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Bioactive chemical constituents of *Duboscia macrocarpa* Bocq., and X-ray diffraction study of 11β, 12β-epoxyfriedours-14-en-3α-ol

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ABSTRACT

A new γ-lactone triterpenoid, Evodoulolide (**1**) and a new triterpenoid Duboscic acid B (**2**), along with five known compounds, maslinic acid (**3**), arboreic acid (**4**), (*E*)-3-(4-hydroxyphenyl)-N-[2-(4-hydroxyphenyl) ethyl] prop-2-enamide (**5**), (*E*)-heptacos-19-enoic acid (**6**) and 11β,12β-epoxyfriedours-14-en-3α-ol (**7**) were isolated from the trunk wood of *Duboscia macrocarpa*. Their structures were elucidated from extensive ^{1}D- and ^{2}D-NMR and MS and by comparison of their spectra with published data. Compounds **1**, **3**, **5** and **6** exhibited significant α-glucosidase inhibitory activity. Compound **5** was found to be a potent inhibitor (IC_{50} = 5.1 ± 0.1 μM) of α-glucosidase as compared to acarbose (IC_{50} = 625.0 ± 1 μM) used as standard drug. These compounds did not show anti-glycation activity using the BSA-MG glycation model or inhibition against the α-chymotrypsin enzyme. The chemotaxonomic connotation of the isolated secondary metabolites is also herein described. The single-crystal X-ray and absolute configuration diffraction analysis of 11α, 12α-epoxyfriedours-14-en-3-ol (**7**) is also described here for the first time.

1. Introduction

Duboscia macrocarpa Bocq. is a tall tree that flourishes in the tropical rain forests of Africa. The aqueous decoction of the seeds is used in African folk medicine to treat tuberculosis while, its fruits are used for dental treatment and abdominal problems [1,2]. The decoction of the stem bark is also used to treat diabetes mellitus [3]. To the best of our knowledge, except our recent letter on *Duboscia macrocarpa* [4], no plants of this genus have been investigated. However, other members of the Tiliaceae family are known to contain triterpenoids, saponins, flavonoids, alkaloids and cardenolide glycosides as major secondary metabolites [5–8]. Because of their ethnopharmacological uses and the interesting class of compounds from this genus, the phytochemical study of *Duboscia macrocarpa* was undertaken. Herein the isolation and structure elucidation of two new triterpenes (**1 and 2**) along with five known compounds is reported. The spectroscopic analysis of MS and 1D-and 2D-NMR data were helped to elucidate their structures (Fig. 1). Known compounds were identified by comparison of their spectroscopic data with those present in the literature [8,9–12]. Also, in line with the local use of *Duboscia macrocarpa* and previous investigations [1–4], the alpha-glucosidase and alpha-chymotrypsin inhibition and anti-glycation were also investigated. The X-ray diffraction study of 11α, 12α-epoxyfriedours-14-en-3-ol (**7**) for the determination of its absolute configuration was performed.

2. Results and discussion

The methanol-methylene chloride (1:1) extract of trunk woods of *D. macrocarpa* was subjected to repeated column chromatography on silica gel and lead to the isolation of two new (**1–2**) (Fig. 1) and five known (**3–7**) compounds. Known compounds were described by comparison of their spectroscopic data with the previously reported result [8,9–12].

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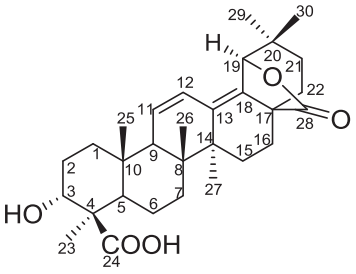
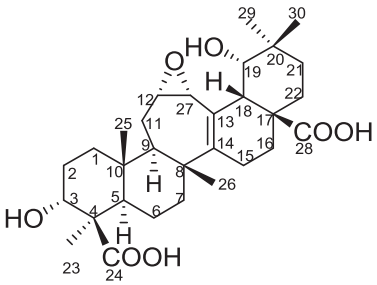
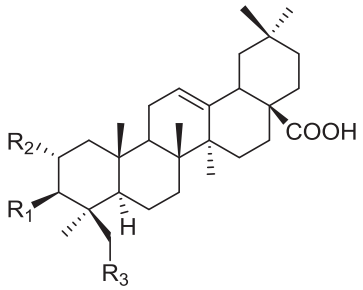


Evodoulolide (**1**)

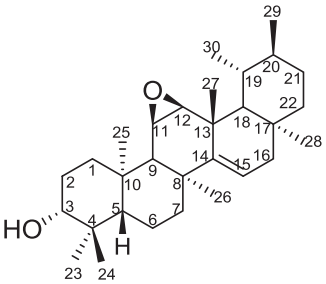


Duboscic acid B (**2**)

