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Three labdane diterpenoids from *Aframomum sceptrum* (Zingiberaceae)

Christabel Tomla[a], Pierre Kamnaing[a], Godfred A. Ayimele[a], Eric A. Tanifum[a], Apollinaire Tsopmo[a], Pierre Tane[a], Johnson F. Ayafor[a,†], Joseph D. Connolly[b,*]

[a]*Department of Chemistry, University of Dschang, PO Box 67, Dschang, Cameroon*
[b]*Chemistry Department, The University of Glasgow G12 8QQ, Scotland, UK*

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Abstract

Three labdane diterpenoids, 8β,17-epoxy-3β,7β-dihydroxy-12(*E*)-labden-16,15-olide (**1**), methyl 8β,17-epoxy-3β,7β,15-trihydroxy-12(*E*)-labden-16-oate (**2**) and 3β,7β,8β,12ζ,17-pentahydroxylabdan-16,15-olide (**3**) have been isolated from the seeds of *Afromomum sceptrum* K. Schum (Zingiberaceae) and their structures assigned on the basis of their spectroscopic properties. Nerolidol, and the known flavonoids 3-acetoxy-4′,5,7-trihydroxyflavanone, and 3,4′,5,7-tetrahydroxyflavanone were also obtained. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Aframomum sceptrum*; Zingiberaceae; Labdane diterpenoids

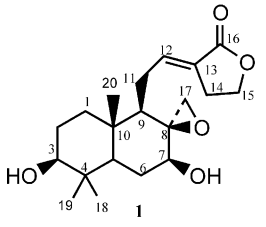
1. Introduction

Over 20 species of the genus *Aframomum* occur in Cameroon (Badré, 1972) where they are widely used for medicinal, ethnodietary and spiritual purposes. Several compounds with interesting bioactivity, including the hot-tasting non-toxic antifungal agent, aframodial (Kimbu et al., 1979; Morita and Itokawa, 1988) and other diterpenoids (Ayafor et al., 1994) and flavonoids (Ayafor and Connolly, 1981; Tsopmo et al., 1996), have been isolated in recent years from some of the species. This paper describes an investigation of the seeds of an unusually bitter species, identified as *Aframomum sceptrum*. These seeds are used in traditional medicine.

2. Results and discussion

Three new labdane diterpenoids, namely 8β,17-epoxy-3β,7β-dihydroxy-12(*E*)-labden-16,15-olide (**1**), the bitter principle, methyl 8β,17-epoxy-3β,7β,15-trihydroxy-12(*E*)-labden-16-oate (**2**), and 3β,7β,8β,12ζ,17-pentahydroxy-labdan-16,15-olide (**3**) have been isolated and characterized along with the known nerolidol, 3-acetoxy-4′,5,7-trihydroxyflavanone, and 3,4′,5,7-tetrahydroxyflavanone (Ayafor and Connolly, 1981). Compound **1** exhibits slight trypanocidal activity. All the *Aframomum* species examined thus far contain labdane diterpenoids.

The dry powdered seeds of *A. sceptrum* were macerated with acetone and the solvent evaporated under reduced pressure. The crude extract was then subjected to repeated column chromatography, gel permeation chromatography through Sephadex LH-20, and the chromatotron to afford compounds **1**–**3**.



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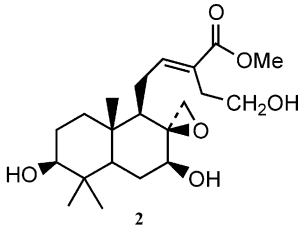

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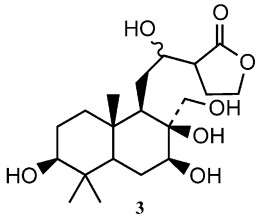

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† Deceased.

* Corresponding author. Tel.: +44-141-330-5499; fax: +44-141-330-4888.

E-mail address: joec@chem.gla.ac.uk (J.D. Connolly).

