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Trypanocidal labdane diterpenoids from the seeds of *Aframomum aulacocarpos* (Zingiberaceae)

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Abstract—Two novel labdane type diterpenoids, 8β(17)-epoxy-14,15,16-trihydroxylabd-12(*E*)-ene (aulacocarpin C) and 15,16-epoxy-14ξ,16ξ-dimethoxylabda-8(17),12-(*E*)-diene (aulacocarpin D) together with the known aulacocarpin A and B; 14,15-epoxy-8(17),12(*E*)-labdadien-16-al, coronarin E, and 15,16-epoxy-12β-hydroxy-labda-8(17)-13(16),14-triene were isolated from the seeds of *Aframomum aulacocarpos*. To the best of our knowledge, the last compound was isolated from a natural source for the first time. Acid hydrolysis of aulacocarpin D led to another new labdane type diterpenoid, 15,16-epoxy-12β-methoxylabda-8(17)-13(16),14-triene. The structures of all compounds were established on the basis of their spectroscopic data. These new compounds exhibit moderate trypanocidal activity.

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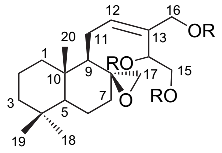
1. Introduction

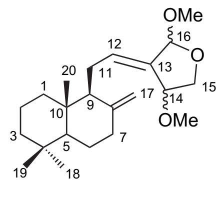
The genus *Aframomum* K. Schum belongs to the economically and medicinally important family Zingiberaceae. Over 20 species of this genus are found in Cameroon where they are widely used for medicinal, ethno dietary, and spiritual purposes.[1] The seeds of *Aframomum aulacocarpos* (Pellegr. Ex. J. Koechlin) are widely used as a food spice.[2] A previous study reported the isolation of three diterpenes, aulacocarpinolide, aulacocarpin A, and aulacocarpin B from these seeds. The two later compounds showed antibacterial activity.[3] In our search for larger quantities of these compounds for bioactivity tests, we have isolated seven labdane diterpenoids from the seeds of this plant. This paper describes the isolation and structural elucidation of three novel compounds, aulacocarpin C (**1**) and D (**2**), and 15,16-epoxy-12β-methoxylabda-8(17)-13(16),14-triene.

2. Results and discussion

The dried powdered seeds of *A. aulacocarpos* were macerated with acetone and the solvent was removed under reduced pressure. The crude extract was separated on silica gel column chromatography to give several fractions, which were each further purified by open column chromatography, gel permeation chromatography through Sephadex LH-20, chromatotron, preparative TLC or re-crystallization, to afford compounds **1** and **2** together with the previously described aulacocarpin A and B,[4] 14,15-epoxy-8(17),12(*E*)-labdadien-16-al,[5] coronarin E,[6–8] and 15,16-epoxy-12β-hydroxylabda-8(17)-13(16),14-triene,[9–11] which was isolated for the first time from a natural source.



1 R=H
1a R=Ac



2

Compound **1** was obtained as brown needles from EtOAc, mp 160–161 °C. Its EIMS showed a pseudo-molecular ion $[M-H_2O]^+$ at m/z 320 consistent with the molecular formula

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Keywords: *Aframomum aulacocarpos*; Zingiberaceae; Labdane diterpenoids; Aulacocarpin; Trypanocidal.

