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Laevifins A–G, clerodane diterpenoids from the Bark of *Croton oblongus* Burm.f.

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ABSTRACT

A phytochemical investigation of the stem barks of the Malaysian *Croton oblongus* Burm.f. (Syn. *Croton laevifolius* Blume) (Euphorbiaceae) yielded seven previously undescribed *ent-neo*-clerodane diterpenoids, laevifins A - G and the known crovatin (**3**). Structures were established by a combination of spectroscopic methods including HRESIMS, NMR spectroscopy and X-ray crystallography. The absolute configuration of crovatin and laevifins A-G was established by comparison of experimental ECD and theoretical TDDFT ECD calculated spectra. This is the first report on the occurrence of the sesquiterpenoid cryptomeridiol in a *Croton* species. *In vitro* cytotoxicity assays on laevifins A, B and G showed moderate activities against the MCF-7 cancer cell line (IC_{50} 102, 115 and 106 μM, respectively) while β-amyrin and acetyl aleuritolic acid showed good anti-inflammatory activity on the LPS-induced NF-κB translocation inhibition in RAW 264.7 cells assay with IC_{50} values of 23.5 and 35.4 μg/mL, respectively.

1. Introduction

The genus *Croton* is the second largest of the Euphorbiaceae family, comprising about 1300 species distributed in tropical and subtropical world regions (Salatino et al., 2007). Several species are used worldwide as traditional medicines for various conditions, including gastric diseases (Craveiro et al., 1980), snake bites (Lima et al., 2010) wound healing (Pieters et al., 1993; Rao et al., 2007), rheumatism and ulcers (Rao et al., 2007; Nardi et al., 2003), diarrhoea and cancer (Rao et al., 2007), malaria (Thuong et al., 2012), diabetes, gastrointestinal disturbances and high cholesterol (Campos et al., 2002). In East Africa, Croton species are used traditionally as dietary additives and the smoke is inhaled to treat chest complaints (Aldaher et al., 2017). The genus *Croton* is known to produce a range of compounds including terpenoids, alkaloids and flavonoids, some of which have been shown to possess anti-cancer, anti-inflammatory, anti-ulcer, anti-malarial and anti-oxidant activities (Salatino et al., 2007).

Croton oblongus Burm.f. (Syn. *Croton laevifolius* Blume) (Euphorbiaceae) is a small tree found throughout the Malaysian peninsula, Borneo and Thailand (Corner, 1988; Whitmore, 1972). In the Malaysian Peninsula it is also known as "*tapin batu*", "*kayu meroyan*", "*melokan*", "*mawai*" and "*kemesak*" and is traditionally used as a postpartum medication and for the treatment of ulcers (Corner, 1939; Burkill, 1935). Two triprenylated dihydrostilbenes have been reported from the flowering parts of this species (Ahmat et al., 2007). In this study, the stem bark was investigated in order to isolate bioactive constituents.

2. Results

The *n*-hexane and CH_2Cl_2 extracts were separated using chromatographic methods. The CH_2Cl_2 extract yielded seven previously undescribed *ent-neo*-clerodane-type diterpenoids (**1, 2, 4–8**) and the known crovatin **3** (Moulis et al., 1992), acetyl aleuritolic acid, β-

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Table 1
^{1}H NMR spectroscopic data (500 MHz, $CDCl_3$) of compounds **1–4** (*J* in Hz).

