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Received November 17, 1999
Accepted February 22, 2000

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Aframodial, a labdane diterpene showing selective *in vitro* antileukemic activity

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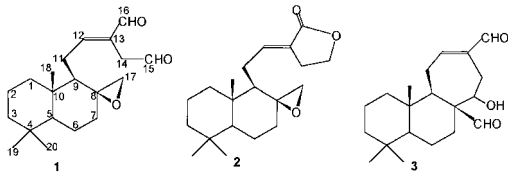
The seeds of *Aframomum daniellii* (Hook f.) K. Schum (Zingiberaceae) known in the Western Province of Cameroon as "Achoh" are the most medicinally used part of this species and are believed to be eaten by snakes to facilitate sloughing. A hot-tasting diterpene, (*E*)-8β(17)-epoxylabd-12-ene-15,16-dial (aframodial, **1**) first isolated from this species in our laboratory in 1979 [1] has since been obtained from other sources albeit in relatively smaller yields [2, 3]. Aframodial displays a wide spectrum of biological activities [2–5]. Of particular significance is its antifungal activity [4]. Aframodial also exhibits strong cytotoxic activity towards KB cells [2] and towards L1210 (ED_{50} 2.5 μg/ml) cells [4]. Tanabe et al. [3] have also shown the antihypercholesteromic effect of aframodial which resulted from its inhibitory activity on hydroxyglutaryl-coenzyme A (HMG-CoA) reductase.



1 2 3

As a continuation of our programme aimed at the isolation of naturally occuring compounds with potential anticancer, antiviral and antiprotozoal activities, we have submitted aframodial to the NCI anticancer screening programme. We now report its *in vitro* antileukemic activity as well as the identification of two minor antifungal diterpenoids [2], galanolactone (**2**) and galanal B (**3**) not previously isolated from any Aframomum species.

In contrast to previous results from our laboratory [4–6], aframodial (**1**) was isolated in a lower yield from the hexane extract of the fine powdered seeds of *Aframomum daniellii* together with two other minor components **2** and **3**. Aframodial, with a 50 mg/kg toxicity index, showed reproducible activity in the NCI *in vitro* human cell line screen [7] against the whole leukemia cell line panel (CCRF-CEM, HL-60, K-562, MOLT-4, RPMI-8226, SR lines), and the NCI-H522 non-small cell cancer lung (NSCLC) line at concentrations of 10^{-4} to 10^{-5} molar. It was advanced into the NCI in vivo hollow fiber assay [8] where, on intraperitoneal (IP) administration, it exhibited cell kill against NCI-H522 cells contained in IP implanted cancer lung (NSCLC) and OVCAR-3 ovarian cells contained in fibers implanted subcutaneously (SC). On the basis of the cell kill observed against the NCI-H522 cell lines in this assay, aframodial was approved for testing against a range of human tumor xenograft systems. However, no significant activity was observed against SC implanted NCI-H522 and NCI-H23 tumors on IP administration of the drug on an intermittent schedule (QDx5 and Q4Dx3, respectively, both starting on day 16) at doses ranging from 2.7 to 10 mg/kg nor against the SC implanted OVCAR-3 tumor on intermittent IP (Q4Dx3 starting on day 7) and IV (QDx5 and Q4Dx3, both starting on day 8) administration at doses from 2.7 to 18 mg/kg. In view of the lack of a significant *in vivo* activity against

the xenograft systems, some synthetic modifications in the structure of aframodial are currently carried on for subsequent testings by the NCI and these results will be reported later.

Experimental

1. General experimental procedures

M.p's. were determined on a Gallenkamp apparatus and are not corrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60 F_{254}) with the mobile phase hexane/EtOAc 85 : 15. TLC spots were visualized under UV light and preferentially by 50% sulfuric acid spray and subsequent heating (black spots). CC was carried out on Merck Kieselgel 60 (70–230 mesh). NMR spectra were recorded on a JEOL JMN EX 400 spectrometer in ~5% solution at 25 °C. IR by a Mattson Polaris FTIR spectrometer in the solid state (KBr).

2. Plant material

The seeds of A. daneillii were collected in Mbalmayo in the Centre Province of Cameroon in February 1997 and identified by Dr Sonke. A voucher specimen of this plant has been deposited in the National Herbarium, Yaounde.

3. Extraction and isolation

Dried seeds (1 kg) of A. daneillii were powdered and extracted with hexane in a Soxhlet apparatus for 24 h. The hexane extract was concentrated and the residue (89.0 g) was first flash-chromatographed on a silica gel pad. Fractions obtained when eluting with Hexane/EtOAc 95 : 5 gave pure aframodial (**1**, 220 mg), galanolactone (**2**, 68 mg) and galanal B (**3**, 72 mg).

