International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 1428-1431

Received: 09-09-2017
Accepted: 10-10-2017

Luciano Zaralahy
(A). Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar
(B). Mention Chimie, Faculty of Sciences, University of Antsiranana, B.P 00, Antsiranana 201, Madagascar

Manitriniaina Rajemiarimiraho
Mention Sciences et Ingénerie des Matériaux, Ecole Supérieure Polytechnique Antananananarivo, University of Antananarivo, Antananarivo 101, Madagascar

Anne Wadouachi
Laboratoire Glycochimie, des Antimicrobiens et des Agroressources CNRS UMR 7378 - Institut de Chimie de Picardie CNRS FRE 3085. Université de Picardie Jules Verne, France

Amélie Raharisololalao
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Léa Herilala Rasoanaivo
Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar

Correspondence
Luciano Zaralahy
(A). Laboratoire de Chimie des Substances Naturelles et Chimie Organique Biologique (LCSN/COB), Faculty of Sciences, University of Antananarivo, BP 906 Ankatso, Antananarivo 101, Madagascar
(B). Mention Chimie, Faculty of Sciences, University of Antsiranana, B.P 00, Antsiranana 201, Madagascar

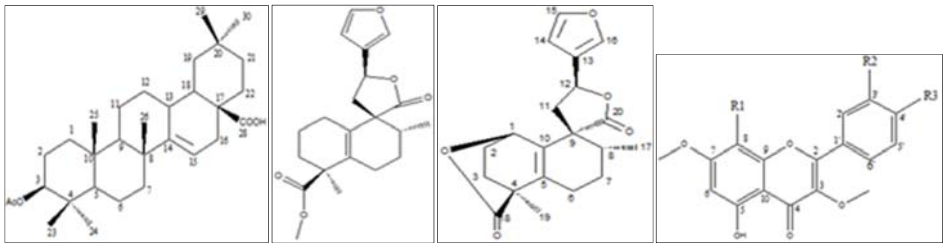
A new halimane from *Croton argyrodaphne* H. Baillon

Luciano Zaralahy, Manitriniaina Rajemiarimiraho, Anne Wadouachi, Amélie Raharisololalao and Léa Herilala Rasoanaivo

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 "lazalaza". 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).



Acetyl aleuritolic acid 1 penduliflaworosin 2 argyrodaphnin 2 ayanin 4 : R1=H R2=OH R3=OCH_3 ternatin 5: R1= R2= OCH_3 R3=OH

Fig 1: Compounds isolated from stem bark of *Croton argyrodaphne*

2. Materiels 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 (^{1}H, ^{13}C, DEPTQ) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C 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 blades. Thin layer chromatography was performed on precoated TLC plates (silica 60 F_{254}; silica 60 RP-18F_{254}) and visualized by UV light and by spraying with vanillin in H_2SO_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_2O at 40°C (500 mL) and then partitioned sequentially using hexane, CH_2Cl_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 → 0:100) and then of ethyl acetate-methanol (100:0 → 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 → 0:100) and ethyl acetate-methanol (100:0 → 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_2O (80/20) to obtain compound 4 (5.2 mg; Rf 0.4) and compound 5 (6.2 mg; Rf 0.5).

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-15), 81.2 (C-3), 55.5 (C-5), 51.4 (C-17), 49.2 (C-9), 41.5 (C-18), 40.8 (C-7), 39.0 (C-8), 37.9 (C-10), 37.7 (C-4), 37.4 (C-13), 37.3 (C-1), 35.3 (C-19), 33.7 (C-12), 33.3 (C-21), 31.9 (C-29), 31.4 (C-16), 30.8 (C-22), 29.3 (C-20), 28.7 (C-30), 28.0(C-23), 26.2 (C-26), 23.5 (C-2), 22.4 (C-27), 18.7 (C-6), 17.3 (C-11), 21.3 (C-2'), 16.6 (C-24), 15.6 (C-25).

Compound 2: white powder; δ(ppm) ^{1}H NMR ($CDCl_3$, 600.19 MHz): 7.48 (H-15), 7.48 (H-16), 6.43(H-14), 5.48(H-12), 3.69(OCH_3-18), 2.83(H-11β), 2.23(H-11α), 1.74(H-8), 1.99(H-2 β), 1.64(H-2 α), 1.22(H-19), 1.05(H-17); δ(ppm) ^{13}C NMR ($CDCl_3$, 125.78 MHz): 177.7(C-18), 176.7(C-20), 143.9(C-15), 143.9(C-16), 134.6(C-5), 128.2(C-10), 107.8(C-14), 72.1(C-12), 53.1(C-9), 52.0(OCH_3-18), 47.3(C-4), 41.4 (C-11), 37.9(C-8), 34.9(C-3), 29.7(C-1), 26.2(C-7), 24.5(C-6), 22.8(C-19), 18.9(C-2), 16.0(C-17).

Compound 3: white powder; δ(ppm) ^{1}H NMR ($CDCl_3$, 600.19 MHz): see table 1 δ(ppm) DEPT Q ($CDCl_3$, 125.78 MHz): see table 1.