Proton position	1	2	3	4
1α	2.45 m	1.78 m	1.84 m	1.82 m
1β	2.62 m	2.17 m	2.32 m	2.48 m
2α	6.02 m	1.83 m	1.56 m	1.52 m
2β	–	1.97 m	1.95 m	1.93 m
3	6.03 m	4.10 dd (5.0, 2.0)	4.48 brt ($W_{1/2}$ = 11.8)	4.53 t (5.0)
4	–	3.72 brt ($W_{1/2}$ = 4.7)	2.83 dd (5.7, 1.3)	2.91 d (5.0)
6α	2.20 m	1.64 dd (13.0, 4.5)	1.43 ddd (13.5, 5.5, 1.5)	1.57 m
6β	2.36 m	1.78 m	2.02 dt (13.5, 6.0)	2.04 d (5.8)
7α	1.28 m	1.70 m	1.34 m	2.41 m
7β	1.85 m	1.26 m	1.70 m	2.41 m
8	1.83 m	1.81 m	1.71 m	–
10	2.21 m	2.27 dd (13.5, 3.0)	2.38 m	2.48 m
11α	2.34 m	2.30 m	2.13 m	2.40 m
11β	2.34 m	2.30 m	2.23 m	2.40 m
12	5.34 t (8.0)	5.35 t (8.5)	5.31 dd (9.0, 7.0)	5.36 t (8.1)
14	6.33 b ($W_{1/2}$ = 3.9)	6.34 t (1.0)	6.37 b[a]	6.37 m
15	7.39[a]	7.38[a]	7.36 t (2.0)	7.36 m
16	7.39[a]	7.38[a]	7.37 b[a]	7.39 m
17a	1.00 d (6.5)	0.99 d (6.5)	0.93 d (7.0)	4.85 s
17b	–	–	–	4.78 s
19	–	–	5.11 d (1.0)	5.23 s
20	5.66 s	5.60 brs	5.26 brs	5.29 s
21 -OCH_3	3.82 s	3.68 s	3.69 s	3.69 s
4-OH	2.96 b			

[a] Peaks superimposed.

amyrin, β-amyrenone, cryptomeridiol, pachypodol and β-sitostenone from the *n*-hexane extract. The structures and absolute configurations of the compounds were established using spectroscopic methods including HRESIMS, NMR spectroscopy, X-ray crystallography and ECD measurements coupled with TDDFT ECD calculations and structures of previously reported compounds were confirmed by comparison against literature data.

Compound **1**, laevifin A, was isolated as colourless crystals. HRESIMS showed a $[M + H]^+$ peak at *m/z* 389.1609 (calcd. 389.1600) establishing the molecular formula as $C_{21}H_{24}O_7$ with ten degrees of unsaturation. The IR spectrum showed absorption bands for a hydroxyl group (3504 cm^{-1}), carbonyl (1749 and 1714 cm^{-1}), C—O of esters (1249 and 1163 cm^{-1}) and cyclic ethers (1096 and 1030 cm^{-1}) and a β-substituted furan ring (3145, 1505, 875 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed the characteristic resonances of a β-substituted furan ring at δ_H 7.39 (bs, 2H) and δ_H 6.33 (bs, $W_{1/2}$ = 3.9 Hz). The ^{13}C NMR spectrum (Table 3) showed twenty-one carbon resonances including one methyl (δ_C 16.4), one methoxy (δ_C 53.0), four methylene (δ_C 25.3, 29.7, 29.8, 38.8), two methine (δ_C 36.8, 39.3), an oxymethine (δ_C 74.9), a fully substituted oxygenated carbon (δ_C 73.2), two quaternary carbons (δ_C 49.1 and 50.6), two carbonyls (δ_C 171.2 and 174.8), a β-substituted furan ring (δ_C 108.1, 127.4, 139.1 and 143.9), a disubstituted alkene (δ_C 126.2 and 129.4) and one unusual downfield signal of a dioxygenated methine carbon at δ_C 105.0. The ^{1}H and ^{13}C NMR chemical shifts were in good agreement with those of furoclerodane diterpenoids with a 12, 20-ether linkage and a 19, 20-lactone ring, accounting for the downfield shift of the C-20 resonance (δ_C 105.0), as reported for compounds such as croverin (Fujita et al., 1980), crotonolide D (Liu et al., 2014) and sonderianin (Craveiro et al., 1981). The HMBC spectrum (Fig. 1a) showed correlations between the H-12 (δ_H 5.34, t, *J* = 8.0 Hz) and C-13 (δ_C 127.4), C-14 (δ_C 108.1), C-16 (δ_C 139.1) and C-9 (δ_C 49.1) resonances. In addition, C-12 (δ_C 74.9) showed a correlation with H-20 (δ_H 5.66, brs) which, in turn, correlated with C-8 (δ_C 36.8), C-9 (δ_C 49.1), C-10 (δ_C 39.3) and C-19 ester carbonyl (δ_C 171.2) resonances. The carbonyl carbon (C-19) also showed correlations with both H-6 and H-10, confirming the presence of the 12,20-ether and 19,20-lactone rings. The COSY spectrum (Fig. 1a) showed the following coupled systems: H-10/H_2-1/H-2 (δ_H 6.02)/H-3 (δ_H 6.03), indicating the presence of an alkene double bond at C-2; two H-6/two H-7/H-8/3H-17; and two H-11/H-12. Finally, the HMBC spectrum confirmed the presence of a methyl ester and hydroxy group at C-4 by correlations observed between the C-18 and H-3 and the downfield C-4 (δ_C 73.2) and H-2 resonances. Compound **1** has a similar structure to that of croverin, an *ent-neo*-clerodane from *C. levatii* (Fujita et al., 1980) but the alkene double bond occurs at C-3 in croverin as compared to C-2 in **1**. Moreover, **1** has an additional hydroxy group at C-4. The relative configuration of C-12 was deduced to be *R** by the NOESY correlation observed between H-12 and H_2-1 (Fig. 1b). The H-20 resonance showed correlations with the H-7β and Me-17 resonances indicating that the methyl group is in the β-orientation which placed H-8 in α-orientation, enabling assignment of the configuration at C-20 as *S**. Furthermore, H-8 showed correlations with H-7α and H-10 proton resonances, while C-10 also showed a correlation with the C-4-OH proton which determined the configurations at C-10 and C-4. The relative configuration was confirmed by single-crystal X-ray diffraction. The ORTEP perspective drawing is shown in Fig. 2. The absolute configuration of **1** was determined by ECD studies. The measured ECD spectrum of **1** compares well with the theoretical calcd. spectrum for *ent-neo*-clerodane, with a negative Cottons' effect at λ (Δε) 236 (−0.3) and a positive Cottons' effect at λ (Δε) 196 (+20.2), thus establishing the configurations at the chiral centres as 4R,5R,8*S*,9*S*,10*S*,12R,20*S* and confirming the structure as an *ent-neo*-clerodane (Fig. 3).

