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Xylopioxyde and other bioactive kaurane-diterpenes from *Xylopia aethiopica* Dunal (Annonaceae)

Désiré SOH[1], Ernestine NKWENGOUA[1], Igor NGANTCHOU[1,2], Barthélemy NYASSE[1,*], Colette DENIER[2], Véronique HANNAERT[3], Kamel H. SHAKER[4] and Bernd SCHNEIDER[4]

[1]Laboratory of Medicinal Chemistry & Pharmacognosy, Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, Box 812 Yaoundé, Cameroon. [2] UMR 5068, Groupe de Chimie Organique- LSPCMIB, Université Paul Sabatier, Bât II RI, 118 Route de Narbonne, 31062 Toulouse, Cedex4, France. [3]Unité de Recherche pour les Maladies Tropicales, Institut International des Pathologies Cellulaires & Moléculaires et Unité de Biochimie, Université de Louvain, Avenue Hippocrate 74-75, B-1200, Bruxelles, Belgique. [4] Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll Str. 8, D-07745 Jena, Germany.

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ABSTRACT

A new cytotoxic *ent*-kaurane-type diterpene named xylopioxyde (16,17-epoxy-15-oxo-*ent*-kauran-19-oic acid) has been isolated from the fruits of *Xylopia aethiopica Dunal* (Annonaceae) together with three known compounds, namely 15α-acetoxy-*ent*-kaur-16-en-19 oic acid (xylopic acid), 15-oxo-*ent*-kaur-16-en-19-oic acid and *ent*-kaur-16-en-19-oic acid. Xylopic acid, obtained in a good amount, has been successively converted in moderate to good yields into 15-hydroxy-*ent*-kaur-16-en-19-oic acid, 15-oxo-*ent*-kaur-16-en-19-oic acid and two new selective trypanocidal stereoisomers of 15-acetoxy-16,17-*ent*-epoxy-kauran-19-oic acid, respectively. All the compounds except the synthetic epoxides displayed cytotoxic effects on the mammalian fibroblast cell line MRC-5 as well as inhibitory effects on the growth of the bloodstream forms of *Trypanosoma brucei brucei* cells (strain 241).

INTRODUCTION

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a vector-borne parasitic disease which is conducted through protozoa belonging to the *Trypanosoma* genus. The parasites are transmitted to humans by tsetse fly (*Glossina* genus) bites. For reasons that are so far unexplained, there are many regions where tsetse flies are found, but sleeping sickness is not. However, rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse fly and therefore to the disease (WHO, 2012). There exist parasite species and sub-species of the *Trypanosoma* genus which are pathogenic to animals and cause trypanosomiasis in wild and domestic animal species. Animals can host the pathogen parasites, *Trypanosoma brucei* (*T. b.*) *rhodesiense* and *T. b. gambiense*, thus contributing to an important parasite reservoir. However, the precise epidemiological role of such a reservoir is not yet well known (WHO, 2012). The management of HAT is still inefficient despite the availability of drugs such as Pentamidine and Suramine for the first stage of the disease, and Melarsoprol or Eflornithine for the second stage.

These drugs, discovered in 1941, 1921, 1949 and 1990, respectively, present serious and undesirable side effects. More recently, in 2009, a combination treatment of Nifurtimox and Eflornithine was introduced (Baker et al., 2011), but unfortunately it is not effective against *T. b. rhodesiense*. The above-mentioned shortcoming, coupled with the resistance of the parasites to commercially available drugs, is demanding for new and cost effective trypanocidal drugs. To contribute to this end, and as a continuation of our ongoing search for trypanocidal compounds from Cameroonian medicinal plants (Nyasse et *al*., 2002, 2004; Nkwengoua et *al*., 2009; Ngantchou et *al*., 20103), the fruits of *Xylopia aethiopica* have been selected for further studies.