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Compound **1** was obtained as white crystals, mp 139–140 °C, from hexane–EtOAc. The EIMS of **1** showed a molecular ion peak at *m/z* 350 compatible with the molecular formula $C_{20}H_{30}O_5$. The IR spectrum revealed the presence of hydroxyl and lactone groups characterized by absorptions at ν_{max} 3441 and 1747 cm^{-1}, respectively. The ^1H NMR spectrum of **1** (Table 1), which simplified on D_2O exchange, showed three tertiary methyl groups δ 1.05 (3H-18), 0.90 (3H-20), and 0.85 (3H-19)] and a deshielded olefinic signal at δ 6.55 (H-12, *m*) typical of labdanes (Ayafor et al., 1994; Tsopmo et al., 1996). The presence of an 8(17)-epoxide in this spectrum was indicated by the chemical shifts at δ 2.90 (H-17b, *d*, $J = 4.0$ Hz) and 2.40 (H-17a, *d*, $J = 4.0$ Hz). A detailed analysis of the ^1H–^1H COSY spectrum in conjunction with the HMQC spectrum established the presence of several spin systems, namely two (–CH–CH_2–CH–), a–CH–CH_2–CH_2–, a–CH_2–CH_2–, and an isolated –CH_2. In the first spin system, the olefinic proton resonating at δ 6.55 (H-12) correlated with two protons at δ 1.90 and 2.15 (2H-11) which were in turn coupled to the signal at δ 1.60 (H-9). The ^{13}C NMR spectrum (Table 1), analyzed with the aid of the HMQC spectrum, showed a carbonyl group at δ 171.4 (C-16), a trisubstituted double bond at δ 142.0 (C-12) and 125.8 (C-13), two oxymethines at δ 78.8 (C-3) and 69.4 (C-7), and an oxymethylene at δ 65.7 (C-15). The presence of the 8(17)-epoxide was characterized by the carbon signals at δ 59.5 (C-8) and 45.1 (C-17) and the tertiary methyl signals by shifts at δ 28.6 (C-18), 15.0 (C-20), and 15.8 (C-19). Correlations in the HMBC spectrum (Table 2) enabled the above part structures to be assembled to give the gross structure **1**. Major correlations were observed between H-7 and C-5, C-6, C-8, C-9, and C-17 as well as between H-9 and C-7, C-10, C-11, and C-12.

The stereochemistry at C-3 and C-7 was deduced from the ^1H NMR spectrum. The magnitude of the observed coupling constants of H-3 (δ 3.27), $J_{3,2ax} = 11.4$ Hz and $J_{3,2eq} = 4.6$ Hz and H-7 (δ 3.70), $J_{7,6ax} = 11.6$.Hz and $J_{7,6eq} = 5.1$ Hz, showed that both hydroxyl groups were equatorial and therefore β-oriented. The β-orientation of the C-8, C-17 epoxide was deduced by comparison of the ^1H NMR shifts of the epoxide protons H-17 with those reported for aulacocarpin A and B (Ayafor et al., 1994) and aframodial (Morita and Itokawa, 1988). The correlations observed in the difference NOE spectra of **1** also confirmed the proposed stereochemistry 8β,17-epoxy-3β,7β-dihydroxy-12(*E*)-labden-16,15-olide for **1**.

Compound **2** was obtained as white crystals. The CIMS of **2** showed a pseudo-molecular ion peak at *m/z* 400 $[M + NH_4]^+$, compatible with the molecular formula $C_{21}H_{34}O_6$. The IR, ^1H and ^{13}C NMR spectra of **2** (Table 1) were similar to those observed for **1** suggesting that it was a close analogue. In the IR spectrum, the C=O absorption band observed at ν_{max} 1747 cm^{-1} in **1** was shifted to 1707 cm^{-1} in **2** revealing that the lactone

Table 1
^1H NMR data (400 MHz) and ^{13}C NMR data (100 MHz) for compounds **1**, **2** and **3**

