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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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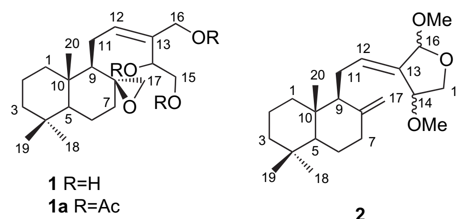
## 1. Introduction

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.




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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$C_{20}H_{34}O_4$, which accounted for four degrees of unsaturation. Absorption of hydroxyl groups ($\nu_{max}$ 3474 $cm^{-1}$) and double bond ($\nu_{max}$ 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 $\delta$ 0.95, 0.90 and, 0.85 and a deshielded olefinic proton at $\delta$ 5.46 (dd, $J$=7.8, 4.7 Hz) typical of labdanes type skeleton.[4,12] This spectrum also showed an oxymethine at $\delta$ 4.60 (dd, $J$=7.4, 4.6 Hz) and signals for protons geminated to two primary alcohols at $\delta$ 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 $\delta$ 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 $\delta$ 66.5 (C-15), 64.3 (C-16), and 50.7 (C-17), one trisubstituted double bond at $\delta$ 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 $\delta$ 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] | — |

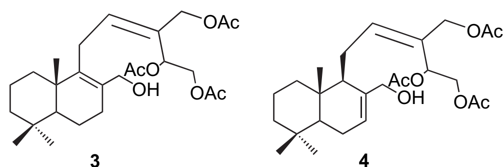
(ov)—Overlap; (m)—multiplet.
[a] Spectra recorded in $CD_3OD$, 400 MHz.
[b] Spectra recorded in $CDCl_3$, 400 MHz.
[c] Spectra recorded in $CDCl_3$, 500 MHz.
[d] The values are interchangeable in the same column.

**Table 2**. $^{13}$C NMR data for compounds **1**–**5** and **1a**

| Carbon | **1**[a] | **2**[b] | **1a**[b] | **3**[b] | **4**[b] | **5**[c] |
|---|---|---|---|---|---|---|
| 1 | 40.7 | 39.5 | 39.6 | 36.9 | 35.6 | 39.0 |
| 2 | 21.6 | 19.7 | 19.0 | 19.3 | 17.8 | 19.3 |
| 3 | 43.6 | 42.5 | 42.3 | 41.9 | 41.1 | 42.1 |
| 4 | 34.9 | 33.9 | 33.9 | 33.7 | 35.6 | 33.6 |
| 5 | 54.9 | 55.3 | 53.7 | 52.1 | 51.6 | 55.3 |
| 6 | 20.1 | 24.6 | 20.5 | 19.3 | 17.8 | 24.4 |
| 7 | 37.5 | 38.4 | 36.3 | 30.3 | 126.1 | 38.3 |
| 8 | 59.7 | 148.6 | 57.7 | 132.6 | 136.7 | 149.3 |
| 9 | 56.6 | 55.3 | 55.4 | 143.0 | 48.7 | 52.7 |
| 10 | 41.3 | 39.9 | 40.2 | 39.5 | 35.6 | 39.2 |
| 11 | 21.2 | 24.5 | 20.6 | 25.8 | 24.1 | 31.9 |
| 12 | 133.7 | 134.7 | 140.1 | 139.9 | 139.4 | 74.1 |
| 13 | 139.2 | 138.5 | 129.1 | 128.0 | 126.7 | 127.1 |
| 14 | 72.3 | 78.2 | 70.5 | 69.9 | 69.2 | 108.6 |
| 15 | 66.5 | 70.7 | 64.5 | 64.7 | 64.9 | 143.3 |
| 16 | 64.3 | 104.8 | 65.4 | 65.6 | 64.3 | 139.4 |
| 17 | 50.7 | 108.1 | 49.4 | 63.6 | 63.1 | 106.2 |
| 18 | 34.5 | 34.0 | 33.9 | 33.6 | 32.1 | 33.3 |
| 19 | 22.6 | 22.1 | 22.1 | 22.0 | 20.9 | 21.7 |
| 20 | 15.7 | 14.7 | 15.0 | 20.2 | 13.0 | 14.5 |
| 12-OMe | — |  | — | — | — | 56.4 |
| 14-OMe |  | 56.9 | — | — | — |  |
| 16-OMe |  | 55.7 | — | — | — |  |
| 14-$COCH_3$ | — | — | 170.9[d], 21.4[d] | 171.0[d], 21.5[d] | 169.7[d], 20.0[d] |  |
| 15-$COCH_3$ | — | — | 170.9[d], 21.2[d] | 171.0[d], 21.3[d] | 169.6[d], 19.9[d] |  |
| 16-$COCH_3$ | — | — | 170.3[d], 21.2[d] | 170.7[d], 21.2[d] | 169.5[d], 19.8[d] |  |

[a] Spectra recorded in $CD_3OD$, 100 MHz.
[b] Spectra recorded in $CDCl_3$, 100 MHz.
[c] Spectra recorded in $CDCl_3$, 125 MHz.
[d] The values are interchangeable in the same column.