* *Corresponding Author*
E-Mail: bnyasse@yahoo.com (B. N.); desiresoh@yahoo.fr (D. S.) Phone: (237) 77 88 88 77 / 79 61 31 95 Fax: (237) 22 22 13 20

Xylopia aethiopica (Dunal) is a tree of 20 meter high or more with a clear straight bole to 75cm girth (Burkill, 1995, 1985). It is reported that, there are between 100 and 150 species of Xylopia distributed throughout the tropical regions of the world, particularly in Africa. The various extracts from Xylopia spp. have been shown to possess antiseptic and analgesic properties, and insecticidal activity against adult mosquitoes, several leaf-eating insects and houseflies. Various parts of the plant have been traditionally employed in different therapeutic preparations (Konnning et al., 2004). A fruit extract, a decoction of the bark or that from the fruit is useful in the treatment of bronchitis and dysenteric conditions. In Congo, it is used against the attacks of asthma, stomach aches and rheumatism (Burkill, 1995, 1985; Choumessi et al., 2012; Woode et al., 2012). *Xylopia aethiopica* has been reported to be recommended to women who have newly given birth as a tonic in the Ivory Coast as a woman remedy. It is taken also to encourage fertility and for ease of childbirth (Burkill, 1995, 1985). Sometimes, a combination of X. aethiopica with other plants or a combination of different parts of X. aethiopica is used to achieve the desired effects (Fall et al., 2003). Among the conditions treated with X. aethiopica in traditional medicine are cough (fruits and roots of the plant) bronchitis, dysentery and biliousness (fruits and stem bark) and boils and sores (leaves and bark) (Ghana Herbal Pharmacopoeia 1992, Mshana et al., 2000). The bark of the plant is used to make doors and partitions. The wood is known to be resistant to termites' attacks and is used in hut construction: posts, scantlings, roof-ridges and joists. The wood is also used for boat construction: masts, oars, paddles and spars. In Togo, Gabon and Cameroon, the wood was traditionally used to make bows and crossbows for hunters and warriors (Burkill, 1995, 1985). The fruit is traded in some many parts of Africa and sold between 0.5 to 1 USD/kg (Aiyeloja et al., 2012).

X. aethiopica is a medicinal plant known to contain kaurane-type diterpenoids (Ekong and Ogan, 1968; Ekong et *al*., 1969; Harrigan et *al*., 1994; Hasan et *al*., 1982). This plant also displays plant growth properties as well as antimicrobial, antiparasitic, antitumoral, insect antifeedant and immunomodulatory activities (García et *al*., 2007; Lopez et *al*., 2009).

Here, we report the isolation of known kaurane diterpenes **1**-**3** and xylopioxyde (**4**), a new cytotoxic kaurane diterpene from the fruits extract of *Xylopia aethiopica* . New synthetic stereoisomers of 15-acetoxy-16,17-ent-epoxy-kauran-19-oic acid (**6** and **7**) obtained from the epoxidation of **3** and their biological activities are also reported.

Experimental

General experimental procedures

IR spectra (film) were registered on a Shimadzu FT-IR 8300 spectrophotometer. Melting points were determined in a capillary on a Büchi melting point apparatus B-540 and are uncorrected. 1D (^{1}H, ^{13}C, DEPT) and 2D NMR spectra (^{1}H,^{1}H-COSY, HSQC, HMBC, ROESY) were recorded using a Bruker DRX 500 spectrometer in $CDCl_3$, C_6D_6, or CD_3OD at 300 K. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are given in δ and coupling constants (*J*) in Hz. The mass spectra were obtained at 70 eV in a VG Autospec apparatus. HRMS were measured in the positive ionization mode on a Micromass Quattro II tandem quadrupole mass spectrometer. The fragments were described as a relation between atomic mass units and charge (*m/z*) and the relative abundance in a percentage of the base peak intensity. TLC analyses were carried out on 0.25 mm thick precoated Merck TLC silica gel plates (Silica 60 F254). Plates were developed with the mobile phase hexane/ethyl acetate 2/8. Spots on TLC plates were visualized under UV light (254 and 365 nm), exposure to iodine vapour, or spraying with 50% sulphuric acid in water, followed by gentle heating. Column chromatography was performed using silica gel (0.063-0.2 mm / 70-230 mesh) from Merck.

Plant material

Fruits of *Xylopia aethiopica* (Annonaceae) were collected at Nkongsamba in February 2004. They were identified by M. Kitio Etienne by comparison to available samples at the National Herbarium of Cameroon (Yaounde). Samples have been deposited under reference number 39485/HNC.