Compound **1** (colourless needles): m.p. 91–92 °C; ^{13}C NMR δ (^{13}C): 39.56 (C1); 18.34 (C2); 41.88 (C3); 33.64 (C4); 52.17 (C5); 19.88 (C6); 39.56 (C7); 57.51 (C8); 55.22 (C9); 40.03 (C10); 22.41 (C11); 161.18 (C12); 136.05 (C13); 36.01 (C14); 198.00 (C15); 194.07 (C16); 48.81 (C17); 33.64 (C18); 21.62 (C19); 14.81 (C20).

Compound **2** (colourless needles): m.p. 125–126 °C; ^{13}C NMR δ (^{13}C): 39.19 (C1); 18.67 (C2); 41.77 (C3); 33.40 (C4); 52.42 (C5); 19.93 (C6); 35.66(C7); 57.36 (C8); 55.01 (C9); 39.56 (C10); 39.56 (C11); 142.80 (C12); 124.70 (C13); 25.24 (C14); 65.42 (C15); 171.06 (C16); 48.66 (C17); 33.39 (C18); 21.62 (C19); 14.83 (C20).

Compound **3** (colourless needles): m.p. 134–135 °C; δ (^{13}C): 38.96 (C1); 18.73 (C2); 41.77 (C3); 33.46 (C4); 55.58 (C5); 19.15 (C6); 34.42 (C7); 55.42 (C8); 55.70 (C9); 38.96 (C10); 24.20 (C11); 157.92 (C12); 140.89 (C13); 28.74 (C14); 78.86 (C15); 193.72 (C16); 208.31 (C17); 33.36 (C18); 21.44 (C19); 15.96 (C20).

Acknowledgements: This work is partly supported by the grant F2626-2 from the International Foundation of Science (IFS, Stockholm) to B.N. The authors wish to thank the United States National Cancer Institute (NCI) for conducting the *in vitro* and *in vivo* antitumor testing.

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Received January 18, 2000
Accepted March 14, 2000

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Analgetic properties of glycosyl diclofenac derivatives

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Diclofenac, natrium salt of *o*-(2,6-dichloroanilino) phenylacetic acid (**1**, as free acid) has been widely used in therapy for over 20 years as a powerful nonsteroid antiinflammatory drug (NSAID) [1, 2]. Similarly to other NSAID, it causes numerous adverse effects in the gastrointestinal system such as: nausea, vomiting, abdominal pain and also leads to the formation of ulcers [3]. There are at least two distinct mechanisms of gastric damage by these agents: the inhibition of the synthesis of prostaglandins PGE2 and PGI2, which are protective towards the stomach mucosa, and the direct irritation of the mucosa [4], which depends on the chemical structure of the drug, and in particular, on the presence of a carboxyl group. In order to decrease the irritating effect of this compounds, attempts have been made to hide the carboxyl group [5–7]. Hussain [8] obtained a sugar derivative of aspirin and he concluded that, at pH values between 3 and 9, aspirin is released from this prodrug by hydrolysis. In numerous laboratories the synthesis of derivatives of **1** is conducted as such compounds are expected to have smaller ulcerogenic properties e.g. In fact, methyl esters of **1** showed less ulcerogenic activity [7]. We obtained several glycosyl derivatives of acid **1** as potential prodrugs [9]. Preliminary *in vitro* experiments on these derivatives (by means of the polarymetric method) showed that in 0.1N HCl, acid **1** is released [10]. We also found that the release of this compound may take place at pH values between 3 and 7 [11]. In this work we studied the analgetic activity of glycosyl derivatives of acid **1** containing glucose **2**, galactose **3**, 2-deoxy-glucose **4** and 2-deoxy-galactose **5**. The acute toxicity of the new diclofenac derivatives **2**–**5** after intraperitoneal administration was found to be about 2.5–3 times lower than that of diclofenac as free acid (**1**) (Table 1). The glucose derivative (**2**) at doses of 0.1 and 0.5 ALD_{50} showed analgetic effects both in the hot plate (Table 1) and tail flick (Table 2) tests. After administration of the galactose derivative **3** in doses of 0.1 and 0.5 ALD_{50}, the reaction lag time to pain was also longer in the tail flick test, and was seen after 120 min of the observation (Table 2). In the hot plate test this effect was already present 60 min after administration of the drug at a dose of 0.1 ALD_{50} (Table 1). The 2-deoxy-glucose derivative **4** showed a weaker analgesic effect, whereas the 2-deoxy-galactose derivative **5** did not show any effect (Tables 1 and 2). Diclofenac as free acid (**1**) showed analgetic effects in both tests and this effect was stronger at the higher dose in the hot plate test (Table 1). A comparison of the analgetic effects of the new sugar derivatives of diclofenac **2**–**5** and **1** showed that only the glucose derivative **2** had an effect in the tail flick test similar to that of **1** at both concentrations tested. In the hot plate test the effect of **2** is similar to that of **1** at a dose of 0.1 ALD_{50}, whereas at 0.5 ALD_{50}, it was smaller. This may be associated with the surplus of the substrate for the reaction of disintegration which takes place.

In the near future larger scale pharmacological studies on the glucose derivative **2** will be conducted.