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A norbislabdane and other labdanes from *Aframomum sulcatum*

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Abstract—Extracts of the seeds of *Aframomum sulcatum* (Oliv. & Hanb) K. Schum (Zingiberaceae) yielded a novel norbislabdane derivative, sulcanal (**1**) and the new labdane (12*E*)-8β,17-epoxy-11-hydroxy-12-labden-15,16-dial-11,15-hemiacetal (**2**) (as a 1:1 C-15 epimeric mixture), along with known labdane diterpenes.

1. Introduction

The seeds of *Aframomum sulcatum* (Oliv. & Hanb) K. Schum are widely used as an anthelmintic and to treat fevers in many medicinal preparations in Cameroon.[1] In the late seventies, the hot-tasting dialdehyde aframodial (**3**) was isolated from the seeds of *Aframomum daniellii*.[2] Aframodial (**3**) has later been shown to possess a broad spectrum of biological activities.[3–5] In an effort to discover other bioactive substances of similar structures and to discover other natural sources of this potential therapeutic agent, several species of *Aframomum* that grow in Cameroon have been investigated.[6–8] In the present study, one of these, *A. sulcatum* has yielded the two novel diterpenoids **1** and **2** (see Figure 1 for structures).

2. Results and discussion

Compound **1**, named sulcanal, crystallized from an hexane–EtOAc mixture. Its elemental composition $C_{39}H_{56}O_4$, was deduced from its high resolution MS and 1D NMR data

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ; multiplicity; *J*) for **1** in $CDCl_3$ with the solvent signals (7.26 and 77.0 ppm) as reference

No.	δ_C	δ_H	No.	δ_C	δ_H
1	39.0	1.07; m; 1H, 1.83; m; 1H	5′	54.5	1.06; m; 1H
2	18.5	1.45; m; 1H, 1.60; m; 1H	6′	20.0	1.64; m; 1H, 1.71; m; 1H
3	42.1	1.23; dd; 3.3, 13.2; 1H, 1.44; m; 1H	7′	35.2	1.45; m; 1H, 2.03; m; 1H
4	33.6	–	8′	58.7	–
5	55.1	1.11; m; 1H	9′	57.7	2.34; d; 9.8; 1H
6	20.1	1.64; m; 1H, 1.71; m; 1H	10′	39.6	–
7	35.9	1.39; m; 1H, 1.97; m; 1H	11′	132.3	5.96; dd; 9.8, 15.9; 1H
8	57.5	–	12′	130.5	7.22; d; 15.9; 1H
9	48.4	1.87; dd; 8.1, 12.4; 1H	17′	48.9	2.32; d; 4.6; 1H, 2.56; d; 4.6; 1H
10	39.5	–	18′	33.6	0.95; s; 3H
11	31.5	1.18; m; 1H, 1.55; m; 1H	19′	22.0	0.92; s; 3H
12	74.1	4.54; dd; 2.5, 10.2; 1H	20′	15.7	1.13; s; 3H
17	49.0	2.39; d; 4.0; 1H, 2.58; d; 4.0; 1H	1″	131.6	–
18	33.5	0.93; s; 3H	2″	140.5	–
19	21.7	0.87; s; 3H	3″	124.5	7.41; d; 1.4; 1H
20	14.7	0.89; s; 3H	4″	151.0	–
1′	40.6	1.09; m; 1H, 1.55; m; 1H	5″	124.4	7.32; d; 1.4, 8.0; 1H
2′	18.5	1.45; m; 1H, 1.60; m; 1H	6″	132.0	7.76; d; 8.0; 1H
3′	42.0	1.23; dd; 3.3, 13.2; 1H, 1.44; m; 1H	7″	191.9	10.23; s; 1H
4′	33.4	–			

Keywords: labdanes; sulcanal; *Aframomum sulcatum*; norbislabdane.
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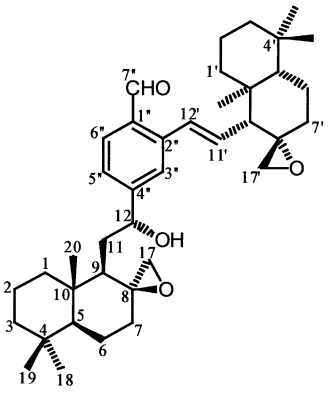

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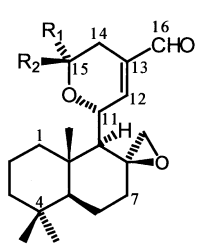

2a R_1 = H, R_2 = OH
2b R_1 = OH, R_2 = H
2c R_1 = H, R_2 = OAc

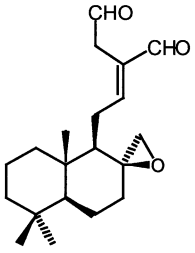

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Figure 1.