Extraction and isolation of natural products

Dried powder of fruits (1 kg) was extracted with hexane during 48 h at room temperature. After evaporating the solvent under reduced pressure at 40°C, crude extract (150 g) was obtained. A part of this extract (75 g) was chromatographed over silica gel (Merck 70-230 mesh) using hexane-EtOAc mixtures for elutions. Fractions eluted with hexane-EtOAc (95:5) yielded 700 mg of 15α-acetoxy-e*nt*-kaur-16-en-19-oic acid (xylopic acid, **3**) and 10 mg of *ent*-kaur-16-en-19-oic acid (**1**). Fractions eluted with hexane-EtOAc (9:1) yielded 15-oxo-*ent*-kaur-16-en-19-oic acid **2** (150 mg). Fractions eluted with hexane-EtOAc (85:15) yielded 40 mg of 16,17-epoxy-15-oxo-*ent*-kauran-19-oic acid (xylopioxyde, **4**).

Chemical modifications

Synthesis of 15α-hydroxy-ent-kaur-16-en-19-oic acid (5)

15α-Acetoxy-*ent*-kaur-16-en-19-oic acid (**3**) (1.38 mmol) was added to 15 ml of methanolic KOH (5%) and refluxed for 4 h. The reaction was monitored by TLC. After 4 h of reaction, the reaction medium was treated with distilled water and extracted with ethyl acetate. The reaction medium was adjusted to pH 4 and the organic layer obtained was evaporated to dryness. The precipitate (350.7 mg) obtained was purified by column chromatography (silica gel, hexane/EtOAc of increasing polarity as the eluent) to give compound **5** (232.7 mg, 53%) (in agreement with (Nagashima et al., 2002)). mp: 172-174°C. ^{13}C NMR data are in agreement with published data (García et *al*., 2007).

Synthesis of 15-oxo-ent-kaur-16-en-19-oic acid (2)

15α-Hydroxy-*ent*-kaur-16-en-19-oic acid (**5**) (0.25 mmol) was dissolved in acetone (10 ml). Ten drops (~1 ml) of

Jones Reagent (8 N CrO_3/H_2SO_4) were rapidly added. The mixture was thoroughly stirred for 6 h and reaction progress was monitored by TLC. The reaction mixture was subjected to liquid-liquid distribution between H_2O/CH_2Cl_2 (3 × 50 ml) and the organic layer was separated, evaporated and purified by column chromatography (silica gel, hexane/EtOAc of increasing polarity as eluent) yielding compound **2** (29.3 mg, 37%) (Hanson, 1966).

*Synthesis of 15α-acetoxy-16,17β-epoxy-ent-kauran-19-oic acid (**6**) and 15α-acetoxy-16,17α-epoxy-ent-kauran-19-oic acid (**7**)*

15α-Acetoxy-*ent*-kaur-16-en-19-oic acid (**3**) (225 mg, 0.623 mmol) and mCPBA (170 mg; 0.985 mmol) were dissolved in CH_2Cl_2 (15 ml). The mixture was stirred at room temperature and the reaction was monitored by TLC. After 24 h, the reaction mixture was subjected to liquid-liquid distribution between H_2O/CH_2Cl_2 (3 × 50 ml). The organic layer was separated, dried with $MgSO_4$ and evaporated under reduced pressure to give a residue which was further purified by column chromatography (silica gel, hexane/EtOAc as eluent) yielding compounds **6** (174.4 mg, 74%) and **7** (28.2 mg, 12%) (Bruno et *al.*, 2001).

*15α-Acetoxy-16,17β-epoxy-ent-kauran-19-oic acid (**6**)* (174.4 mg): White powder, m.p. 128-130°C, molecular formula $C_{22}H_{32}O_5$. ^{1}H-NMR (500 MHz, CD3OD) and ^{13}C-NMR (125 MHz, CD3OD) see tableau 1HRMS: $[M]^+$ found: 376.22650, calc.: 376.22497.

*15α-Acetoxy-16,17α-epoxy-ent-kauran-19-oic acid (**7**)* (28.4 mg): White powder, m.p. 116-118°C, molecular formula $C_{22}H_{32}O_5$. ^{1}H-NMR (500 MHz, CD3OD) and ^{13}C-NMR (125 MHz, CD3OD) see table 1, HRMS: $[M]^+$ found: 376.22623, calc.: 376.22497.