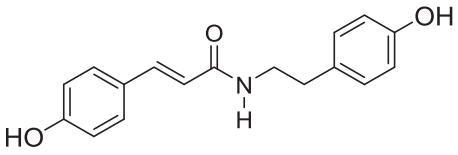


R_1=R_2 = OH, R_3 = H, Maslinic acid (**3**)

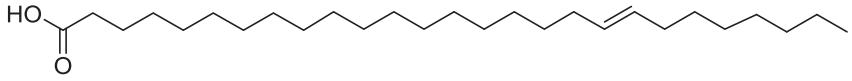
R_1 = R_2 = OH, R_3 = OH, Arboreic acid (**4**)



11β,12β-epoxyfriedours-14-en-3α-ol (**7**)



N-*p*-Trans-coumaroyltyramine (**5**)



(*E*)-Heptacos-19-enoic acid (**6**)

Fig. 1. Structure of compounds **1–2**, **7**.

Compound **1**, was isolated as a white powder, which gave a positive Liebermann-Burchard test indicative of terpenoids. The positive mode HRFAB–MS of **1** showed a pseudo molecular ion $[M + H]^+$ at *m/z* 483.3032, consistent with the molecular formula $C_{30}H_{42}O_5$, corresponding to ten double bond equivalents. The EI-MS of **1** corroborated the above structure with important fragments at *m/z* 439.2 [M + H-CO_2]. The IR spectrum indicated the presence of *gem*-dimethyl (1390 and 1375 cm^{-1}), γ-lactonic C═O (1765 cm^{-1}), carbonyl of an ester stretch (1724 cm^{-1}), hydroxyl (3511 cm^{-1}) and carboxylic acid (3191 and 1734 cm^{-1}) groups. The UV spectrum displayed characteristics absorptions at 240 (1.90), 247 (2.52), and 253(3.41) nm for hetero annular conjugated diene ester, and 261(3.70) nm for carboxylic functionality [13]. The analysis of 1D and 2D NMR spectra depicted that **1** possessed 30 carbons, including six angular methyls [δ_H/δ_C 0.84/16.5; 0.95/14.6; 0.98/22.4, 1.21/27.1; 1.10/18.5 and 1.27/23.7 (each 3H, s)], two oxy methines at [δ_H/δ_C 4.01 (brt, J = 2.8 Hz, J = 10.3 Hz, H-3)/70.6 and 4.85 (s, H-19)/85.5, four unsaturated carbons [δ_H/δ_C 5.85 (dd, J = 10.2, J = 2.1 Hz, H-11)/129.9, 6.26 (dd, J = 10.2 Hz, J = 3.1 Hz, H-12)/123.1, 135.6 and 132.7] as well as two carbonyl [δ_C 179.9 and 179.2], eight methylenes at [δ_H/δ_C 1.72 (m, H-1α)/34.5, 1.78 (m, H-1β)/34.5; 1.65 (m; H-2α)/33.8, 1.40 (m, H-2β)/33.8; 1.95 (m, H-6α)/19.7, 1.75 (m, H-6β)/19.7; 1.62 (m, H-7α)/25.6, 2.27 (m, H-7β)/25.6; 1.63 (m, H-15α)/22.0, 1.42 (m, H-15β)/22.0; 2.29 (m, H-16α), 1.65 (m, H-16β)/24.3; 1.52 (m, H-21)/33.2; 1.55 (m, H-22α)/32.7, 1.48 (m, H-22β)/32.7], two methines at [δ_H/δ_C 1.59 (m, H-5)/48.4 and 2.28 (dd, H-9)/52.3] appearing as doublet of doublet du to long range COSY coupling with H-12 and six quaternary carbons at δ_C 48.4 (C-4), 40.8(C-8), 37.4(C-10), 41.1(C-14), 44.3 (C-17), and 35.7 (C-20). The above spectroscopic data suggested that **1** might be a pentacyclic triterpenoid. With the help of ^{1}H-^{1}H COSY, HSQC, HMBC, and ROESY experiments, the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 1. The ^{1}H-^{1}H COSY spectra revealed the presence of spin-coupling systems in bold as shown in Fig. 2. The HMBC cross-correlations between CH_3-23 (δ_H 1.27) and C-3 (δ_C 70.6), C-24 (δ_C 179.9) and C-5 (δ_C 48.5); CH_3-25 (δ_H 1.27) and C-1 (δ_C 34.5), C-9 (δ_C 52.3) and C-5 (δ_C 48.5); CH_3-26 (δ_H 0.84) and C-9 (δ_C 52.3), C-14 (δ_C 41.1) and C-7 (δ_C 25.6); CH_3-27 (δ_H 1.10) and C-8 (δ_C 40.8), C-13 (δ_C 135.6) and C-15 (δ_C 26.0) and between CH_3-29 (δ_H 1.21), CH_3-30 (δ_H 0.98) and C-19 (δ_C 85.5) and C-21 (δ_C 33.2) allowed to fixed angular methyls on the pentacyclic skeleton. The exhaustive interpretation of HMBC cross correlations (Fig. 2) allowed the establishment of the planar structure of **1** (Fig. 1). The hydroxyl methine proton appearing at δ_H 4.01 (brt, $J_{H3\alpha\text{-}H2\alpha}$ = 2.8 Hz, $J_{H3\alpha\text{-}H2\beta}$ = 10.3 Hz, H-3α) was assigned to C-3 position and his coupling pattern suggested *α* orientation [14], whereas the second oxymethine appearing as a singlet at δ_H/δ_C 4.85 (H-19)/85.5 (C-19) was also identified as an allylic proton due to the presence of C-13(18) double bond and its HMBC correlation with the carbon at δ_C 179.2 (Fig. 2) helped to infer the substitution of the δ-lactone ring at C-28 in ring E. To figure out the relative configuration of **1**, the NOESY spectrum was exhaustively analyzed, and the following cross-peaks were observed between H-1 and H-5, H-5 and H-9, CH_3-25 and CH_3-26, H-16 and H-19 and between H-7α and CH_3-27 and CH_3-30. The above correlations also indicate that **1** possessed comparable A/B trans, B/C trans, and D/E cis junctions as typical oleanan-11,12 (18)-diene (Fig. 3, Suppl. Mat. S4) [15]. Additionally, the NOESY correlations amongst H-3 and H-10, between H-16α and H-19, and between H-19 and H-29β suggested that the configuration at C-3 and C-19 are alpha (Fig. 3 Suppl. Mat. S4). Based on above data, compound (**1**) represents a new pentacyclic triterpenoid derived from oleanolic acid name 3α-hydroxyoleana-11,13(18)-dien-19α-28-olid-24-oic acid (**1**) and, the trivial name "Evodoulolide" was proposed.