(see Table 2). This revealed the presence of a second double bond, a primary alcohol as well as the three acetoxy functions and the absence of the epoxide. The $^1$H NMR spectrum of **4** indicated the presence of a second olefinic proton, which was absent in that of **3**. Further analyses of their HMBC spectra led to the unambiguous positioning of the double bond in the two compounds.




HMBC correlations were observed between H-9 ($\delta$ 1.95) and the methyl carbon at $\delta$ 13.0 (C-20) for **4** but no correlation from this methyl group to H-9 proton was observed for compound **3**. Their structures were thus established to be 17-hydroxy-14,15,16-triacetoxylabda-8(*E*),12(*Z*)-diene (**3**) and 17-hydroxy-14,15,16-triacetoxylabda-7(*E*),12(*Z*)-diene (**4**).

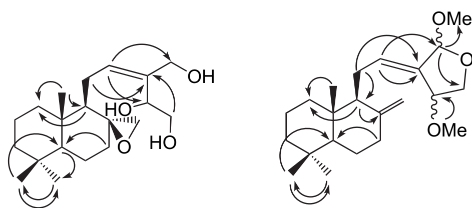
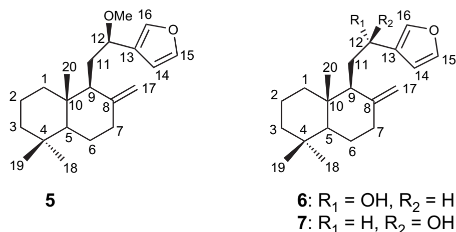



**Figure 1**. HMBC correlations for compounds **1** and **2**.

Compound **2** was obtained as white needles in methanol, mp 114–115 °C. Its EIMS showed a pseudo-molecular ion peak at *m*/*z* 316 [M−MeOH]$^+$ compatible with the molecular formula $C_{22}H_{36}O_3$, which accounted for one more degree of unsaturation compared to **1**, indicating one additional ring or double bond. The IR, $^1$H, and $^{13}$C NMR spectra of **2** (Tables 1 and 2) showed some similarities with those of **1**, suggesting that they were analogs. In the $^1$H NMR data of **2** (Table 1), olefinic proton signals were observed at $\delta$ 5.92 (dd, $J$=7.2, 6.0 Hz, H-12), and at $\delta$ 4.87 (d, $J$=1.2 Hz, H-17a) and 4.63 (d, $J$=1.2 Hz, H-17b) characteristic of the exomethylene moiety at C-8 found in labdane diterpenoids.[15,16] This spectrum also showed two methoxyl groups at $\delta$ 3.39 (s, 16-OMe), $\delta$ 3.34 (s, 14-OMe), a deshielded ketalic proton at $\delta$ 5.26 (s, H-14); an oxymethylene [$\delta$ 4.07 (d, $J$=10.3 Hz, H-15b), 3.95 (dd, $J$=10.3, 3.8 Hz, H-15a)], and one oxymethine at $\delta$ 4.45 (d, $J$=3.8 Hz, H-16). The $^{13}$C NMR spectrum (Table 2) indicated the presence of one ketal carbon atom at $\delta$ 104.8 (C-16), one oxymethine carbon at $\delta$ 78.2 (C-14), one disubstituted double bond [$\delta$ 108.1 (t, C-17) and 148.6 (s, C-8)], and one trisubstituted double bond [$\delta$ 134.7 (d, C-12) and 138.5 (s, C-13)]. The rest of the data were in agreement with those published for labdane skeletons.[13] The two methoxyl groups were positioned at C-14 and C-16 on the basis of correlations observed on the HMBC spectrum (Fig. 1). Further correlations were observed between 2H-11 and C-9, H-12, C-13 and C-14. The $^1$H–$^1$H COSY clearly demonstrated the connectivities H-9/2H-11/H-12. From the above evidence, the trisubstituted double bond was located at C-12/C-13. The *E*-configuration of the *hitherto* mentioned double bond was deduced from the connectivities observed between H-12 and H-16. A judicious analyses of the HMBC (Fig. 1), $^1$H–$^1$H COSY, DEPT 135, NOESY (Fig. 2), and

HMQC data led to the unambiguous assignment of structure **2**, 15,16-epoxy-14ξ,16ξ-dimethoxylabda-8(17),12-(*E*)-diene. Compound **2** was trivially named as aulacocarpin D.