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$C_{20}H_{34}O_4$, which accounted for four degrees of unsaturation. Absorption of hydroxyl groups (ν_{max} 3474 cm^{-1}) and double bond (ν_{max} 1640 cm^{-1}) was observed in the IR spectrum. The ^{1}H NMR spectrum of **1** (Table 1) displayed signals of three tertiary methyl groups at δ 0.95, 0.90 and, 0.85 and a deshielded olefinic proton at δ 5.46 (dd, *J*=7.8, 4.7 Hz) typical of labdanes type skeleton.[4,12] This spectrum also showed an oxymethine at δ 4.60 (dd, *J*=7.4, 4.6 Hz) and signals for protons geminated to two primary alcohols at δ 4.15 (d, *J*=12.4 Hz), 4.05 (d, *J*=12.4 Hz) and 3.60 (dd, *J*=11.3, 7.4 Hz), 3.52 (dd, *J*=11.3, 4.6 Hz). Chemical shifts at δ 2.70 (d, *J*=3.8 Hz, H-17β) and 2.30 (d, *J*=3.8 Hz, H-17α) suggested an 8(17)-epoxide in **1**. The ^{13}C NMR spectrum (Table 2) confirmed the presence of these functions with signals of three oxymethylenes at δ 66.5 (C-15), 64.3 (C-16), and 50.7 (C-17), one trisubstituted double bond at δ 139.2 (C-13) and 133.7 (C-12). The rest of the data (Table 2) being comparable to those reported in the literature for similar compounds.[13] The ^{1}H–^{1}H COSY and HMQC spectral analysis revealed spin systems of –CH–CH$_2$–CH–, –CH$_2$–CH$_2$–CH$_2$–, –CH–CH$_2$–CH$_2$–, –CH–CH$_2$–, and two isolated –CH$_2$–. HMBC correlations (Fig. 1) enabled us to complete the structural elucidation of **1**. Pertinent correlations were observed between H-19 and C-18, C-5; H-20 and C-1, C-9; H-9 and C-1; H-11 and C-13 as well as between H-12 and C-16, C-14.

The stereochemistry of **1** was deduced from its NOESY spectrum. Spatial connectivities were observed between H-19 and H-20, H-5 and H-18, H-9. Further connectivity between H-16a and H-12 indicated the double bond being in the *E*-configuration. The β-orientation of the 8(17)-epoxide was deduced by comparison of the ^{1}H NMR spectroscopic shifts of the epoxide proton H-17 with those reported for aulacocarpin A and B.[4,14] Compound **1** was thus established to be 8β(17)-epoxy-14,15,16-trihydroxylabd-12(*E*)-ene and was trivially named as aulacocarpin C.

Acetylation of (**1**) (in acetic anhydride–pyridine (1:1) at room temperature, 24 h) and work-up with 10% HCl solution led to the isolation of the triacetate (**1a**) as the major product and compounds **3** and **4**, being artefacts resulting from the opening of the oxonium ion formed by protonation of the epoxide at C-8 (17) to give the tertiary carbocation and subsequent elimination of β-protons in the acidic work-up medium.

The triacetate (**1a**) was obtained as syrup. The CIMS of **1a** showed an [M+NH$_4$]$^+$ peak at *m/z* 482 indicating an increase of 126 units attributed to the three acetate groups picked up by **1** to form **1a**. The ^{1}H NMR spectral data (Table 1) of **1a** were comparable with that of **1** except the increases observed in the chemicals shifts of the protons at positions 14, 15, and 16 due to the greater deshielding effect of the acetoxy group; and three tertiary methyl signals at δ 2.0, 2.0, and 1.9, attributed to the methyl groups of the acetyl functions. According to the data above, compound **1a** was found to be 8β(17)-epoxy-14,15,16-triacetoxylabd-12(*E*)-ene.

The two other triacetates, **3** and **4** were also obtained as syrups. The CIMS spectra of these showed the same [M+NH$_4$]$^+$ peak at *m/z* 482 as **1a**. However, some differences were observed in their ^{1}H NMR (see Table 1) and ^{13}C NMR

Table 1. ^{1}H NMR data for compounds **1**–**5** and **1a** (*J* in Hz)