	1[a]		**2**[a]		**3**[b]	
	δ_c	δ_H, mult. (*J*)	δ_c	δ_H, mult. (*J*)	δ_c	δ_H, mult. (*J*)
1	37.7	1.80[c], 1.65[c]	37.7	1.80[c], 1.15 *dd* (11.0,3.8)	37.0	1.45[c], 0.8[c]
2	27.4	1.65[c]	27.5	1.70[c]	27.2	1.40[c]
3	78.7	3.27 *dd* (11.4, 4.6)	78.9	3.30 *m*	77.2	3.00 *m*
4	39.4	–	39.3	–	38.7	–
5	51.7	1.06[c]	51.8	1.10[c]	52.3	0.80[c]
6	30.5	2.05 [c], 1.50 [c]	30.6	2.10[c], 1.50[c]	27.0	1.55[c]
7	69.4	3.70 *dd* (11.6, 5.1)	69.5	3.70[c]	73.2	3.11[c]
8	59.5	–	60.0	–	70.7	
9	51.4	1.60[c]	51.8	1.60[c]	52.4	0.95 *d* (10.2)
10	39.3	–	39.5	–	36.2	–
11	23.3	2.15[c], 1.90[c]	21.9	2.20[c], 2.00[c]	22.1	1.85 *m*, 1.10 *m*
12	141.3	6.55 *m*	146.7	6.75 *dd* (11.7, 11.7)	77.9	3.55, *m*
13	125.8	–	129.5	–	43.8	2.85, *ddd* (3.8, 3.8, 3.8)
14	25.7	2.85 *m*	31.4	2.60 *m*	25.2	2.30 *m*, 2.15 *m*
15	65.7	4.40 *t* (7.4)	62.0	3.75[c]	67.2	4.25 *m*, 4.15 *m*
16	171.4	–	168.7	–	177.1	–
17	45.1	2.95 *d* (4.1), 2.8 *d* (4.1)	45.2	2.95 *d* (4.1), 2.96 2.45 *d* (4.1)	76.2	3.80 *d* (11.4), 3.81 3.10 *d* (11.4)
18	28.6	1.05 *s*	27.5	1.10 *s*	28.7	0.92 *s*
19	15.8	0.85 *s*	15.8	0.85 *s*	16.4	0.70 *s*
20	15.0	0.90 *s*	15.1	0.95 *s*	16.0	0.90 *s*
OMe	–	–	52.4	3.80 *s*	–	–

[a] Spectra recorded in $CDCl_3$.
[b] Spectra recorded in DMSO-d_6.
[c] Multiplicity not determined due to overlap.

Table 2
HMBC (H to C) correlations observed for **1**, **2** and **3**

Proton position	**1**	**2**	**3**
1	5, 9, 10	–	5, 10
2	–	–	1, 3, 4, 10
3	19	–	–
4	–	–	–
5	–	–	–
6	4, 5, 7, 8, 10	4, 5, 8	8, 10
7	6	–	–
8	–	–	–
9	5, 7, 10, 11, 12, 20	10, 11, 12, 20	5, 10, 20
10	–	–	–
11	8, 9, 12, 13	8, 9, 12, 13	9, 12
12	14, 16	9, 14, 16	16
13	–	–	11, 12, 16
14	12, 13, 15, 16	12, 13, 15, 16	12, 13, 16
15	13, 14, 15	13	14, 16
16	–	–	–
17	7, 8, 9	8	8, 9
18	3, 4, 5, 19	3, 4, 5, 19	3, 4, 5, 19
19	3, 4, 5	3, 4, 5, 18	3, 4, 5
20	9, 10	9, 10	1, 5, 10
OMe	–	16	–

function was not present in **2**. In the ^{1}H NMR spectrum of **2** (Table 1), the chemical shift of the oxymethylene protons at position 15 were shifted upfield from δ 4.40 in **1** to δ 3.75 in **2** with a subsequent correlation in the HMQC spectrum to the carbon at δ 62.0. A methoxy signal appearing at δ 3.80, with an HMBC correlation to a carbonyl carbon at δ 168.7, revealed the presence of a methyl ester function in **2**. Further analyses of the DEPT-135, HMQC, ^{1}H–^{1}H COSY, NOE difference and HMBC spectra (Table 2) of **2** led to the unambiguous assignment of all protons and carbons and to structure **2**, methyl 8β,17-epoxy-3β,7β,15-trihydroxy-12(*E*)-labden-16-oate to this compound. Similar labdanes with a methyl ester at position 16 have been reported before from *Aframomum aulacocarpus* (Ayafor et al., 1994) and *A. daniellii* (Kimbu et al., 1987).

