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Aframodial and other bioactive diterpenoids from *Aframomum* species

J. F. Ayafor[a*], M.H.K Tchuendem,[a] B. Nyasse,[a] F. Tillequin,[b] and H. Anke[c]

[a]Department of Organic Chemistry, University of Yaounde 1, Box 812, Yaoundé, Cameroon, [b]URA Associé au CNRS No 3010, Université René Descartes, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006, Paris, France,and [c]Department of Biotechnology, University of Kaiserslautern, Paul-Ehrlich Str. 23 D-6750, Kaiserslautern Germany.

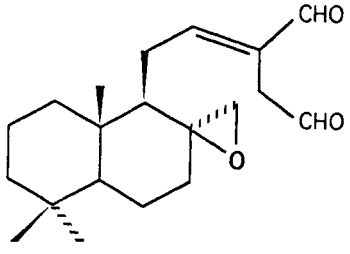
Abstract: The biological activity of the potential therapeutic agent, 8ß,17-epoxy-12E-labdene-15,16-dial (aframodial) **1**, has been briefly reviewed. Ten other Cameroonian *Aframomum* species have been investigated as potentially improved sources for **1** and their aframodial content analyzed. In the course of these investigations three novel diterpenoids, *aulacocarpinolide, aulacocarpin A*, and *aulacocarpin B*, were isolated from *A. aulacocarpos* and their structures determined as **2**, **3**. and **4**, respectively. In the structural elucidation, particular use was made of high-field **1D** **2D** and **3D** NMR spectroscopic techniques.

The seeds of *Aframomum daniellii* (Hook, F.) K. Schum. (Zingiberaceae) are widely used medicinally and as a food spice in Cameroon. A hot-tasting diterpene, 8ß,17-epoxy-12E-labdene-15,16-dial (aframodial) **1**, was isolated from this species in our laboratory in 1979(1). Aframodial has since been obtained from two other sources albeit in relatively smaller yields: *Alpinia galanga* (L) Willd(0.0018%) (2) and *Zingiber Officinale Roscoe* (0.04%) (3). Aframodial **(1)** displays a wide spectrum of biological activity. Of particular significance is its antifungal activity. The minimum inhibitory concentrations (MIC) of this compound against a variety of microorganisms are presented in Table 1. **1** particularly exhibited strong activity against *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Hansenula anomala, and Candida utilis*. Aframodial **1** is slightly more active than the commercially available antifungal amphotericin B, which is one of several antifungal drugs currently used to stop the evolution of numerous deep-seated mycoses (*candidiasis, aspergillosis*...), although its high toxicity limits its wide use. These antifungal data obtained on **1** since 1982 parallel those recently published by Morita and Itokawa(2). At a time systemic infections caused by filamentous fungi have become increasingly serious especially when the host's defence mechanism is weakened, the above results suggest that **1** is a promising therapeutic antimicrobial agent. Compound **1** also exhibits strong cytotoxic activity (ED_{50} 2.5 µg/ml) towards L1210 cells {present investigation and towards KB cells (2)}. Furthermore, recent studies by Japanese workers (3) have demonstrated the anti-hypercholesteromic effect of Aframodial. According to these researchers, **1** could be considered to be an effective hydroxylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor.

Kilogramme quantities of **1** will certainly be required in the future for further testing. Thus, to avert a possible drug supply crisis, the aframodial content of the seeds of ten related *Aframomum* species growing in Cameroon have been investigated. Three of them, A. polyanthum, A.masuianum, and *A. kayserianum* contain amounts (5g/kg dry weight of seeds) comparable to those found in *A. daniellii*, while six species, *A. melegueta, A. citratum, A.giganteum, A. hanburyii, A. pruinosum, and A. letestuianum* are completely devoid of **1**. It must however be noted that the content of **1** varied considerably with the geographical location of the *Aframomum* species. The tenth species, *A. aulacocarpos*, Pelleg. ex. J. Koechlin, contain somewhat lower amounts (less than O.5g/kg) of **1** in addition to relatively larger quantities of three related novel diterpenoids. The structural elucidation of the 3 diterpenoids is outlined below.