Acid hydrolysis of (**2**) and work-up with solid $NaHCO_3$ led to the isolation of a new labdane diterpenoid (**5**) in 2% yield and the known compounds (**6**): 15,16-epoxy-12α-hydroxylabda-8(17)-13(16),14-triene[9–11] (21% yield) and the previously isolated (**7**): 15,16-epoxy-12β-hydroxylabda-8(17)-13(16),14-triene[9–11] (44% yield). The furanolabdane (**5**) was obtained as colorless oil. Its CIMS showed an $[M+NH_4]^+$ peak at *m*/*z* 334 indicating an increase of 14 units as compared to **6** and **7**. The obtained **5** also exhibited spectral properties very similar to those reported for **6** and **7**[9–11] except for the chemical shifts at $\delta$ 3.17 in $^1$H NMR and $\delta$ 56.4 in $^{13}$C NMR attributed to a methoxy group. The positioning of this methoxy group at C-12 was in agreement with the decrease observed in the chemical shift of the H-12 proton due to the shielding effect of the methoxy group; and the increase in that of C-12. Its β-orientation was deduced by comparison of the $^1$H NMR shifts of the methylene protons H-17 with those reported for compounds **6** and **7**.[9–11] All these data, together with the analyses of its $^1$H–$^1$H COSY, HMBC, and HMQC spectra led to the structure of **5**: 15,16-epoxy-12β-methoxy-labda-8(17)-13(16),14-triene.




Aulacocarpin A and B,[4–6] coronarin E,[5,6] 14,15-epoxy-8(17),12-(*E*)-labdadien-16-al,[14] and 15,16-epoxy-12β-hydroxylabda-8(17)-13(16),14-triene[9–11] spectral data were in agreement with those reported in the literature.

Compounds **1** and **2** exhibited inhibitory activity against four strains of bloodstream trypomastigotes *Trypanosoma brucei rhodesiense* with $IC_{50}$ values in the range 15–29 μg/ml. The tests were carried out by Professor Cyrus Bacchi, Pace University, Haskins Laboratory, New York, NY 10038 (see Section 3).

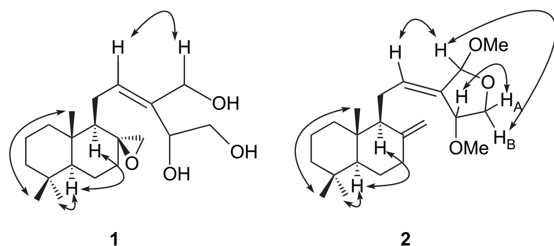



**Figure 2**. Important NOESY correlations for compound **1** and **2**.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were measured on an AA Series Automatic Polarimeter Polaar-2000 at 22 °C. Melting points were determined on a Reitcher Nr-229 micro-melting point apparatus and were uncorrected. Mass spectra (70 eV) were recorded in the EI and CI modes on a Jeol JMS 700 apparatus and no fragments below *m*/*z* 40 were registered. The UV spectra were obtained with a Shimadzu 3101 PC instrument and the IR spectra determined with a JASCO FTIR 410 apparatus. $^1$H NMR (400.6 and 500 MHz) and $^{13}$C NMR (100.13 and 125 MHz) spectra with DEPT program were recorded in $CDCl_3$ (with its signal at $\delta$ 7.25 and 77.0 ppm as standard reference) or in $CD_3OD$ (with its signals at $\delta$ 3.21 and 49.4 ppm as standard reference), unless otherwise stated, on Brükers DPX 400 and 500 apparatus. The coupling constants (*J*) are given in hertz. NMR data acquisition and processing were performed with the aid of the XWIN NMR software package. $^1$H–$^1$H COSY, HMBC, and HMQC experiments were recorded with gradient enhancements using sine shaped gradient pulses. NOESY experiments were carried out using a Brüker AM 360 instruments. For the MPLC, the chromatotron ser. no. 36B connected to a FMI pump QD (flow rate 10 ml/mn) was used with the plates (e 2 mm) prepared with silica gel $PF_{254}$ containing $CaSO_4$. Column chromatographies were run with Merck silica gel 60 and Sephadex LH-20, while TLC was carried out on silica gel 60 $GF_{254}$ pre-coated plates with detection accomplished by spraying with 50% $H_2SO_4$ followed by heating at 100 °C, or by visualizing with an UV lamp at 254 and 366 nm.