Compound **2** is a white amorphous powder. It showed a positive Liebermann-Burchard test characteristic of triterpenoids. The MS and NMR data supported its molecular formula as $C_{30}H_{44}O_7$. The APCI-MS

Table 1
^{1}H- and ^{13}C-NMR chemical shift data of compounds **1–2** [CD_3OD, δ ppm (J = Hz)].

C. No	**1**		**2**	
	δ_C (ppm)	δ_H (*mult. J* in Hz)	δ_C (ppm)	δ_H (*mult. J* in Hz)
1	34.5	α 1.72, m β 1.78, m	34.9	α 1.45, m β 1.35, m
2	33.8	α 1.65, m β 1.40, m	27.3	α 1.54, m β 2.22, m
3	70.6	4.01 (brt, 2.8, 10.3)	71.8	3.97, brt
4	48.2		48.7	
5	48.4	1.59, m	49.9	1.52, m
6	19.7	α 1.95, m β 1.75, m	21.1	1.67, m
7	25.6	α 1.62, m β 2.29, m	39.8	1.15, m; 1.88, m
8	40.8		40.8	
9	52.3	2.28,(dd, 2.1, 3,1)	52.0	1.29, m
10	37.4		36.8	–
11	129.9	5.85, dd, (10.2, 2.1)	22.3	1.54, m; 2.39, m
12	123.1	6.26, dd, (10.2, 3.1)	48.8	3.52, brt (10.4, 1,99)
13	135.6		127.3	
14	41.1		149.6	
15	26.0	α 1.63, m β 1.42, m	22.6	α 2.02, β 2.07
16	24.3	α 2.29, m β 1.65, m	25.4	1.75
17	44.3		46.7	
18	132.7		45.2	2.67 d (2.8)
19	85.5	4.85, s	78.8	3.44 d (2,8)
20	35.7		35.8	
21	33.2	1.52, m	29.6	α 1.78, m; β 1.10, m
22	32.7	α 1.55, m β 1.48, m	31.8	α 1.42, m; β 1.91, m
23	23.7	1.27, s	24.8	1.22, s
24	179.9	–	180.2	
25	14.6	0.95, s	14.5	0.82, s
26	16.5	0.84, s	19.6	0.96, s
27	18.5	1.10, s	48.5	3.83, dd (10.4, 1.99)
28	179.2		180.7	
29	27.1	1.21, s	29.1	α 1.05, s
30	22.4	0.98, s	25.6	β 0.99, s

α and β mean diastereotopic protons attached to the same carbon.

of **2** showed the molecular ion peak at m/z 519 $[M + H]^+$ and the ^{1}H, ^{13}C NMR and DEPT spectra (300 MHz, CD_3OD) (Table 1) indicated thirty carbons including five methyl groups at [δ_H/δ_C 0.82/14.5; 0.99/25.6; 1.05/29.1; 1.22/24.8; and 0.96/19.6]; two quaternary olefinic carbons resonated at [δ_C 127.3 (C-13) and 149.6 (C-14)]; two oxymethines resonated at [δ_H/δ_C 3.97/71.8 (C-3), 3.44/78.4 (C-19); two acids carbonyl carbons resonated at [δ_C 180.2 (C-24) and 180.7 (C-28)] and two oxirane carbons at [δ_H/δ_C 3.52/48.8(C-12) and 3.83/48.5 (C-27)]. The above spectroscopic data show that **2** could be a pentacyclic triterpenoid. With the aid of ^{1}H-^{1}H COSY, HSQC, HMBC, NOESY experiments and literature, the ^{1}H and ^{13}C NMR signals of **2** were assigned as shown in Table 1 (Suppl. Mat. S1).