TABLE 1. Antimicrobial activity of Aframodial 1

Microorganisms Tested	MIC (μg/ml)
Staphyloccus aureus NCTC 8530	12.5
Bacilus subtilis K-49	12.5
Escherichia coli IFO 3545	>100
Pseudomonas aeruginosa IAM 1007	>100
Saccharomyces cerevisiae IFO 0203	0.78
Schizosaccharomyces pombe IFO 0342	0.39
Hansenula anomala IFO 0136	1.56
Candida utilis ATCC 42402	1.56
Sclerotinia libertiana ss	3.13
Mucor mucedo IFO 7684	25
Rhizopus chinensis IFO 4745	12.5
Aspergillus niger ATCC 6275	12.5
Penicillium crustosum Thom	6.25



1

Compound 2, trivial name aulacocarpinolide, mp 150-151°, $[\alpha]_D^{20}$-615° (c 0.02; MeOH), $C_{20}H_{28}O_4$, had intense ir absorption bands at max 3510, 1760, and 1660 cm^{-1} indicative of hydroxyl, carbonyl, and olefinic functionalities, respectively. The ^1H nmr spectrum of **2** revealed signals for three tertiary methyls [δ 0.83 and 1.02 (6H)], an epoxide methylene [4.06 and 4.03 (ABd, J 15.3 Hz), a secondary hydroxyl group [δ 3.23 (dd, J 4.2 and 11.6 Hz)], and a vinyl proton [δ 7.39 (tt, 1.5 Hz, H-14)]. The ^{13}C nmr spectrum confirmed the existence in **1** of two double bonds, one tetrasubstituted [δ 135.72 (s) and 134.86 (s)], and the other trisubstituted [δ 124.87 (s) and 144.86 (d)]. An examination of the ^{13}C nmr carbonyl resonance at δ 172.22 in conjunction with the proton signals at δ 4.83 (dt) and δ 7.39 suggested that the carbonyl group was part of an unsaturated δ-lactone moiety. The chemical shifts of the latter signals and their splitting pattern were reminiscent of a 16,15-olide (5) rather than a 15,16-olide. The above data account for the functionality of **2** which is, therefore, tetracyclic.

The ^{13}C-nmr spectrum of 2 was assigned (Table 2) with the aid of DEPT 135, ^1H - ^{13}C HETCOR, HMQC-TOCSY and off-resonance decoupling techniques and revealed the presence of 20 carbon atoms which included 3 methyls, 7 methylenes. 4 methines, and 6 quaternary carbons. A detailed analysis of the ^1H -^1H COSY spectrum in conjunction with one-bond correlation experiments established the presence of two isolated five proton (-CH -CH_2 - CH_2) spin systems, a five carbon (-CH_2 - CH = C - CH - CH_2-) spin network, and an isolated CH_2 system. The connectivities defined in the above spin systems were further confirmed by HMQC-TOCSY experiments(6) run with mixing times of 12ms and 54ms, respectively. These spectral features, along with the co-occurrence of **2** with aframodial(**1**) in *A. aulacocarpos* suggested that **2** was a labdane diterpene. On the basis of spectral data of model compounds(7) and some diagnostic heterocorrelations, the four spin systems above were assembled to give gross structure **2.** This structure is fully compatible with the ^{13}C- and ^1H-nmr spectral data (Table 2).

The relative stereochemistries at C-3 and C-12 were deduced from the ^1H-nmr spectrum. The magnitude of the observed coupling constants of H-3 at δ 3.23 ppm, J 11.6 and 4.2 Hz indicates that the C-3 OH group must be axial and therefore ß-oriented. Also, H-12 at δ 4.24 displayed two coupling constants with H-11, one large (9.8 Hz) and the other relatively smaller (1.3 Hz). Inspection of models revealed that these couplings required a conformation with an equatorial 16, 15-olide moiety. The values of the coupling constants compared well with those reported (5) for a closely related compound with the same stereochemistry at C-12. Aulacocarpinolide (**2**) is thus 12α,17-epoxy-3ß-hydroxy-8(9),13-labdadien-16,15-olide and to our

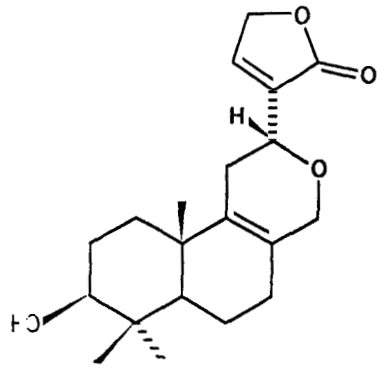
knowledge is the first labdane derivative with both a 12,17-ether linkage and a 15,16-olide residue at C-12.