### 3.2. Plant material

The fruits of *Aframomum aulacocarpos* were collected at Bamenda, North-West province, Cameroon in March 2000. Authentication was done by Mr Paul Mezili, a retired botanist of the Cameroon National Herbarium, Yaoundé. Voucher specimen (BUD 0513) was deposited at the Herbarium of the Botany Department of the University of Dschang, Cameroon.

### 3.3. Extraction and isolation

The seeds (500 g) obtained from the fresh fruits of *A. aulacocarpos* were air-dried, ground into a fine powder, and macerated three times with acetone at room temperature. The solvent was removed under reduced pressure to afford 103 g of crude extract. This extract was subjected to open column chromatography on silica gel and eluted with a step gradient of hexane–EtOAc to yield five major fractions (A–E): A (5 g) eluted with *n*-hexane, B (1.60 g) eluted with *n*-hexane–EtOAc (9:1), C (2.5 g) eluted with *n*-hexane–EtOAc (7:3), D (23.2 g) eluted with *n*-hexane–EtOAc (1:1), and E (4.2 g) eluted with EtOAc. A portion of fraction A (800 mg) was subjected to gel permeation chromatography through Sephadex LH-20 [(*n*-hexane–EtOAc (99:1)] to give three sub-fractions ($A_1$–$A_3$). $A_1$ was further chromatographed on silica gel using gradients of *n*-hexane–acetone to yield compounds **2** (75 mg) and 15,16-epoxy-12β-hydroxylabda-8(17)-13(16),14-triene (6 mg). Compound **1** (137 mg) crystallized out of fraction E. Fraction B (1.60 g) was passed through a chromatotron, using an isocratic system of *n*-hexane–EtOAc (97:3) to yield two sub-fractions ($B_1$ and $B_2$). $B_2$ was then purified by preparative TLC plates using *n*-hexane–acetone (95:5) and extracted with $CH_2Cl_2$ solvent to yield 14,15-epoxy-8(17),12-(*E*)-labdadien-16-al (18 mg).

Fraction C was treated on a silica gel column chromatography using *n*-hexane–EtOAc (95:5) as eluant to yield coronarin E (72 mg), while fraction D treated in the same manner gave aulacocarpin A (3.71 g) [*n*-hexane–EtOAc (7:3)] and aulacocarpin B (5.05 g) [*n*-hexane–EtOAc (6:4)]. Some of the compounds were further purified by re-crystallization in appropriate solvent systems before analyses.

**3.3.1. Aulacocarpin C (1): 8β(17)-epoxy-14,15,16-trihydroxylabd-12-(*E*)-ene.** Brown needles from EtOAc; mp 160–161 °C; $[\alpha]_D^{22}$ +13.71° (*c* 2.27, $CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$); no absorption above 210. IR (KBr) $\nu_{max}$ 3474 (OH), 1603 (C=C), 1445, 1204, 1093, 1027, 962, 914 and 545 $cm^{-1}$; for $^1H$ NMR ($CD_3OD$, 400 MHz) data see Table 1. For $^{13}C$ NMR ($CD_3OD$, 100 MHz) data see Table 2. HRCIMS *m/z* 339.4939 $[M+H]^+$ (calcd for $C_{20}H_{35}O_4$, 339.4941); EIMS (70 eV) *m/z* (rel int.) $[M-H_2O]^+$ 320 (1), 302 (9), 284 (13), 277 (22), 259 (42), 203 (20), 189 (18), 131 (83), 117 (78), 63 (100), 61 (96).

**3.3.2. Aulacocarpin D (2): 15,16-epoxy-14ξ,16ξ-dimethoxylabda-8(17),12-(*E*)-diene.** White needles from MeOH; mp 114–115 °C; $[\alpha]_D^{22}$ +36.8° (*c* 0.38, $CH_2Cl_2$); UV ($CH_2Cl_2$) $\lambda_{max}$ nm (log $\varepsilon$) no absorption above 210. IR (KBr) $\nu_{max}$ 3454, 1685 (C=C), 1642 (C=C), 1459, 1387 (C–O), 1100, 1038, 979, 929, 675 and 578 $cm^{-1}$; for $^1H$ NMR ($CDCl_3$, 400 MHz) data see Table 1. For $^{13}C$ NMR ($CDCl_3$, 100 MHz) data see Table 2. HRCIMS *m/z* 349.5321 $[M+H]^+$ (calcd for $C_{22}H_{37}O_3$, 349.5325); EIMS (70 eV) *m/z* (rel int.) $[M]^+$ 348 (2), 316 (100), 285 (52), 269 (17), 215 (8), 179 (40), 137 (68), 119 (33), 81 (48), 69 (54), 55 (36).