The ^{1}H and ^{13}C NMR signals assigned to rings A, B, and E were similar to those of oleanolic acid (Scheme 1) [14]. The presence of oxirane carbons, two carbonyl functionalities and the absence of an angular methyl in comparison with the usual oleanane suggested that **2** should possess five methyl and seven-membered ring C which was similar to the skeleton of duboscic acid and ilelic acids [4,14,16]. The ^{1}H-^{1}H COSY spectrum revealed apart from the individual angular methyls, the presence of six spin-coupling systems in bold as shown in Fig. 2 (Suppl. Mat. S4). The HMBC correlations between H-27 (δ_H 3.83) and C-11 (δ_C 22.3), C-14 (δ_C 146.6), and C-18 (δ_C 45.2) confirmed that C-27 was inserted between C-12 and C-13 to form a seven-membered ring C. The detailed interpretation of HMBC correlations Fig. 2 (Suppl. Mat. S4) allowed the establishment of the planar structure of **2**. Compared to duboscic acid, compound **2** possesses a seven-membered ring with only a methoxy group being absent Fig. 1 and the ^{1}H-NMR spectrum revealed additional signals at δ_H 3.52 (1H, brt, J = 10.4 Hz, J = 2.0 Hz, H-12) and 3.83 (dd, J = 10.4 Hz, J = 2.0 Hz, H-27) attributed to the proton of an oxirane ring which was fixed on C-12 and C-27 due to their HMBC correlation with carbons at δ_C 52.0 (C-9), 127.3 (C-13), and $_C$ 22.3 (C-11), 146.6 (C-14), 45.2 (C-18) respectively. The coupling constant of H-12 and H-11 (J = 2.0 Hz) suggested an alpha-orientation of the ether linkage [17]. Additional evidence for *endo*-configuration of epoxide was obtained from NOESY spectra Fig. 3 (Suppl. Mat. S5). To figure out the relative configuration of **2**, the NOESY spectrum was extensively analyzed, and the NOESY correlations between H-3/CH_3-23; H-5/H-9, Me-25/Me-26/H-12; Me-26/H-27; H-18/Me-30, H-16/Me-29 and H-27/Me-30 were established. Further analysis by molecular modelling resulted in the flipped, *E*-ring conformation indicated that **2** possessed comparable A/B trans, B/C trans, D/E cis ring junctions as normal oleanane. Moreover, the NOESY cross peaks between H-3 and H-25, H-16β and H-19 and between H-19 and H-30 suggested the presence of 3α-OH and 19α-OH. Compound (**2**) characterizes a new pentacyclic triterpenoid derived from oleanolic acid name 12α-27α-epoxy-3α-19α-dihydroxydubos-13-ene-24, 28-dioic acid (**2**) with the trivial name of duboscic acid B.

Duboscic acid B (**2**) represents an example of a new class of triterpenoids for which the name “dubosane” was proposed [4]. Its plausible biogenesis is shown in Scheme 1 (Suppl. Mat. S6) [18]. In fact, the cycloheptane ring may arise through expansion of the six-membered rings C of oleanene, promoted by homoallylic participation. Such type of modification has been studied by Tadanier in homoallylic rearrangements of 19-substituted steroids by using C-19 functionalized Δ^5 steroids as substrates [19]. The first step involves the enzyme-oxidation of C-27 follows by the monomolecular elimination who lead to the

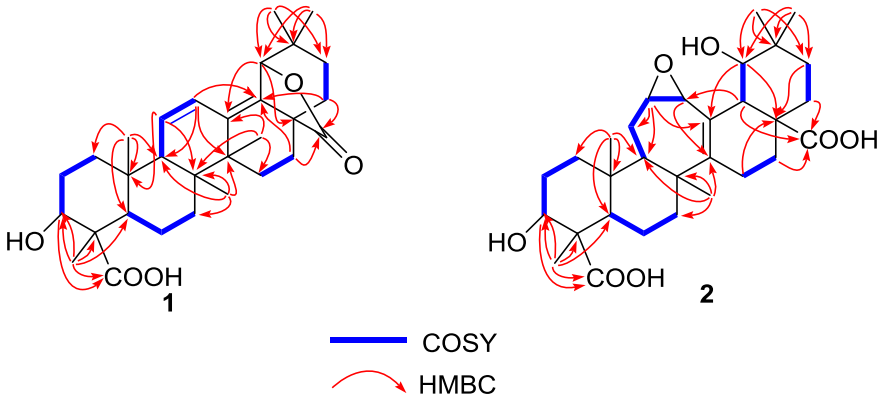


Fig. 2. Key HMBC and COSY connectivities for compounds **1** and **2**.