Two further new labdane diterpenoids, aulacocarpin A and B, were also obtained from the extracts of *A. aulacocarpos*. Aulacocarpin A **(3)**, $C_{21}H_{32}O_5$, mp 102-103°, $[\alpha]_D^{20}$ + 11°, was spectroscopically elucidated as methyl 3ß-hydroxy-8ß,17:14 ,15-diepoxy-12E-labden-16-oate while aulacocarpin B **(4)**, $C_{21}H_{32}O_6$, mp 140 - 141°, $[\alpha]_D^{20}$ + 21° is its 6ß-hydroxy congener, methyl 3ß, 6ß-dihydroxy-8ß,17:14 ,15-diepoxy-12E-labden-16-oate. The ß-OH substitution at C-3 of **3** and **4** respectively, was evident from the chemical shifts and J values of the axial proton at C-3 {δ 3.25(dd J.4.6 and 11.5 Hz); **3**} and {δ 3.18 (dd, J 6.5 and 9.8 Hz; **4**)}. The ß-configuration of the C-6 hydroxy group in **4** was also deduced from the well-resolved signal of the equatorial C-6 proton (δ 4.50, d, J = 2.3 Hz). The stereochemistry of the C-8, C-17 epoxide in **3** and **4** was obtained by comparison of the proton nmr shifts of the Ha-17 and Hb-17 with those reported for aframodial **(1)** (1) and its synthetic 8-α-isomer **(5)** (2). In **3**, for example, Ha-17 and Hb-17 resonate at δ 2.53 and 2.32, respectively, and at δ 2.42 and 2.27 respectively, in **1**. In **5** which has the opposite stereochemistry at C-8, the two protons were observed at δ 2.70 and 2.56, respectively. The ^{13}C resonances of C-8 and C-17 in **3** (δ 48.80 and 57.30) also agreed better with those reported for **1** (48.80, 57.60) than those published for the synthetic 8-α-isomer **(5)** (2). No direct evidence was obtained for the stereochemistry of the 12, 13-double bond in **3** and **4**, but the co-occurrence of **3** and **4** with **1** with very identical ^1H and ^{13}C spectral features for H-12, C-12, and C-13 suggested the same E configuration for the three diterpenoids. Finally, the relative stereochemistry of the 14,15-epoxy group could not be determined.

TABLE 2: ^{13}C-Nmr Data (500 and 125 MHz) of the Diterpenoids **2, 3 and 4.**

	2		**3**	**4**
Position	δ ^{13}C	δ ^1H, m, J, in Hz	$\delta^{13}C$	$\delta^{13}C$
1....	33.69,t	1.20(m);1.72(m)	35.68	39.76
2....	27.32,t	1.61(m);1.77(m)	27.09	27,21
3....	78.73,d	3.23(dd,4.2,11.6)	78.55	78.69
4....	36.96(s)		38.95	39.58
5....	50.76,d	1.15 (dd, 1.8, 12.6)	55.08	55.80
6....	17.98,t	1.53(m);1.76(m)	19.64	68.21
7....	26.93t	1.89(m)	37.12	43.67
8....	135.72,s		57.30	58.62
9....	134.88,s		52.55	52.50
10...	38.67,s		39.45	39.72
11...	28.10,t	1.89(m);2.42(m)	20.83	21.40
12...	69.95,d	4.24(dd,1.2, 9.8)	149.81	149.02
13...	124.87,s		127.41	127.84
14...	144.6,d	7.39(tt,1.5)	48.30	48.38
15...	70.48,d	4.83(dt,1.8)	47.57	47.57
16...	172.22,s		166.36	166.29
17...	68.91,t	4.06 and 4.03 (ABd, 15.3)	48.78	47.10
18...	27.91,q	1.02(s)	28.19	16.91
19...	18.98,q	1.02(s)	15.26	28.00
20...	15.37,q	0.83(s)	14.51	16.42

Compounds **2, 3,** and **4** moderately inhibited the growth of the pathogenic bacteria *Bacillus subtilis* (MIC 25 μg/ml. The compounds were also weakly active against *Mucor miehei* (MIC 50 μg/ml). Aulacocarpinolide **(2)** further showed cytotoxicity against L1210 cells at a concentration of 12.5 μg/ml.



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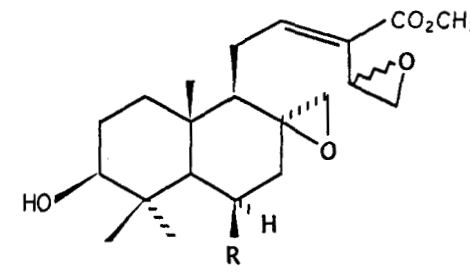
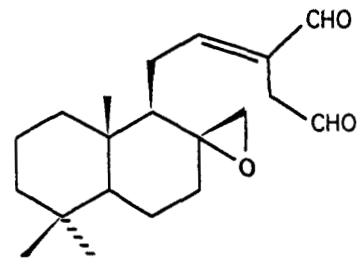


3 R= H
4 R= OH



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