Biological assay procedure

Cell culture

Trypanosoma brucei brucei (strain 427) bloodstream forms were cultured in Iscove's modified medium (HMI-9) containing 10% heat-inactivated fetal calf serum and bloodstream form supporting factors: 0.05 mM bathocuproine sulfonate, 1.5 mM L-cysteine, 1 mM hypoxanthine, 0.2 mM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.16 mM thymidine known as HMI 9 (Batista et *al.*, 2007). The mammalian fibroblast cell line MRC-5 was grown in Dulbecco's Modified Eagle Medium (Gibco) supplemented with 10% heat-inactivated Fetal Bovine Serum and extra glutamine. Cells were grown at 37 °C in humidified incubators in an atmosphere of 5% CO_2 (Hirumi and Hirumi, 1994).

Antitrypanosomal and cytotoxicity assays

The assay performed, the long incubation low inoculation test (LILIT), was previously described (Brun and Lun, 1994) with minor modifications (Räz et *al.*, 1997). The LILIT method was also used to facilitate the comparison between our samples and the control. To evaluate the selectivity of the *in vitro* antitrypanosomal activity, *in vitro* cytotoxicity tests were also performed. The compounds were initially dissolved in DMSO at a concentration of 20 mg/ml and diluted with the medium. Series of dilutions were carried out in 96-well plates; each dilution was tested in duplicate. Cells in suspension were subsequently added to each well at a density similar to that reached after 72 h of incubation in control wells by cultures at the end of the logarithmic growth phase (*T. brucei*), or in adhesive cells that have formed a confluent monocellular film (mammalian cells MRC-5). The highest concentration of DMSO was 0.5%. After 72 h of incubation, Alamar Blue, a marker of cell viability, was added in each well and fluorescence was quantified after a total incubation time of 76 h at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. ED_{50} values were calculated by linear interpolation. Assays with commercial drugs were also carried out in order to have reference values. All results represent average values obtained in at least three independent experiments, each experiment comprising a duplicate set of tests.

RESULTS AND DISCUSSION

Identification of natural products

Compound **1** was obtained as a white powder (m.p. 172-174°C) upon purification of the hexane extract of the *Xylopia aethiopica* fruits on a silica gel column (eluent: hexane-ethyl acetate 8:2, v:v). The ^{13}C NMR and DEPT spectra revealed the presence of 20 carbon atoms corresponding to two methyl groups, ten methylenes including one exocyclic methylene, three methines and five quaternary carbon atoms including a carboxyl group. All these are suggestive of structural features of kaurane-type diterpenoids. Following the reported occurrence of kaurane-type natural products in *Xylopia aethiopica* (Ekong and Ogan, 1968; Ekong et *al*., 1969; Harrigan et *al.*, 1994; Hasan et *al*., 1982), the presence of an exocyclic methylene at C-16 and a carboxyl group at C-3 as inferred from NMR data, compound **1** was identified as *ent*-kaur-16-en-19-oic acid (Fig. 1). The ^{13}C NMR chemical shifts of compound **1** are in agreement with reported data (Hutchison et *al*., 1984) and the molecular mass (EIMS: *m/z* 302 $[M]^+$) further confirmed the suggested structure.

Compound **2** was also obtained as a white powder (m.p. 192-194°C) upon elution of the hexane extract of the fruits (eluent: hexane-ethyl acetate 9:1, v:v). When compared to compound **1**, the molecular mass of **2** (EIMS: *m/z* 316 $[M]^+$) suggested that a carbonyl functional group has been introduced in place of a methylene group in **1**. Upon further comparison of the NMR chemical shifts of **2** with those of **1** and reported data (Hutchison et *al*., 1984), compound **2** was identified as 15-oxo-*ent*-kaur-16-en-19-oic acid (Fig. 1).