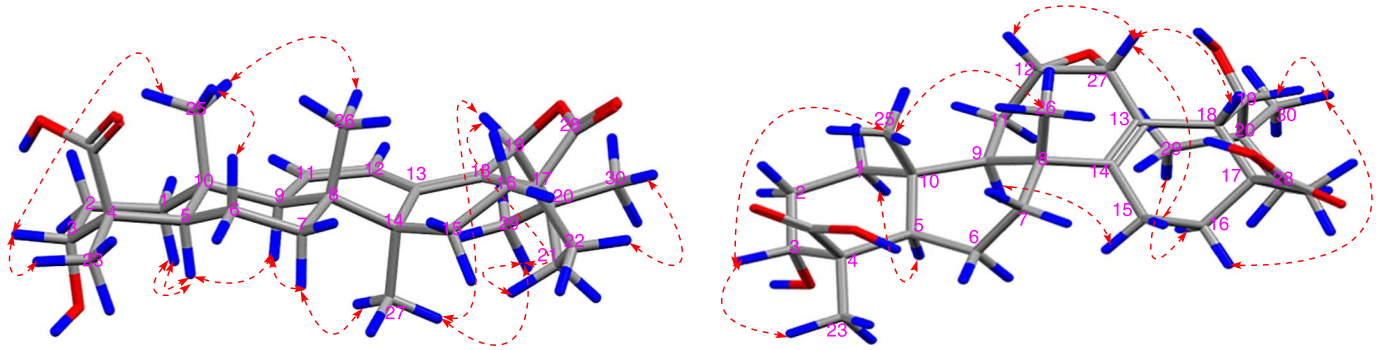


Fig. 3. Key NOESY connectivities for compounds **1** and **2.**

formation of an intermediate with a primary carbocation which undergoes rearrangement into a cycloheptene with the allylic stable carbocation. Scheme 1 (Suppl. Mat. S6). The carbocation under the thermodynamic control form illelic acid B who undergoes a series of eliminations, rearrangements, and epoxidation to give duboscic acid B. Scheme_1 (Suppl. Mat. S6). The fragmentation of duboscic acid B is similar to that of duboscic acid previously reported [20]. (Schemes 3,4 Suppl. Mat. S3).

The single-crystal X-ray diffraction analysis of 11β,12β-epoxyfriedours-14-en-3α-ol (**7**) is also described. Compound **7** crystallized from the mixture n-hexane-ethyl acetate (3:7) in the non-centrosymmetric monoclinic space group $P2_1$ without any crystal solvent molecules. The molecular structure is shown in Fig. 4. The absolute configuration of **7** was unambiguously determined to be *R* at C3, *S* at C5, *S* at C8, *S* at C9, *R* at C10, *R* at C11, *S* at C12, *R* at C13, *S* at C17, *S* at C18, *S* at C19 and *S* at C20 (Fig. 3a) by using Cu-Kα radiation. Analysis of the absolute structure using likelihood methods was performed with PLATON [21,22]. The method calculated for a two-hypothesis model the probability that the absolute structure is correct as 1.00 P2(true) for a pure enantiomer. The method calculated for a three-hypotheses model the probability that the absolute structure is right as 1.00 P3(true), that the structure is a 50% inversion twin as $0.4{\cdot}10^{-5}$ P(rac-twin) and the probability that the structure is wrong as $0.4{\cdot}10^{-13}$ P3(false). Thus, the results indicate that the absolute structure has been correctly assigned. We note that the CH_3 group with C29, which is bonded to C20, was refined in a major equatorial position (C29A) with 73.4% occupancy giving an *S*-configuration on C20 and it was also refined in a minor axial position (C29B) with 26.6% occupancy giving an *R*-configuration on C20. Crystallographic data for this structural analysis has been deposited with the Cambridge Crystallographic Data Center (CCDC 1543499). These data can be download free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the CCDC, 12 Union Road, Cambridge CB2, 1EZ; Fax: + 44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk.