(Table 1), and the presence of hydroxyl and conjugated aldehyde functions was indicated by the intense absorption bands at ν_{max} 3449, 1686, and 1601 cm^{-1} in the IR spectrum. The ^1H NMR spectrum of **1** indicated the presence of six methyl groups, all appearing as singlets around 1 ppm, a 1,2,4-trisubstituted benzene as indicated by the signals of three aromatic protons with a characteristic coupling pattern, and an aldehyde group. Comparison of NMR data of **1** with those of the known labdanes 8,17-epoxy-12-labden-15,16-dial, or aframodial, (**3**)[5] and 8,17-epoxy-12-labden-16,15-olide,[3] which were also isolated, led to the conclusion that **1** was a drimane or labdane derivative (Fig. 1). The analysis of the ^{13}C NMR spectrum of **1** with the aid of the HMQC spectrum revealed the presence of 39 carbon atoms including six methyls, 13 methylenes, 11 methines, and nine quaternary carbons. The complete structural elucidation of **1** was achieved by analysis of the HMBC and COSY spectra. Pertinent correlations (shown in Fig. 2) were observed between 11-H_2 and C-8, C-9, C-10, C-12 and C-4″, as well as between 11′-H_2 and C-8′, C-9′, C-10′, C-12′ and C-2″. 12-H correlated with C-3″, C-4″ and C-5″, in addition to C-9 and C-11, and the corresponding correlations were also observed for 12′-H. 20-H_3 correlate to C-1, C-5, C-9 and C-10, while 18-H_3 and 19-H_3 correlate to C-3, C-4 and C-5 in addition to each other's carbons. 17-H_2 correlate to C-7, C-8 and C-9. The corresponding correlations were observed in the other decalin system, and, complemented with data from the COSY experiment, established the two drimane partial structures.

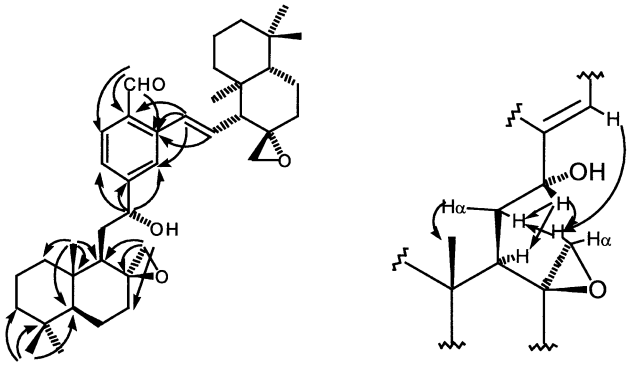

Figure 2. Pertinent HMBC correlations observed with sulcanal (**1**) (left), and NOESY correlations observed with its central parts (right).

The relative configuration of sulcanal (**1**) was suggested by correlations observed in a NOESY spectrum (Fig. 2). The configurations of C-5, C-8, C-9 and C-10 (as well as of C-5′, C-8′, C-9′ and C-10′) shown in the structure were indicated by NOESY correlations observed between, for example, 1-Hα and 5-H as well as 9-H, 17-Hβ and 11-Hβ, 17-Hα and 7-Hβ, and 20-H_3 and 6-Hβ. 11-Hα correlates with 1-Hβ and 20-H_3, while 12-H correlates with 9-H, 17-Hβ and 11-Hβ. Clear NOESY correlations between 17-Hβ and the aromatic protons 3″-H and 5″-H suggest that the configuration of C-12 should be as shown in Fig. 1. The 11′–12′ double bond is *E*, as judged by the coupling constants between 11′-H and 12′-H, but upon standing in chloroform for days at room temperature sulcanal (**1**) slowly isomerised to a *E*/*Z* mixture (approximately 2:1).

Sulcanal (**1**) may possibly arise by a Diels–Alder reaction of aframodial (**3**) and an appropriate diene, followed by decarbonylation and aromatisation, or by the condensation of two molecules of **3** and decarbonylation/aromatisation. Aframodial (**3**) was found to be a metabolite of *A. sulcatum* as well, and is therefore possible that it is the biosynthetic precursor. However, we could not detect sulcanal (**1**) as a chemical product formed from any of the labdane diterpenes isolated from *Aframomum* sources, and **1** is consequently believed to be a natural product.