Compound **3** was obtained as a white powder (m.p. 230-232°C) from a column chromatographic separation (eluent: hexane-ethyl acetate 95:5, v:v). The difference in the molecular mass between compound **3** (EIMS: *m/z* 360 $[M]^+$) and compound **2** (*m/z* 316) suggested the addition of a 44 Da molecular unit that could stem from an acetyl group. The presence of such a group was also supported by the fragment ion *m/z* 300 corresponding to the loss of 60 mass units. Such a loss accounted for an acetate

moiety. The ^{13}C NMR data of compound **3** (Fig. 1) are consistent with those of 15α-acetoxy-*ent*-kaur-16-en-19-oic acid, exception made on C-5 and C-9 signals which could be interchanged in a previous work (Hanson et *al.*, 1976). Configurational assignment of the 15-hydroxy- or 15-acetoxy group, in *ent*-kaur-16-enes, is inconsistent in the literature. The chemical shift difference of Δδ~8 ppm of C-15 has been used to distinguish 7β,15α- and 7β,15β-hydroxy *ent*-kaur-16-enes (Buchanan et *al.*, 1996), but no significant shift difference was observed at C-15 for 15α- and 15β-hydroxy-*ent*-kaur-16-enoic acids (Hutchison et *al.*, 1984). In both of the latter two isomers, C-15 resonates at about δ 80-82. Chemical shift values in the same range were also observed for 7α,15β,18-trioxygenated *ent*-kaur-16-enes (Halfon et *al.*, 2011) and compound **3** (δ_{C-15} 81.6). Hence, it became difficult to assign the configuration at C-15 solely on the basis of those chemical shifts. However, correlations of H-15 (δ 5.44) in the 2D ROESY spectrum of compound **3** were observed with the two H-14 protons (δ 0.94 and 1.83) but not with H-9 (δ 1.45), suggesting a □-orientation for H-15 and α-orientation for the 15-*O*-acetyl group. From these data, compound **3** was identified as 15α-acetoxy-*ent*-kaur-16-en-19-oic acid (Fig. 1). Compound **4** was obtained as a white powder (m.p. 190-192°C) upon a column chromatographic separation (eluent: hexane-ethyl acetate 85:15, v:v) of the hexane extract of the *Xylopia aethiopica* fruits. Its molecular ion appeared at *m/z* 332 [M^+] in the positive ionization EIMS, suggesting an additional oxygen atom in the structure of **4** as compared to **2** (*m/z* 316 [M^+]). HRMS (found: *m/z* 332.19896 [$M]^+$) suggested the molecular formula as $C_{20}H_{28}O_4$ (calculated: *m/z* 332.19876). The ^{13}C NMR spectrum of compound **4** displays signals similar to those of compound **2** without the exocylic methylene group signals. Characteristic signals of the exocyclic methylene group at C-16 (δ 149.5) and C-17 (δ 114.5) of **2** are replaced by the signals of oxygenated carbon atoms at δ 50.1 and 63.9, which suggested an epoxide bridge between C-16 and C-17 in **4**. Complete assignment of ^1H and ^{13}C NMR signals of **4** was carried out using HMBC correlations. Correlations between the oxymethylene protons (δ 3.12, 3.00) and C-13 (δ 37.0), C-15 (δ 220.8) and C-16 (δ 63.9) are important in order to establish the epoxide. The β spatial position of the epoxide group in **4** was suggested from a comparison of the coupling constants of its H-17a proton (δ_{H-17a} 3.00 d 5.9H_z) with those of compounds of **6** (δ_{H-17a} 2.99, d 6.2H_z) and **7** (δ_{H-17a} 2.84 d 4.5H_z). Based on these NMR (Table 1) and MS data, the structure of compound **4** was suggested as 16,17β-epoxy-15-oxo-*ent*-kauran-19-oic acid and named xylopioxyde (Fig. 1). It's worth of mention that the stereochemistry of the epoxide cannot be unequivocally assigned in this context.