Compound 4: yellow powder; δ(ppm) ^{1}H NMR ($CDCl_3$, 600.19 MHz): 12.64(OH-5), 7.71(H-6'), 7.68(H-2'), 7.05(H-5'), 6.45 (H-8), 6.36 (H-6), 3.98(OCH_3-4'), 3.88(OCH_3-7), 3.86(OCH_3-3); δ(ppm) ^{13}C NMR ($CDCl_3$, 125.78 MHz): 179.0(C-4), 165.6(C-7), 162.2(C-9), 156.9(C-5), 156.1(C-3'), 148.5(C-4'), 146.5 (C-2), 139.0(C-3), 122.6(C-6'), 122.8(C-1'), 111.0(C-5'), 114.7(C-2'), 106.2(C-10), 98.0(C-6), 92.3(C-8), 60.3(OCH_3-3), 56.3(OCH_3-7), 56.0(OCH_3-4').

Compound 5: yellow powder; δ(ppm) ^{1}H NMR ($CDCl_3$, 600.19 MHz): 12.47(OH-6), 7.80(H-2'), 7.79(H-6'), 7.06(H-5'), 6.42 (H-6), 3.98(OCH_3-3'), 3.95(OCH_3-7), 3.92(OCH_3-8), 3.88(OCH_3-3); δ(ppm) ^{13}C NMR ($CDCl_3$, 125.78 MHz): 179.1(C-4), 158.5(C-7), 157.4(C-9), 156.0(C-5), 155.8(C-2), 148.7(C-4'), 146.5(C-3') 138.8(C-3), 128.9(C-8), 123.0(C-6'), 122.8(C-1'), 114.9(C-5'), 111.0(C-2'), 105.5(C-10), 95.6(C-6), 61.6(OCH_3-8), 60.3(OCH_3-3), 56.4(OCH_3-7), 55.7(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 (3β-acetoxy taraxer-14-en-28-oic acid)[4,5] reported to exhibit antimicrobial activity against *S.aureus* and *S. typhimuriu*, 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 penduliflaworosin previously isolated from *Croton 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 penduliflaworosin 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_H 7.49, br s; 7.48, br s and 6.42, br s), two oxygenated methines (δ_H 5.62, dt and 4.99, t), a diastereotopic methylene (δ_H 2.89, dd and 2.34, dd), a secondary methyl (δ_H 0.99, d) and a tertiairy methyl (δ_H 1.42, s). The DEPT Q spectrum showed 20 carbons, which were confirmed by HSQC experiment to be two methyls (δ_C 15.5 and 15.7), five methylenes (δ_C 20.9, 26.0, 26.8, 28.6 and 40.0), six methines (δ_C 35.7, 72.1, 75.4, 107.8, 139.3 and 144.2), and seven quaternary carbons (δ_C 45.9, 49.5, 125.3, 133.2, 141.1, 176.5 and 176.6). Signals at δ_C 107.8 (C-14), 125.3 (C-13), 139.3 (C-16) and 144.2 (C-15) confirmed the presence of furan rung at C-12 (δ_C 72.1) [9, 10]. This was supported by the HMBC correlations between the oxymethine protons H-12 (δ_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_H 5.62 (H-12) showed cross peaks with the methylene at δ_H 2.34/2.89 (H-11), the methyl at δ_H 0.99 (15.5) correlated with the methine at δ_H 1.74 (35.7), the methylene at δ_H 1.63/1.83 (26.0) exhibited correlations with the methylene at δ_H 2.09/2.17 (20.9), and the methylene at δ_H 1.78/2.15 (26.8) correlated with the methylene at δ_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-19,6β;20,12-diolide [11]. However, the HMBC correlation (figure 2) from oxymethine at δ_H 4.99 (δ_C 75.4) to C-10 (δ_C 133.2), C-9 (δ_C 49.5) and C-18 (δ_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.

Table 1: ^{1}H, ^{13}C spectra data and HMBC correlation of argyrodaphnin 2 (600.19 MHz/125.78 MHz; $CDCl_3$)

H/C	δ_H	δ_C	HMBC	H/C	δ_H	δ_C	HMBC
1	4.99	75.4	2, 3, 5, 9, 10, 18	11	2.34/2.89	40.0	8, 9, 10, 12, 13, 20
2	1.78/2.15	26.8		12	5.62	72.8	9, 11, 13, 14, 16, 20
3	1.53/1.68	28.6	1, 2, 4, 18	13		125.3	
4		45.9		14	6.42	107.8	
5		141.1		15	7.49	144.2	
6	2.09/2.17	20.9	5, 7, 8, 10	16	7.48	139.3	
7	1.63/1.83	26.0	5, 6, 8, 9, 17	17	0.99	15.5	7, 8, 9
8	1.74	35.7	7, 9	18		176.6	
9		49.5		19	1.42	15.7	3, 4, 5, 18
10		133.2		20		176.5	

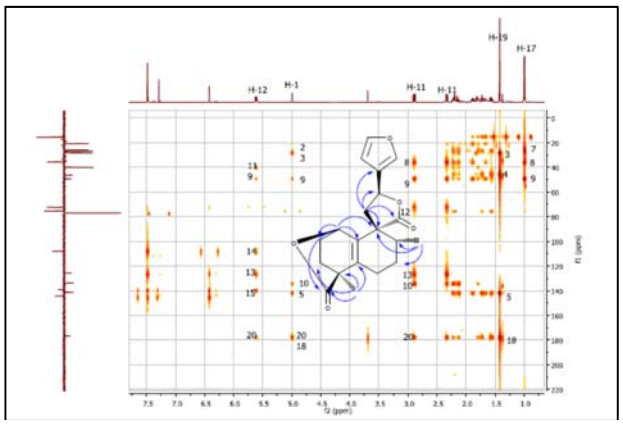


Fig 2: Selected HMBC correlations of argyrodaphnin **2**

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 cardiovascular properties (hypotensor), as well as 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].

4. Conclusion

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

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