Compound **2** was obtained as white crystals from a mixture of hexane–EtOAc, and again the presence of a hydroxyl and a conjugated aldehyde group was indicated by the IR data. As the signals in the ^1H and ^{13}C NMR spectra (Table 2) were doubled, we initially believed that **2** is a dimer similar to sulcanal (**1**), but the apparent molecular ion at *m*/*z* 334 in the EI mass spectrum suggested instead that **2** is a 1:1 mixture of the two epimers **2a** and **2b**. The molecular formula ($C_{20}H_{30}O_4$) could then be deduced from the HREIMS and 1D NMR spectral data. The structure of **2** was determined from 2D NMR data, and again the HMBC correlations from the three methyl groups as well as from 11-H to C-8, C-9, C-10, C-12 and C-13 were very informative. The two protons at C-17 correlated with C-7, C-8 and C-9, and the aldehyde proton correlated with C-12, C-13 and C-14, confirming that the aldehyde group is conjugated, HMBC correlations between 11-H and C-15 as well as between 15-H and C-11 indicated an ether linkage between the two carbons. COSY correlations between the remaining protons

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ; multiplicity; J) for **2a/2b** in $CDCl_3$–CD_3OD 19:1 with the $CHCl_3$/$CDCl_3$ signals (7.26 and 77.0 ppm) as reference

No.	δ_C	δ_H	No.	δ_C	δ_H
1	39.9	1.14; m; 1H, 1.86; dd; 8.3, 13.4; 1H	1′	40.0	1.15; m; 1H, 1.78; br d; 12.0; 1H
2	18.5	1.47; m; 1H, 1.60; m; 1H	2′	18.5	1.47; m; 1H, 1.60; m; 1H
3	41.4	1.15; m; 1H, 1.43; br d; 13.4; 1H	3′	41.5	1.15; m; 1H, 1.43; br d; 13.4; 1H
4	33.4	–	4′	33.4	
5	55.1	0.99; dd; 2.4, 11.8; 1H	5′	55.3	1.01; dd; 2.4, 11.8;
6	20.0	1.60; m; 1H, 1.65; m; 1H	6′	20.2	1.60; m; 1H, 1.65; m; 1H
7	36.6	1.22; m; 1H, 1.91; dd; 8.3, 13.4; 1H	7′	36.7	1.22; m; 1H, 1.91; dd; 8.3, 13.4; 1H
8	58.6	–	8′	58.7	–
9	56.4	1.96; br s; 1H	9′	56.7	2.11; br s; 1H
10	39.7	–	10′	39.3	–
11	63.8	4.91; br s; 1H	11′	71.9	4.66; br s; 1H
12	152.2	7.13; s; 1H	12′	152.8	7.07; s; 1H
13	132.4	–	13′	134.7	–
14	26.5	2.29; dd; 3.0, 17.6; 1H, 2.38; m; 1H	14′	28.6	2.11; m; 1H, 2.54; dt; 2.9, 16.9; 1H
15	89.9	5.39; d; 3.3; 1H	15′	93.8	4.79; dd; 3.0, 9.0; 1H
16	193.6	9.35; s; 1H	16′	192.9	9.39; s; 1H
17	49.8	2.19; d; 4.5; 1H, 2.65; d; 4.5; 1H	17′	50.1	2.20; d; 4.4; 1H, 2.78; d; 4.4; 1H
18	33.8	0.87; s; 3H	18′	33.7	0.87; s; 3H
19	21.7	0.87; s; 3H	19′	21.7	0.87; s; 3H
20	16.8	1.18; s; 3H	20′	16.9	1.20; s; 3H

established the labdane skeleton unambiguously. In the NOESY spectrum of **2**, strong NOESY correlations were observed between 11-H to 9-H and 15-H in one of the epimers, which consequently was assigned the structure **2b**. In the other epimer, **2a**, no NOESY correlation between 11-H and 15-H was observed, indicating that the point of epimerisation is at C-15. Comparison of NMR data with those published for 11,15-epoxy-15-hydroxy-8(17),12-labdadien-16-al[9] support the suggested structure. Acetylation of **2** gave the monoacetate **2c**, as a pure compound. ^{1}H–^{1}H coupling constants and NOESY correlations observed with **2c** are very similar to those of **2a**, and the relative configuration of C-15 of the two should be the same.

Besides **2** and **3**, two additional labdanes were obtained and identified as the known 8β,17-epoxy-12-labden-16,15-olide[3] and galanal B,[10] by comparison with the published spectroscopic data.