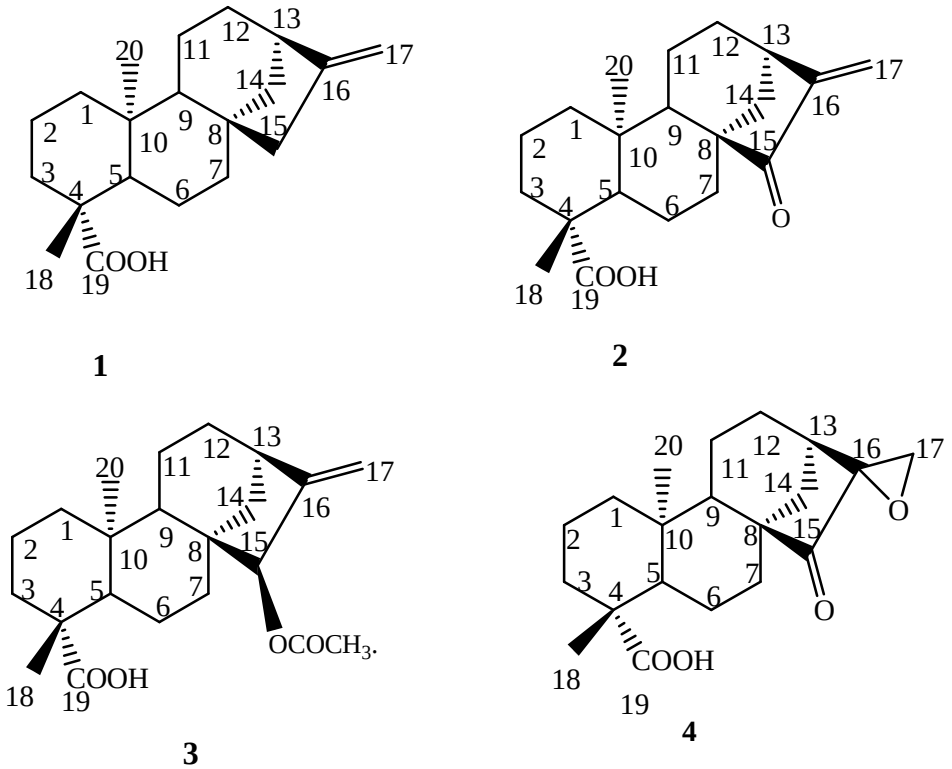
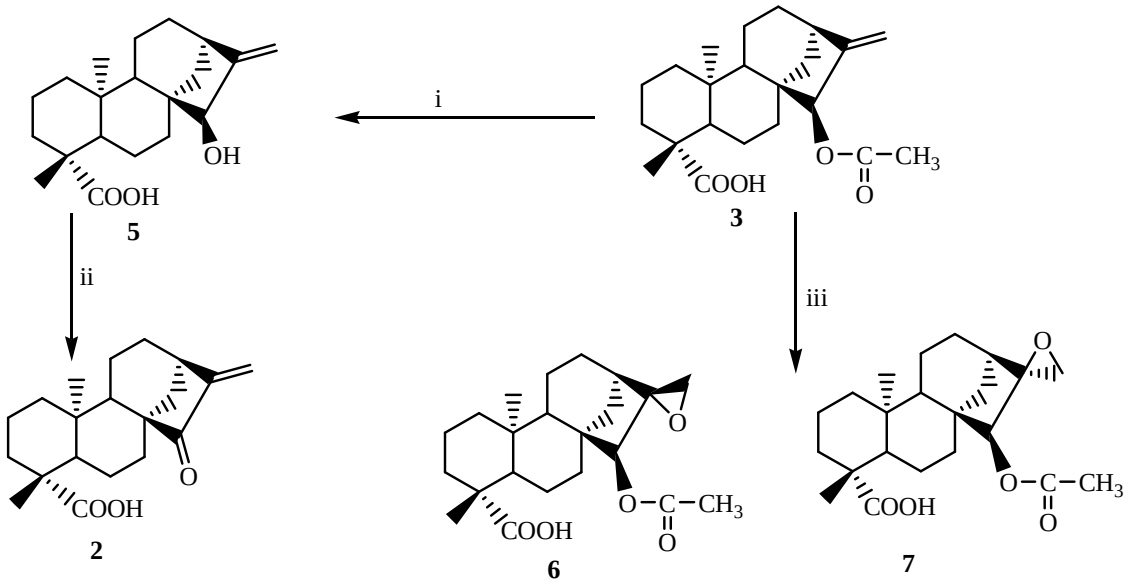


Fig. 1: Structures of compounds **1-4.**



Scheme. 1: Chemical modifications of xylopic acid (**3**)
KOH/MeOH, reflux/64.6°C, 53%; (ii) 8N-CrO_3/H_2SO4, rt, 37%; (iii) mCPBA, CH_2Cl_2, rt, 74% for 6; 12% for 7.

