successful X-ray diffraction analysis [Figure 4, absolute structure parameter: −0.020(8)].[8] Compound **2** represents the first chlorinated member of the clerodane family. Although chlorine-containing compounds are common metabolites of some marine organisms, they are rare in higher plants with very limited reports.[10]

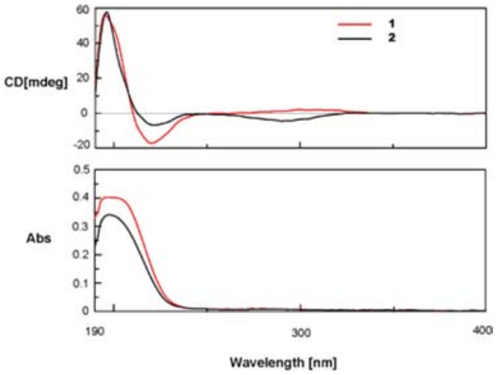

Figure 3. CD spectra for laevinoids A (**1**) and B (**2**).

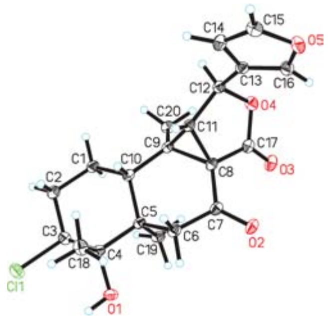

Figure 4. Structure of **2** from X-ray crystallographic analysis.

Laevinoids A (**1**) and B (**2**) are the first examples of a new rearranged *ent*-clerodane skeleton with a unique 3/5 bicyclic ring system in the clerodane family, and here we name this new skeleton laevinane. The plausible biosynthetic origin of the laevinane skeleton could be traced back to an *ent*-clerodanic precursor as shown in Scheme 1.[11] The rearrangement could be initiated by the loss of a suitable C-11 leaving group and migration of the C-8−C-9 bond, which was followed by the loss of H-8 and the formation a

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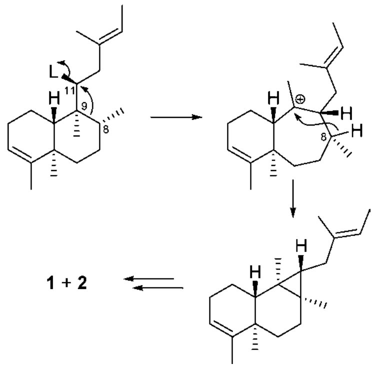
[8] Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.

[9] Laevinoid B (**2**): colorless crystals; mp 220−222 °C $[\alpha]^{23}_D$ 43.9 (*c* 0.167, MeOH); IR (KBr) ν_{max} 3575, 3161, 2956, 1772, 1687, 1504, 1325, 1070, 874 cm^{-1}; CD (MeOH) λ (Δ_ε) 196 (+13.6), 222 (−1.6) nm; ^1H and ^{13}C NMR data, see Table 1; ESI(+)MS *m*/*z* 379.1/381.1 $[M + H]^+$, 779.3/781.2/783.3 $[2M + Na]^+$; HRESI(+)MS *m*/*z* 379.1311 $[M + H]^+$ (calcd for $C_{20}H_{24}O_5Cl$, 379.1312).

[10] (a) Wang, X. N.; Yin, S.; Fan, C. Q.; Wang, F. D.; Lin, L. P.; Ding, J.; Yue, J. M. *Org. Lett.* **2006**, *8*, 3845–3848. (b) Gribble, G. W. *Chemosphere* **2003**, *52*, 289–297. (c) Zhu, Y.; Yang, L.; Jia, Z. J. *J. Nat. Prod.* **1999**, *62*, 1479–1483.

[11] Esquivel, B.; Cardenas, J.; Toscano, A.; Soriano-Garcia, M.; Rodriguez-Hahn, L. *Tetrahedron* **1985**, *41*, 3213–3217.

Scheme 1. Plausible Biosynthetic Pathway to Laevinane Skeleton



new cyclopropyl ring. Then the new backbone would undergo a series of enzyme-catalytic structural modifications as in the formation of most clerodane diterpenoids[12] to afford **1** and **2**. It is noteworthy that laevinoid A (**1**) bearing the new skeleton was produced by *C. laevigatus* as the major chemical component with a high yield (0.012% of dried plant material).

To evaluate the biological properties of this new chemical skeleton, laevinoids A (**1**) and B (**2**) were tested in an array of bioassays (Bioassay section in the Supporting Information). They showed neither cytotoxicity toward A549 and HL-60 tumor cell lines nor an inhibitory effect against protein tyrosine phosphatase 1B (PTP1B). They were also inactive in the assays to explore their potential immunosuppressive activity.

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Supporting Information Available. Experimental section (general experimental procedures, plant material, extraction and isolation, X-ray crystallographic analyses, and bioassays) and full spectroscopic data (NMR, MS, and IR) for **1** and **2** were provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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[12] (a) Merritt, A. T.; Ley, S. Y. *Nat. Prod. Rep.* **1992**, *9*, 243–287. (b) Hanson, J. R. *Nat. Prod. Rep.* **2006**, *23*, 875–885.

The authors declare no competing financial interest.