Compound **3** was obtained as white powder (CH_2Cl_2), mp 189–190 °C. Its EIMS showed no $[M]^+$ peak, but showed an $[M\text{-}H_2O]^+$ ion peak at m/z 368. Analyses of this spectrum together with the ^{1}H and ^{13}C NMR spectra led to the molecular formula $C_{20}H_{34}O_7$. The IR spectrum of **3** revealed the presence of prominent absorption bands at ν_{max} 3439 and 1747 cm^{-1}, attributed to hydroxyl and carbonyl (lactone) functionalities, respectively. In contrast to **1** and **2**, the UV spectrum showed no absorption maximum above 210 nm. The ^{1}H NMR spectrum showed three tertiary methyls at δ 0.92, 0.90, and 0.70 typical of labdanes attributed to C-18, C-20 and C-19, respectively. Two oxymethylene groups [δ_H 4.25 (1H, *m*) and 4.15 (1H, *m*), δ_C 67.2 (C-15): δ_H 3.78 (1H, *d*, J = 11.4 Hz) and 3.10 (1H, *d*, J = 11.4 Hz), δ_C 76.2 (C-17)] and three oxymethines [δ_H 3.00, 3.11, 3.55 (all *m*, H-3, H-7 and H-12 respectively), δ_C 77.2, 73.2 and 77.9] were also present in the molecule. The ^{13}C NMR spectrum of **3** also revealed a carbonyl function at δ 177.1. Comparison of ^{1}H and ^{13}C NMR data of **3** with those of **1** showed the absence of olefinic signals and the appearance of an additional oxymethine signal (δ_H 3.55, δ_C 77.9) in **3**. The epoxy protons in **1** were also replaced in **3** by shifts at δ_H 3.10 and 3.80, which clearly showed the presence of a hydroxyl group at C-17. The rest of the data (Table 1) were comparable with those reported in the literature for similar compounds (Barrero and Altarejos, 1993). Further analyses of the ^{1}H–^{1}H COSY, HMQC, DEPT-135, HMBC (Table 2) and NOE difference spectra of **3** led to the assignment of all protons and carbons and to structure **3**, 3β,7β,8β,12ζ,17-pentahydroxylabdan-16,15-olide. No direct evidence was obtained for the stereochemistry at C-12 and C-13.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were measured on an AA Series Automatic Polarimeter Polaar-2000. The UV spectra were recorded on a Shimadzu UV-3101 PC spectrophotometer, while IR spectra (KBr) were recorded on a Jasco FT-IR-410 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ or in DMSO-d_6 on a Bruker DPX-400 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS δ = 0) as internal standard and coupling constants (J values) are given in Hertz. ^{1}H–^{1}H COSY, HMBC, and HMQC experiments were recorded with gradient enhancements using sine shaped gradient pulses. Mass spectra were recorded in the positive EI and CI modes on a Jeol JMS-700 instrument and no fragments below m/z 40 were registered. Column chromatography, on Merck Si gel 60 and gel permeation chromatography on Sephadex LH-20, were used for isolation and purification. TLC was carried out on Kieselgel 60 F_{254} (Merck) pre-coated plates and spots were visualized by spraying with 50% H_2SO_4 solution followed by heating.

3.2. Plant material

The seeds of *Aframomum sceptrum* K. Schum used for this experiment were collected in Fontem, South-West Province, Cameroon in December 1999. Paul Mezili, a botanist of the Cameroon National Herbarium, authenticated the plant material. Voucher specimens have been deposited in the Herbarium of the Botany Department of the University of Dschang, Cameroon.