Table. 1: ^{1}HNMR (500 MHz) and ^{13}C NMR data (125 MHz) of xylopioxyde (**4**), and compounds **6**, and **7** (CD_3OD).

	4			**6**		**7**	
	δ_H; mult.,*J*, Hz	**δ_C**	**HMBC***	**δ_H; mult.,*J*, Hz**	**δ_C**	**δ_H; mult.,*J*, Hz**	**δ_C**
1a	1.90; m	41.3	C-20	1.97; m	42.2	1.97; m	42.3
1b	0.86; m		C-2, 9, 10, 20	0.93; m		0.93; m	
2a	1.82; m	20.1		1.93; m	20.5	1.92; m	20.5
2b	1.43; m			1.44; m		1.43; m	
3a	2.14; overlap	39.1		2.14; d; 13.9	39.3	2.14; d; 13.5	39.2
3b	1.04; m		C-2, 4	1.03; overlap		1.03; overlap	
4		44.7			44.8		44.8
5	1.14; dd; 12.0, 2.9	57.5	C-4, 10, 20	1.06; overlap	57.8	1.02; overlap	57.8
6a	1.88; m	20.7	C-8, 10	1.87; overlap	22.5	1.87; m	22.7
6b	1.52; m		C-10				
7a	1.81; dd; 12.6, 4.0	34.9	C-5, 6, 8, 14, 15	1.47; m	40.6	1.52; m	40.5
7b	1.40; m		C-5, 9			1.45; m	
8		54.8			46.9		49.0
9	1.16; d; 8.7	52.7	C-1, 8, 10, 12, 20	1.50; d; 8.4	48.5	1.37; d; 7.1	48.2
10		41.6			40.7		40.7
11a	1.93; m	21.3	C-8, 10	1.80; m	19.9	1.76; m	20.5
11b	1.93; m			1.68; dd; 15.2, 5.8		1.76; m	
12a	1.89; m	30.2	C-16	1.61; m	28.1	1.62; m	30.1
12b	1.80; m		C-13	1.51; m			
13	2.14; overlap	37.0		1.94; overlap	39.6	1.79; m	41.1
14a	1.74; dd; 12.3, 4.9	37.3	C-8, 9, 12, 13	2.22; d; 12.6	35.6	2.21; d; 12.3	37.1
14b	2.58; d; 12.3		C-8, 9, 12, 13, 15, 16	1.28; overlap		1.50; overlap	
15		220.8		4.66; s	80.1	4.86; s	83.7
16		63.9			64.7		69.9
17a	3.00; d; 5.9	50.1	C-13, 15, 16	2.99; d; 6.2	55.1	2.84; d; 4.5	48.7
17b	3.12; d; 5.9		C-15, 16	2.74; d; 6.2		2.81; d; 4.5	
18	1.21; s	29.4	C-3, 4, 5, 19	1.19; s	29.6	1.19; s	29.6
19		181.4			181.7		181.7
20	1.08; s	16.4	C-1, 5, 9, 10	1.03; s	16.9	1.03; s	16.9
21					172.9		172.4
22				2.06; s	20.8	2.09; s	20.9

*Main HMBC correlations observed

Table. 2: Cytotoxic and trypanocidal activities (ED_{50}) of compounds **2-7.**

Compound	**Trypanocidal activity**	**Cytotoxic activity**
	***Trypanosoma brucei brucei*(strain 427) bloodstream forms**	**mammalian cells (MRC-5 fibroblasts)**
2	27.04± 0.03µM	22.11± 0.03µM
3	205.34 ± 0.05µM	121.22 ± 0.05µM
4	193.16 ± 0.05µM	105.09± 0.05µM
5	> 100 mM	> 100 mM
6	52.23 ± 0.03µM	> 100 mM
7	127.17± 0.03µM	> 100 mM
Mel	0.20 ± 0.03µM*	-

*Data from (Hutchison et *al.*, 1984). Mel: melarsoprol (as reference drug).

Chemical modifications of xylopic acid (3)

Because compound **3** (xylopic acid) was obtained in good amounts, it was submitted to chemical modifications. Hence, **3** was first transformed into compound **2** which has a greater potential of biological activities. This was achieved by refluxing **3** with 5% of potassium hydroxide for 4 h yielded the free hydroxyl derivative **5** in 55% yield (Etouati et al., 1988). Then, derivative **5** was oxidized by Jones reagent (Millet et *al.*, 2002) for 5 h to give compound **2** in 37% yield (Scheme 1). The analytical data (NMR, MS) of compounds **2** and **5** are in agreement with the suggested structures (Hutchison et *al.*, 1984; Hanson, 1966).