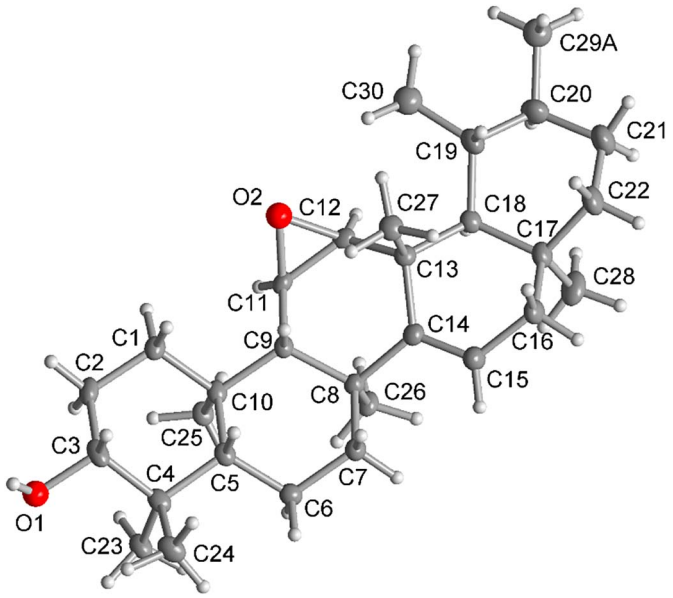


Fig. 4. Molecular structure of **7** (50% thermal ellipsoids except for C29A and C30 with standard radii). Note: Carbon atom C29A belongs to an equatorial methyl group of an anellated six-membered ring which is in a boat conformation. Also the adjacent methyl substituent with C30 on this six-membered ring has larger atomic displacement parameters. This seems to indicate that there is thermal motion in this six-membered ring, perhaps as part of an equilibrium between the boat and the chair conformation. Only the methyl group with C29A is shown in the structure drawing while the methyl group with C29B in the axial position giving an *R*-configuration on C20 has been omitted for clarity. (See Fig. 5 in Suppl. Mater. for a packing diagram.)

The known compounds **3–7** were identified by similarity of their spectroscopic data with previously reported results and identified as maslinic acid (**3**) [23], arboreic acid (**4**) [10], (*E*)-3-(4-hydroxyphenyl)-N-[2-(4-hydroxyphenyl) ethyl] prop-2-enamide (**5**) [24], (*E*)-heptacos-19-enoic acid (**6**) [25], and 11β,12β-epoxyfriedours-14-en-3α-ol (**7**) [26].

Compounds **1** and **3–7** were evaluated for their α-glucosidase, α-chymotrypsin inhibition, and anti-glycation (BSA-MG model system) potential. Compound **2** was excluded for these assays because of insufficient quantity. All tested compounds except **7** exhibited a potent α-glucosidase inhibitory activity. Table 2 (Suppl. Mat. S2), as compared to the standard inhibitor 1-deoxynojirimycin (IC_{50} = 441.0 ± 0.1 μM) and clinically used inhibitor, viz., acarbose (IC_{50} = 625.0 ± 1 μM). Unfortunately, these compounds showed less than 50% inhibition at 500 μM, or lower concentration for non-enzymatic protein glycation assay, therefore, were considered as inactive. All compounds also showed less than 50% inhibition at 500 μM, or lower concentration for the α-chymotrypsin enzyme, hence, these compounds do not inhibit the activity of α-chymotrypsin, a digestive enzyme for proteins [27]. Also, gastrointestinal problems, such as abdominal spasms and diarrhea are expected to be low in case of selective inhibition of α-glucosidase in diabetic subject [28,29]. As α-glucosidase from yeast is known to be different from the enzyme of mammalian origin, the enzyme inhibitory activity cannot be generalized without further studies.

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on a JASCO 302-A spectrophotometer in $CHCl_3$ or in KBr pellets. HRFAB-MS and APCI-MS (ionization voltage 70 eV) were measured on a variant JEOL mass spectrometer (Orbitrap mass analyzer). Hypothetical fragmentation of protonated duboscic acid B checked out by using ACD/MS Fragmentor software (ACD Labs). 1D- and 2D-NMR spectra were registered on Bruker AMX 300 and 600 MHz NMR spectrometer. The chemical shifts and coupling

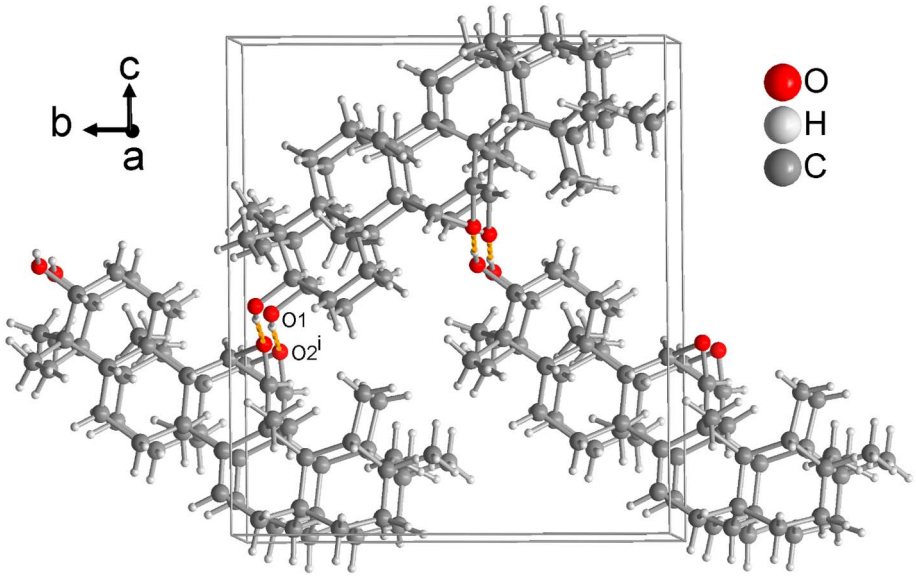


Fig. 5. Section of the packing diagram with hydrogen bonding interaction between the hydroxyl group and the epoxide. Details of H bond H···O2^{i} 2.02 Å, O1···O2^{i} 2.794(3) Å, O1-H···O2^{i} 171°. Symmetry transformation i = $-x + 2, y + 1/2, -z + 1$.