### 3.4. Acetylation of 1

The triol (**1**) (0.074 mmol) was shaken in 2 ml of the acetic anhydride–pyridine (1:1) mixture and allowed to stand at room temperature for 24 h. The reaction mixture was then subjected to a 10% HCl solution treatment followed by a chloroform extraction. The organic phase was further chromatographed on an open silica gel column chromatography using $CH_2Cl_2$–acetone (95:5) to yield the triacetate **1a** (24.6 mg; 71.7%) as the major product and compounds **3** (5.8 mg; 16.9%) and **4** (3.8 mg; 11.0%), 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.

**3.4.1. Compound 1a: 8β(17)-epoxy-14,15,16-triacetoxylabd-12(*E*)-ene.** Syrup; for $^1H$ NMR ($CDCl_3$, 400 MHz) data see Table 1. For $^{13}C$ NMR ($CDCl_3$, 100 MHz) data see Table 2. HRCIMS *m/z* 465.6056 $[M+H]^+$ (calcd for $C_{26}H_{41}O_7$, 465.6057); CIMS *m/z* $[M+NH_4]^+$ 482.

**3.4.2. Compound 3: 17-hydroxy-14,15,16-triacetoxylabda-8(*E*),12(*Z*)-diene.** Syrup; for $^1H$ NMR ($CDCl_3$, 400 MHz) data see Table 1. For $^{13}C$ NMR ($CDCl_3$, 100 MHz) data see Table 2. HRCIMS *m/z* 465.6047 $[M+H]^+$ (calcd for $C_{26}H_{41}O_7$, 465.6057); CIMS *m/z* $[M+NH_4]^+$ 482.

**3.4.3. Compound 4: 17-hydroxy-14,15,16-triacetoxylabda-7(*E*),12(*Z*)-diene.** Syrup; for $^1H$ NMR ($CDCl_3$, 400 MHz) data see Table 1. For $^{13}C$ NMR ($CDCl_3$, 100 MHz) data see Table 2. HRCIMS *m/z* 465.6051 $[M+H]^+$ (calcd for $C_{26}H_{41}O_7$, 465.6057); CIMS *m/z* $[M+NH_4]^+$ 482.

### 3.5. Acid hydrolysis of 2

A solution of **2** (0.328 mmol) in a mixture of dioxane (3 ml) and 1 M HCl (0.66 ml) was stirred at room temperature as reported in the litterature.[17] The reaction was monitored by TLC and found to be completed after 49 h. The reaction mixture was then neutralized with solid $NaHCO_3$, the filtrate being evaporated. The residue was chromatographed on silica gel with *n*-hexane–acetone (95:5) as eluant to give the furanolabdane **5** (2 mg; 1.9%) as the minor product. Compounds **6** (44 mg; 44.5%) and **7** (21 mg; 21.2%) were obtained using *n*-hexane–acetone (88:12).

**3.5.1. Compound 5: 15,16-epoxy-12β-methoxylabda-8(17)-13(16),14-triene.** Colorless oil; $^1H$ NMR ($CDCl_3$, 500 MHz) data see Table 1. $^{13}C$ NMR ($CDCl_3$, 125 MHz) data see Table 2. HRCIMS *m/z* 317.4898 $[M+H]^+$ (calcd for $C_{21}H_{33}O_2$, 317.4905); CIMS *m/z* $[M+NH_4]^+$ 334.

**3.5.2. Compound 6.** Compound **6** was isolated as colorless oil and identified on the basis of its NMR, MS spectra data, and comparison with previously reported data.[9–11]

**3.5.3. Compound 7.** After purification through chromatography **7** was isolated as colorless needles and identified by comparison of its NMR data with literature values as being 15,16-epoxy-12β-hydroxylabda-8(17)-13(16),14-triene.[9–11] This is the first report of **7** from natural source.

### 3.6. Biological tests

The compounds were tested against trypanosome isolates grown in bloodforms in an HMI-18 medium containing 20% fetal bovine serum. Coulter counts were made daily and $IC_{50}$ values determined after 48 h. The strains used in the tests included Lab 110 EATRO, KETRI 243, 269, and 243 As 103.

Compound **1** was weakly active against Lab 110 EATRO ($IC_{50}$ 15.5 μg/ml), KETRI 243 ($IC_{50}$ 29 μg/ml), and KETRI 243 As 103 ($IC_{50}$ 24 μg/ml).

Compound **2** moderately inhibited the growth of the same pathogenic microbes with $IC_{50}$ values of 17.5, 21.0, and 24 μg/ml. No activity was shown against KETRI 269.

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