Furthermore, when compound **3** was treated with *m*-chloroperbenzoic acid (mCPBA) in methylene chloride according to the method described by Bruno et al. (Bruno et *al.*, 2001) (Scheme 1), a mixture of isomeric epoxides **6** and **7**, which showed a different migration on thin layer chromatography (R_f 0.5 and R_f 0.4, respectively), was obtained. The two synthetic compounds were separated by column chromatography, yielding 74% of compound **6** and 12% of compound **7**, respectively.

Identification of the synthetic epoxides 6 and 7

The mass spectra of **6** and **7** allowed the establishment of their molecular formula as $C_{22}H_{32}O_5$ suggestive of an epoxide group in place of the exomethylene group in the parent compound **3**. The ^{1}H NMR and HSQC spectra of compounds **6** and **7** displayed signals of an oxymethylene moiety (CH_2-17; **6**: δ_{1H} 2.74 and 2.99, each 1H, d, J = 6.2 Hz; δ_{13C} 55.1; **7**: δ_{1H} 2.81 and 2.84, each 1H, d, J = 4.5 Hz; δ_{13C} 48.7) similar to that in the spectra of compound **4**. The complete assignment of ^{1}H and ^{13}C NMR data (Table 1) by means of ^{1}H,^{1}H COSY, HSQC and HMBC spectra and comparison with of those of some *ent*-kauranes bearing an epoxide moiety attached to the five-membered ring (Bruno et *al.*, 2001; Nagashima et *al.*, 2002; Batista et *al.*, 2007), identified compounds **6** and **7** as isomeric 15α-acetoxy-16,17-epoxy-*ent*-kauran-19-oic acids. The key correlations in the ROESY spectrum of compound **6** between H-13 and the epoxy ethylene protons (H_2-17) are much stronger than the corresponding cross-signals in the ROESY spectrum of compound **7**. Based on this observation, compound **6** was assigned as 15α-acetoxy-16,17β-epoxy-*ent*-kauran-19-oic acid and compound **7** as 15α-acetoxy-16,17α-epoxy-*ent*-kauran-19-oic acid.

Biological activities: trypanocidal and cytotoxic activities

Compounds **2-7** were assayed for their trypanocidal and cytotoxic activities against *Tryponosoma brucei brucei* cells (strains **241**) and the mammalian fibroblast cells line MRC-5, respectively (Table 2).

Results shown in Table 2 suggest that the epoxides **6** and **7** are potential selective trypanocidal compounds with a similar profile as other *ent*-kaurane compounds described early (Batista et al., 2007; Vieira et al., 2002). The two compounds do not show any toxicity on the mammalian cells when compared to the parent compound **3**. Compound **2** with the α-enone moiety displayed the highest cytotoxic activity on the mammalian cell. Such an activity may result from its non selective interaction with some vital enzymes as it was observed with labdanes containing α-enone isolated from *Entada abyssinica* (Nyasse et al., 2004).

CONCLUSION

From the fruits of *X. aethiopica,* three known diterpenoids (**1**, **2** and **3**) have been isolated along a new derivative named xylopioxyde (**4**). Compounds 1 and 2 displayed cytotoxic effects on MRC-5 fibroblast and could be further studied for their potential to inhibit the growth of other cancer cells. The new synthetic derivatives 15α-acetoxy-16,17β-epoxy-*ent*-kauran-19-oic acid (**6**) and 15α-acetoxy-16,17α-epoxy-*ent*-kauran-19-oic acid (**7**) obtained from the oxidation of xylopic acid (**3**) are selective trypanocidal compounds against *T. brucei* (ED_{50} 52 and 127 µM, respectively) with no detected cytotoxicity on MRC-5 fibroblast.

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Author(s)' Statement(s)

Competing Interests

The author(s) declare(s) no conflict of interest and competing interests in this work.

Informed Consent, Ethical Approvals

Plant material used in this work is commercially available and does not need any informed consent from the local populations.

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