3. Experimental

3.1. General

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature in $CDCl_3$ or in $CDCl_3$–CD_3OD using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts are reported in ppm with the solvent signals (7.26 ppm for $CHCl_3$ and 77.0 ppm for $CDCl_3$) as reference, while the coupling constants (J) are given in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For 2D heteronuclear correlation spectroscopy, the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^nJ_{CH}$=10 Hz. The IR spectra were recorded with a Perkin–Elmer 298 and a Shimazu hyper 1.51 spectrophotometer. EIMS and HREIMS spectra (direct inlet at 70 eV) were recorded with a JEOL SX102 spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin–Elmer 141 polarimeter at 22°C. Column chromatography was run on Merck Si gel 60 and gel permeation on Sephadex LH-20, while TLC were carried out on Si gel 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 a UV lamp at 254 and 366 nm.

3.2. Plant material, extraction and isolation

The seeds of *A. sulcatum* were collected from Nkwen, North West Province, Cameroon, in 1998. Authentication was done by Mr Paul Mezili, a retired botanist of the Cameroon National Herbarium, Yaounde. A voucher specimen (PM 2032) has been deposited at the Botany Department, University of Dschang. The dried powdered seeds of *A. sulcatum* (600 g) were extracted overnight at room temperature by percolation with hexane, followed by acetone. Concentration in vacuo yielded 50 and 45 g of crude extract, respectively. The hexane extract was subjected to column chromatography on silica gel using a gradient of EtOAc in hexane to furnish three fractions which were further purified by gel permeation chromatography on a Sephadex LH-20 column (eluted with CH_2Cl_2–hexane 1:1) affording 8,17-epoxy-12-labden-16,15-olide (230 mg), sulcanal (**1**) (124 mg) and galanal B (29 mg). Column chromatography of the acetone extract on silica gel with mixtures of hexane–EtOAc as the mobile phase yielded, in addition, aframodial (**3**) (546 mg) and compound **2** (5 mg).

3.2.1. Sulcanal (1). Compound **1** was obtained as colourless plates (hexane–EtOAc), mp 122–124°C. $[\alpha]_D^{22}$=+93 (c 0.8, $CHCl_3$). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3449, 2926, 1686, 1601, 1460, 1431, 1389, 1366, 1219, 1201, 758. See Table 1 for ^{1}H and ^{13}C NMR spectral data. EIMS (probe) 70 eV, m/z (rel. int.): 588.4184 (25, M^+, $C_{39}H_{56}O_4$ requires 588.4178), 570 (68), 557 (27), 433 (18), 382 (41), 363 (100), 317 (22), 267 (30), 162 (48).

3.2.2. 8β,17-Epoxy-11,15-epoxy-15-hydroxy-12-labden-16-al (2a/2b). Compound **2a/2b** was obtained as a 1:1 epimeric mixture as white crystals (hexane–EtOAc), mp 224–226°C. $[\alpha]_D^{22}=-55$ (*c* 0.2, $CHCl_3$). IR (KBr) ν_{max} (cm^{-1}): 3294, 1649, 1628, 1369, 1178, 1109, 901. See Table 2 for ^1H and ^{13}C NMR spectral data. EIMS (probe) 70 eV, *m/z* (rel. int.): 334.2146 (4, M^+, $C_{20}H_{30}O_4$ requires 334.2144), 316 (14), 301 (9), 287 (11), 235 (17), 207 (31), 189 (46), 177 (41), 128 (100), 109 (55), 95 (63), 81 (53), 69 (60), 55 (32) 41 (35).

3.2.3. 8β,17-Epoxy-11,15-epoxy-15-acetoxy-12-labden-16-al (2c). Compound **2c** was obtained as a colourless oil after acetylation of **2a/2b** with acetic anhydride in pyridine at room temperature over night. ^1H NMR spectral data ($CDCl_3$, 500 MHz): 0.92 (3H, s, H-19), 1.05 (3H, s, H-18), 1.10 (1H, dd, *J*=2.5, 12.3 Hz, H-5), 1.22 (3H, s, H-20), 2.10 (3H, s, Ac), 2.25 (1H, d, *J*=4.5 Hz, H-17a), 2.40 (1H, dd, *J*=3.5, 17.8 Hz, H-14a), 2.55 (1H, m, H-14b), 2.26 (1H, d, *J*=4.5 Hz, H-17b), 4.80 (1H, br s, H-11), 6.38 (1H, d, *J*=4.4 Hz, H-15), 7.20 (1H, br s, H-12).

Acknowledgements

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