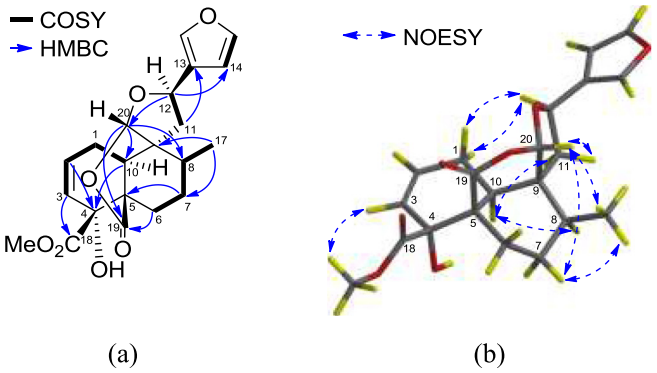

Fig. 1. (a) Selected COSY, HMBC (b) NOESY correlations of compound **1**.

The HRESIMS of compound **2**, laevifin B, isolated as white solid gave a $[M + H]^+$ peak at *m/z* 391.1772 (calcd. 391.1757) indicating a molecular formula of $C_{21}H_{26}O_7$. Compound **2** differed from **1** only in the structure of ring A. In **2**, the C-2 double bond was not present, the C-4 hydroxy group was absent, a 3β-hydroxy group was present and the

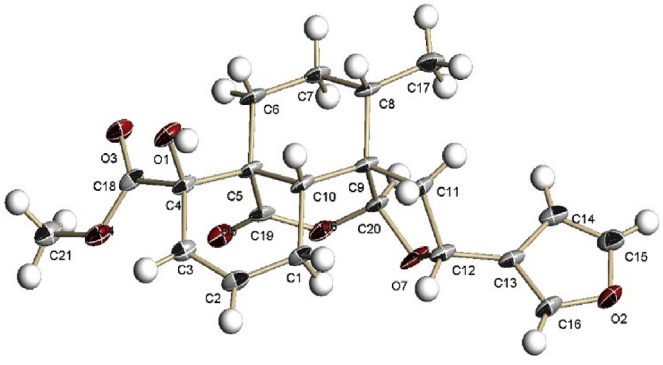

Fig. 2. ORTEP perspective structure of compound **1**.