Proton	**1**[a]	**2**[b]	**1a**[b]	**3**[b]	**4**[b]	**5**[c]
1	1.0 (m), 1.85 (m)	1.05 (dd, 4.0, 12.6), 1.77 (m)	0.90 (m), 1.70 (m)	1.70 (m)	1.80 (m)	1.77 (m)
2	1.55 (m)	1.52 (m), 1.57 (dt, 3.3, 7.0)	1.40 (m), 1.55 (ov)	1.40 (m)	1.50 (m)	1.56 (m)
3	1.25 (m), 1.45 (ov)	1.21 (dd, 4.1, 13.3), 1.42 (m)	1.15 (m), 1.35 (br t)	1.10 (m), 1.30 (m)	1.40 (m)	1.18 (m)/1.58 (m)
5	1.65 (ov)	1.12 (dd, 2.6, 12.7)	1.45 (br s)	1.10 (m)	1.6 (m)	1.20 (dd, 2.9, 12.8)
6	1.50 (m), 1.75 (m)	1.36 (ov) (m), 1.74 (m)	1.60 (ov)	1.50 (m), 1.80 (m)	2.1 (m)	1.75 (m)
7	1.99 (ov), 1.89 (m)	2.03 (m), 2.41 (m)	1.30 (ov), 1.85 (m)	2.1 (m), 2.20 (m)	5.70 (m)	1.93 (m), 2.42 (m)
9	1.1 (m)	1.80 (m)	1.45 (br s)	—	1.95 (m)	2.08 (m)
11	1.75 (m), 2.00 (ov)	2.32 (m), 2.48 (m)	1.80 (m)	2.75 (dd, 12.0, 4.3), 3.10 (dd, 12.0, 3.3)	2.20 (m), 2.35 (m)	1.55 (m), 1.97 (m)
12	5.46 (dd, 7.8, 4.7)	5.92 (dd, 7.2, 6.0)	5.55 (dd, 7.4, 5.0)	5.60 (dd, 4.3, 3.3)	5.85 (m)	4.12 (br d, 10.1)
14	4.60 (dd, 7.4, 4.6)	4.45 (d, 3.8)	5.65 (dd, 8.3, 3.9)	5.85 (dd, 7.8, 3.8)	5.80 (m)	6.39 (br s)
15	3.52 (dd, 11.3, 4.6), 3.60 (dd, 11.3, 7.4)	3.95 (dd, 10.3, 3.8), 4.07 (d, 10.3)	4.1 (ov)	4.15 (m)	3.8 (br d), 4.0 (br d)	7.40 (s)
16	4.05 (d, 12.4), 4.15 (d, 12.4)	5.26 (s)	4.35 (d, 12.2), 4.55 (d, 12.2)	4.30 (d, 12.0), 4.55 (d, 12.0)	4.40 (d, 12.0), 4.60 (d, 12.0)	7.35 (s)
17	2.30 (d, 3.8), 2.70 (d, 3.8)	4.63 (d, 1.2), 4.87 (d, 1.2)	2.20 (d, 3.8), 2.58 (d, 3.8)	3.82 (d, 11.7), 3.95 (d, 11.7)	4.2 (ov)	4.44 (s), 4.84 (s)
18	0.90 (s)	0.91 (s)	0.80 (s)	0.85 (s)	0.83 (s)	0.89 (s)
19	0.85 (s)	0.84 (s)	0.70 (s)	0.7 (s)	0.78 (s)	0.81 (s)
20	0.95 (s)	0.67 (s)	0.85 (s)	0.90 (s)	0.89 (s)	0.66 (s)
12-OMe	—	—	—	—	—	3.17 (s)
14-OMe	—	3.34 (s)	—	—	—	—
16-OMe	—	3.39 (s)	—	—	—	—
14-$COCH_3$	—	—	2.01 (s)[d]	2.05 (s)[d]	2.10 (s)[d]	—
15-$COCH_3$	—	—	2.00 (s)[d]	2.00 (s)[d]	2.00 (s)[d]	—
16-$COCH_3$	—	—	1.99 (s)[d]	1.95 (s)[d]	1.90 (s)[d]	—

(ov)—Overlap; (m)—multiplet.

[a] Spectra recorded in CD_3OD, 400 MHz.

[b] Spectra recorded in $CDCl_3$, 400 MHz.

[c] Spectra recorded in $CDCl_3$, 500 MHz.

[d] The values are interchangeable in the same column.