Table 2
α-Glucosidase, anti-glycation and α-chymotrypsin inhibitory effect of compounds **1**, and **3–6**.

Compounds	IC_{50} ± SEM (μM)		
	α-Glucosidase inhibition	Anti-glycation studies	α-chymotrypsin inhibition
1	245.1 ± 3.2	NC	NC
3	109.0 ± 1.0	NC	NC
4	41.7 ± 0.1	NC	NC
5	5.1 ± 0.1	NC	NC
6	27.7 ± 1.0	NC	NC
Standard inhibitors	441.0 ± 0.1[a] 625.0 ± 1.2[b]	294.5 ± 1.5[c]	5.7 ± 0.1[d]

SEM = Standard Error of Mean, where $n = 3$.
NC. = Not calculated because less than 50% were shown at 500 μM or lower concentration.
[a] 1-Deoxynojirimycin.
[b] Acarbose.
[c] Rutin.
[d] Chymostatin.

constants are given in ppm (δ), and in Hz respectively. Column chromatography was performed on silica gel (70–230 mesh, Merck). TLC was performed on Merck percolated silica gel 60 F_{254} aluminium foil, and spots were detected using a ceric sulphate spray reagent. Microtiter plate reader (SpectraMax M2, Molecular Devices, CA, USA) was used to study the biological activities.

3.2. Plant material

The plant specimen was harvested from Evodoula (Cameroon) in February 2004 and identified by Prof. Sonke at the University of Yaounde-I. The voucher specimen (No. 95919) was conserved at the Cameroon National Herbarium (CNH).

3.3. Extraction and isolation

Trunk wood (5 kg) of *D. macrocarpa* after powdered and air-dried was extracted three times (each for three days) with CH_3OH-CH_2Cl_2 (1:1) (15 L) at room temperature. The resulting extract was evaporated to obtain 100 g of total extract. The crude extract was then subjected to column chromatography over a silica gel column (70 cm × 5 cm) and eluted with a gradient of ethyl acetate: *n*-hexanes (5% to 80%, 5 × 500 mL) to obtain four major sub-fractions (T_1–T_4). Sub-fraction T_1 (30 g), eluted with 10% ethyl acetate- n-hexane was re-chromatographed on silica gel column (30 cm × 3 cm) using the solvent system ethyl acetate-hexanes (10% to 25% ethyl acetate, 30 × 200 mL) to afford five major fractions (T_{1A}–T_{1E}). Sub-fraction T_{1A} was an essential oil, sub-fraction T_{1B} was re-chromatographed by silica gel column (30 cm × 3 cm) using 10% ethyl acetate-n-hexane as eluting solvents system to obtain compound (*E*)-heptacos-19-enoic acid (**6**, 1.0 g) and 11β,12β-epoxyfriedours-14-en-3α-ol (**7**, 3.0 mg). The second fraction T_2 (12 g), obtained with 20% ethyl acetate-hexanes was re-chromatographed on silica gel column (30 cm × 3 cm) by using the solvent system 15% ethyl acetate-hexanes as eluent to afford white powder of compound evodoulolide (**1**, 10.0 mg). The fractions obtained with 40% ethyl acetate-n-hexane afforded a white powder of duboscic acid B (**2**, 1.4 mg). Fraction T_3 (5 g), eluted with ethyl acetate-hexanes (20% to 50%), was subjected to silica column chromatography that yielded two major fractions A and B. The sub-fractions A eluted at 20% to 30% ethyl acetate-n-hexane were combined on the basis of TLC and rechromatographed over silica gel to obtain maslinic acid (**3**, 20.0 mg), arboreic acid (**4**, 50.0 mg), *p*-coumaroyl-tyramine (**5**, 5.0 mg).

3.3.1. Evodoulide (1)

White powder from n-hexane-ethyl acetate 1.5:8.5; Liebermann Buchard Test: positive; UV ($CHCl_3$) λ_{max} nm (logε): 202 (1.90), 220 (2.52), 253(3.41) and 261(3.70); IR (KBr) ν_{max} cm^{-1}: 1390 and 1375 (*gem*-dimethyl), 1765 (γ-lactonic C=O), 1724 (ester), 3511 (hydroxyl), 3191 and 1734 (carboxylic acid groups); for ^{1}H NMR (CD_3OD, 300 MHz) and ^{13}C NMR (CD_3OD, 75 MHz): see Table 1; (empirical formula $C_{30}H_{42}O_5$); The APCI-MS: *m/z* 483.0 $[M + H]^+$, 439.2 $[M + H\text{-}CO_2]^+$.

