A new halimane from Croton argyrodaphne H. Baillon

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Abstract
From the stem barks of a Madagascar endemic plant, Croton argyrodaphne H. Baillon, one new furano-diterpenoid, argyrodaphnin 3, two known terpenoids: acetyl aleuritolic acid 1, penduliflaworosin 2 and two known flavonoids, ayanin 4 and ternatin 5, were isolated and their structures were established by NMR spectroscopic methods. These compounds are described for the first time for this plant.

Keywords: Croton argyrodaphne, furano-diterpenoid, halimane, terpenoids, NMR 3-methoxyflavones

1. Introduction
The Croton genus of the Euphorbiaceae family is the largest genera of flowering plants, with between 1200 and 1300 species of herbs, shrubs, trees, and occasionally lianas. It is distributed in the tropics and subtropics worldwide [1]. About 200 species with 150 endemics have been represented in Madagascar, of which a tree Croton argyrodaphne H. Baillon is endemic to northern Madagascar and popularly known as “lazala”. The stem barks of Croton argyrodaphne is used as folk remedies to cure stomach aches, for the care of measles and jaundice and for the treatment of scabies [2]. Chemical study of Croton species has led to the isolation of alkaloids, flavonoids, triterpenoids, and a large number of diterpenoids [3]. Croton argyrodaphne H. Baillon has not previously been investigated phytochemically. In this paper we report the isolation and identification of one new furano-diterpenoid, argyrodaphnine 3, two known terpenoids: acetyl aleuritolic acid 1, penduliflaworosin 2 and two know flavonoids, ayanin 4 and ternatin 5, from ethanolic extract of the stem barks of Croton argyrodaphne (figure 1).

2. Materials and methods
2.1 Plant material
Croton argyrodaphne H. Baillon, collected in July 2013 from Ambilobe, DIANA’s Region, Madagascar, was identified to the herbarium references at Botanical and Zoological Park Tsimbazaza (PBZT, Antananarivo Madagascar) and a voucher specimen has been deposited in the “Laboratoire de Chimie des Substances Naturelles et Chimie Organique et Biologique” (LCSN/COB).

2.2 General experimental procedures
1D (1H, 13C, DEPTQ) and 2D (1H-1H COSY, 1H-13C HSQC, 1H-13C HMBC) NMR spectra
were recorded on a Bruker Varian 600 NMR operating at 600.19 MHz for $^1$H and 125.78 MHz for $^{13}$C using CDCl$_3$ as solvent. Chemical shifts (in ppm) are given from internal TMS. Column chromatography (CC) was carried out on silica gel 60 F254 (Merck, 70-230 mesh) in glass bls. Thin layer chromatography was performed on precoated TLC plates (silica 60 F$_{254}$ silicone 60 RP-18F$_{254}$) and visualized by UV light and by spraying with vanillin in H$_2$SO$_4$.

2.3 Extraction and isolation
Dried stem barks (350 g) were extracted with 80% aqueous ethanol (1200 ml) at room temperature for 3 days. After concentration under reduced pressure, the hydroalcoholic extract (40 g) was suspended in H$_2$O at 40°C (500 mL) and then partitioned sequentially using hexane, CH$_2$Cl$_2$, AcOEt and n-BuOH (500 mL x 3) furnishing hexanic (3 g), dichloromethanic (6.36 g), ethyl acetate (0.95 g), butanolic (10.70 g) and aqueous (19.98 g) extracts, respectively. Hexanic extract (3 g) is yellow oily containing a precipitated. This extract is recrystallized with petroleum ether to give compound 1 (14.5 mg).

The dichloromethanic extract (1.5 g) was chromatographed over a silica gel column (100 g), eluting successively with a gradient solvent system of cyclohexane-ethyl acetate (100:0 → 100:100) and then of ethyl acetate-methanol (100:0 → 100), to give 350 fractions. Fractions 25 [Hexane/AcOEt (80:20), 15 mg] and 26-48 [Hexane/AcOEt (80:20), 20 mg] were recrystallized with methanol to obtain compound 2 (10 mg) and compound 3 (15 mg) respectively.

The ethyl acetate extract (0.95 g) was chromatographed over a silica gel column (60 g), eluting successively with a gradient solvent system of dichloromethane-ethyl acetate (100:0 → 100) and ethyl acetate-methanol (100:0 → 100), to give 185 fractions. Fractions 105-122 [AcOEt/MeOH (80:20), 76.4 mg] were washed with dichloromethane to obtain a 12.2 mg yellow powder. This powder was separated by preparative TLC using 60 RP-18 F$_{254}$ silica with MeOH/H$_2$O (80/20) to give 1429 fractions. Fractions 105-122 [AcOEt/MeOH (80:20), 76.4 mg] were recrystallized with methanol to obtain compound 2 (10 mg) and 26-48 [Hexane/AcOEt (80:20), 20 mg] were recrystallized with methanol to obtain compound 3 (10 mg) and compound 4 (5.2 mg; Rf 0.4) and compound 5 (14.5 mg).

Compound 1: white powder; δ(ppm) DEPT Q ([125.78 MHz); CDCl$_3$) : 181.6 (C-28), 171.2 (C-1'), 160.4 (C-14), 116.8 (C-13), 17.3 (C-11), 21.3 (C-2'), 16.6 (C-24), 15.6 (C-25). 28.0 (C-23), 26.2 (C-26), 23.5 (C-2), 22.4 (C-27), 18.7 (C-6), 18.0 (C-29), 31.4 (C-16), 30.8 (C-22), 29.3 (C-20), 28.7 (C-30), 18.6 (C-17), 60.3 (OCH$_3$-3), 56.4 (OCH$_3$-4'), 56.0 (OCH$_3$-3').