3.3. Extraction and isolation

The dried and finely powdered seeds (270 g) of *A. sceptrum* were macerated three times with acetone (2 l). Filtration and removal of solvent in vacuo afforded 40 g of crude extract. This extract was subjected to open column chromatography on silica gel and eluted with a hexane–EtOAc gradient to yield five major fractions (A-E): A (6.7 g) eluted with hexane–EtOAc (19:1), B (5.4 g) eluted with hexane–EtOAc (9:1), C (4.6 g) eluted with hexane–EtOAc (1:1), D (12 g) eluted with hexane–EtOAc (3:7), and E (200 mg) eluted with pure EtOAc. A portion (380 mg) of fraction A was purified on a chromatotron with hexane–EtOAc (9:1) as eluent to give nerolidol (125 mg), while fraction C was treated on a silica gel column using CH_2Cl_2–MeOH (49:1) as eluent to yield 3-acetoxy-4′,5,7-trihydroxyflavanone (6 mg). Fraction D (2 g) was subjected to gel permeation chromatography through Sephadex LH-20 (MeOH) to give three sub-fractions (D_1–D_3). D_2 was further chromatographed on Si gel using gradients of CH_2Cl_2–MeOH to yield **1** (1 g) and **2** (3.5 mg), while D_3 treated in the same manner gave 3,4′,5,7-tetrahydroxyflavanone (6 mg). Compound **3** (90 mg) crystallized out of fraction E.

3.3.1. 8β,17-Epoxy-3β,7β-dihydroxy-12(E)-labden-16,15-olide (*1*)

White needles (hexane–EtOAc): mp 139–140 °C; $[\alpha]_D^{22}$ +27.9 (*c* 1.24, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 222 (3.12) nm; IR (KBr) ν_{max} 3441 (OH), 1747 (C=O), 1672, 1632, 1445, 1388, 1204, 1093, 1027, 962, 914, 545 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 1; CIMS *m*/*z* (rel. int.) $[M+NH_4]^+$ 368 (100), 338 (42), 333 (7); EIMS (70 eV) *m*/*z* (rel. int.) $[M]^+$ 350 [(1), 332 (8), 320 (4), 305 (6), 287 (14), 271 (65), 269 (5), 247 (8), 229 (10), 209 (31), 178 (32), 139 (23), 112 (100), 91 (53), 81 (44).

3.3.2. Methyl 8β,17-epoxy-3β,7β,15-trihydroxy-12(E)-labden-16-oate (*2*)

White crystals (hexane–EtOAc): mp 85–86 °C; $[\alpha]_D^{22}$ +13.7 (*c* 0.35, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 220 (3.10), 263 (1.63) nm; IR (KBr) ν_{max} 3435 (OH), 1707 (C=O), 1633 (C=C), 1437, 292, 1209, 1094, 912, 749, 544 cm^{-1}; ^1H NMR and ^{13}C NMR data are reported in Table 1; CIMS *m*/*z* (rel. int) 400 $[M+NH_4]^+$ (15), 368 (100), 350 (16), 338 (68); EIMS (70 eV) *m*/*z* (rel. int.) $[M]^+$ absent, 350 (3), 332 (10), 287 (9), 271 (6), 247 (110, 209 (350), 180 (30), 135 (43), 121 (100), 112 (97), 94 (65), 43 (66).

3.3.3. 3β,7β,8β,12ζ,17-Pentahydroxylabdan-16,15-olide (*3*)

White powder (CH_2Cl_2): mp 189–190 °C, $[\alpha]_D^{22}$−7.14 (*c* 0.49, MeOH); UV (MeOH) no absorption above 210 nm; IR (KBr) ν_{max} 3439 (OH), 1746 (C=O), 1631 (C=C), 1456, 1093, 1024, 950, 874 cm^{-1}; ^1H NMR and ^{13}C NMR data are reported in Table 1; Isobutane-CIMS *m*/*z* (rel. int) $[M+H]^+$ 369 (68), 351 (67), 333 (100), 315 (30), 247 (5); EIMS $[M]^+$ absent, 350 $[M-H_2O]^+$ (4), 336 (100), 318 (8), 274 (14), 233 (17), 211 (22), 135 (13), 121 (20), 81 (21), 55 (23), 41 (24).

Acknowledgements

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