3.3.2. Duboscic acid B (2)

White powder from n-hexane-ethyl acetate (4:6); Liebermann Buchard Test: positive; UV ($CHCl_3$) λ_{max} nm (logε): 208(3.40), 214(2.10); IR (KBr) ν_{max} cm^{-1}: 3555 (OH), 1365–1230 *gem*-diméthyl, 3059 (carboxylic acid); for ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz): see Table 1; (empirical formula: $C_{30}H_{44}O_7$). The HRFAB-MS: *m/z* 517.3085 $[M\text{-}H]^-$; APCI-MS: m/z 501.1 $[M + H\text{–}H_2O]^+$.

3.4. α-Glucosidase inhibition assay

α-Glucosidase (E.C.3.2.1.20) from *Saccharomyces* sp. was obtained from Wako Pure Chemical Industries Ltd., Japan). Inhibitory activity was evaluated using 0.1 M phosphate buffer (pH 6.8) at 37 °C. The enzyme (200 milliunits/mL) in phosphate buffer was incubated with different concentrations of test compounds at 37 °C for 15 min. The substrate, *p*-nitrophenyl-α-D-glucopyranoside (0.7 mM, final concentration) was added, and change in absorbance at 400 nm was monitored up to 30 mins with the spectrophotometer (Spectra Max, Molecular Devices, CA, U.S.A.). The test compound was replaced by DMSO (7.5% final concentration) as a control. 1-Deoxynojirimycin and acarbose were used as the standard inhibitors [12]. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α-glucosidase, was performed continuously with the spectrophotometer (Spectra Max, Molecular Devices, CA, U.S.A.). The percent inhibition was calculated by using the following formula:

$$\%\text{inhibition} = 100 - \left(\frac{\text{OD of the sample}}{\text{OD of the control}}\right) \times 100$$

3.5. α-Chymotrypsin inhibition assay

The α-chymotrypsin (source: Bovine pancreas) inhibition assay was performed in 50 mM Tris-HCl buffer (pH 7.6 with 10 mM $CaCl_2$) according to Cannel with slight modifications [31] α-Chymotrypsin (12 units/mL prepared in the buffer) with the test compound (0.5 mM prepared in DMSO) was incubated at 30 °C for 25 min. The reaction was started by adding *N*-succinyl-L-phenylalanine-*p*-nitroanilide (SPpNA; 0.4 mM prepared in the buffer). The change in absorbance was continuously monitored at 410 nm on a microtiter plate reader (SpectraMax M2, Molecular Devices, CA, USA). The positive control without test compound and the negative control without enzyme or with standard inhibitor were run in parallel and Chymostatin was used as a standard inhibitor [32].

3.6. Antiglycation assay

Bovine serum albumin (10 mg/mL), methylglyoxal (14 mM), different concentrations of the test compounds (prepared in DMSO, final concentration of DMSO was maintained at 10%), and 0.1 M phosphate buffer (pH 7.4) containing sodium azide (30 mM) were incubated under aseptic conditions at 37 °C for 9 days. Then each sample was examined for the development of specific fluorescence (excitation, 330 nm; emission, 440 nm), against the sample blank [31]. Rutin was used as a standard inhibitor. The percent inhibition of the test sample was calculated for each inhibitor compound by using the following formula:

$$\%\text{Inhibition} = \left(1 - \frac{\textit{fluorescence of test sample}}{\textit{florescence of the control group}}\right) \times 100$$

3.7. Statistical analysis

All of the analysis were carried out in 96-well microplate reader (SpectraMax M2, Molecular Devices, CA, USA). The results were analyzed using SoftMaxPro 4.8 and MS-Excel and presented as means ± SEM of three experiments (as indicated in Table 1). IC_{50} Values were determined by using EZ-FIT, Enzyme kinetics software by Perrella Scientific, Inc., USA.

4. Conclusion

D. macrocarpa was found to contain triterpenes (**1–4, 7**), fatty acid (**6**) and alkaloids (**5**) usually present in some members of Tiliaceae family (*Grewia microcos*, *Luechea divaricate*, *Corchorus aestuans*) [7,8,30]. They could be considered as the important chemotaxonomic marker of this genus. Compounds **1**, **2** may be used to differentiate *D. macrocarpa* from other members of the genus. Compound **5** was found to be a potent inhibitor of *α*-glucosidase as compared to the standard drug acarbose. But as α-glucosidase from yeast is known to be different from the enzyme of mammalian origin, this result cannot be generalized without further studies. These compounds did not show anti-glycation activity against BSA-MG glycation model, and no inhibition of the *α*-chymotrypsin enzyme. The single-crystal X-ray and absolute configuration diffraction analysis of 11α,12α-epoxyfriedours-14-en-3-ol (**7**) is also described here for the first time.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2017.12.015.

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