3. Results and Discussion
Purification with petroleum ether of the hexane fraction, obtained from the ethanolic extract of the stem bark of Croton argyrodaphne Baill, resulted in the isolation of triterpenoid 1. The DEPT Q spectroscopic data of 1 was in agreement with those reported for acetyl aleuritolic acid (3-β-acetoxy taraxer-14-en-28-oic acid)[4,5] reported to exhibit antimicrobial activity against S. aureus and S. typhimurium, previously isolated from Croton urucurana, Croton macrostachys, Croton megalocarpus[6-8].

Compound 2 was obtained as white powder. The structure of 2 is similar of penduliflorosorin previously isolated from Croton penduliflorus, Croton crassifolius[22, 23]. A modification was bring for the attribution of the chemical shift of $^1$H and $^{13}$C reported[22] for penduliflorosor in 2 using 2D NMR.

Compound 3 was obtained as white powder. The $^1$H-NMR of this compound exhibited the characteristic signals for a β-substituted furan ring (δ$_{\text{H}}$ 7.49, br s; 7.48, br s and 6.42, br s), two oxygenated methines (δ$_{\text{H}}$ 5.62, dt and 4.99, t), a diastereotopic methylene (δ$_{\text{H}}$ 2.89, dd and 2.34, dd), a secondary methyl (δ$_{\text{H}}$ 0.99, d) and a tertiary methyl (δ$_{\text{H}}$ 1.42, s). The DEPT Q spectrum showed 20 carbons, which were confirmed by HSQC experiment to be two methyls (δ$_{\text{C}}$ 15.5 and 15.7), five methylenes (δ$_{\text{C}}$ 20.9, 26.0, 26.8, 28.6 and 40.0), six methines (δ$_{\text{C}}$ 35.7, 72.1, 75.4, 107.8, 139.3 and 144.2), and seven quaternary carbons (δ$_{\text{C}}$ 45.9, 49.5, 125.3, 133.2, 141.1, 176.5 and 176.6). Signals at δ$_{\text{C}}$ 107.8 (C-14), 125.3 (C-13), 139.3 (C-16) and 144.2 (C-15) confirmed the presence of furan ring at C-12 (δ$_{\text{H}}$ 72.1)[9, 10]. This was supported by the HMBC correlations between the oxymethine protons H-12 (δ$_{\text{H}}$ 5.62, dt) and C-13, C-14 and C-16. These data suggested that compound 3 was a furano-diterpenoid. In the COSY spectrum of 3, the oxygenated methine at δ$_{\text{H}}$ 5.62 (H-12) showed cross peaks with the methine at δ$_{\text{H}}$ 2.34/2.89 (H-11), the methyl at δ$_{\text{H}}$ 0.99 (15.5) correlated with the methine at δ$_{\text{H}}$ 1.74 (35.7), the methine at δ$_{\text{H}}$ 1.63/1.83 (26.0) exhibited correlations with the methylene at δ$_{\text{H}}$ 2.09/2.17 (20.9), and the methylene at δ$_{\text{H}}$ 1.78/2.15 (26.8) correlated with the methylene at δ$_{\text{H}}$ 1.53/1.68 (28.6). The $^1$H-NMR and DEPT Q spectra (table 1) of compound 3 was identical with those of neo-clerodan-5,10-en-19,6-diolide[6-11]. However, the HMBC correlation (figure 2) from oxymethine at δ$_{\text{H}}$ 4.99 (δ$_{\text{C}}$ 75.4) to C-10 (δ$_{\text{C}}$ 133.2), C-9 (δ$_{\text{C}}$ 49.5) and C-18 (δ$_{\text{C}}$ 176.6) permitted to locate this oxymethine at C-1 and replace the lactone between C-6 and C-4 from C-1 and C-4 (figure 2). Thus, compound 3, named here argyrodaphnin, was proposed to be the halima-5(10), 14(15), 16-trien-18(1β),20-12-diolide.
Compound 4 was obtained from ethyl acetate extract as yellow powder. The $^1$H and $^{13}$C NMR spectroscopic data of 4 was in agreement with those reported for ayanin (3',5-dihydroxy-3,4',7-trimethoxyflavone) [12, 13] reported to have antioxidant, anti-inflammatory [19, 20] and antispasmodic and relaxant effects, previously isolated from other Croton species such Croton schiedeanus, Croton adenocalyx, Croton glabellus [14-16].

Compound 5, isolated from ethyl acetate extract, was obtained as yellow powder. It structure was suggested by $^1$H and $^{13}$C NMR spectral data together with the HSQC and HMBC experiments as 4',5-dihydroxy-3,3',7,8-tetramethoxyflavone. Comparison of these spectral data with literature values indicated that compound 5 is identical to ternatin [17, 18]. However, ternatin reported to have biological activity such antioxidant, anti-inflammatory [19, 20]. This work has demonstrated that Croton species is rich of 3-methoxyflavones [21].

Table 1: $^1$H, $^{13}$C spectra data and HMBC correlation of argyrodaphnin 2 (600.19 MHz/125.78 MHz; CDCl$_3$)

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Fig 2: Selected HMBC correlations of argyrodaphnin 2

4. Conclusion

The present study reports to the isolation and identification of one new furano-diterpenoid (argyrodaphnin), two known terpenoids (acetyl aleuritolic acid and penduliflavorosin) and two known 3-methoxyflavones (ayanin and ternatin).

5. References


