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Duboscic Acid: A Potent α-Glucosidase Inhibitor with an Unprecedented Triterpenoidal Carbon Skeleton from *Duboscia macrocarpa*

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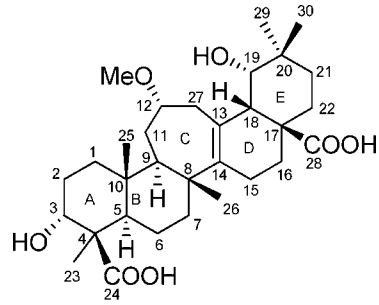
ABSTRACT



Duboscic acid (**1**)

Duboscic acid (1), a triterpenoid with a unique carbon backbone, was isolated from *Duboscia macrocarpa* Bocq. It is the first member of a new class of triterpenoids, for which the name "dubosane" is proposed. Duboscic acid has a potent α-glucosidase inhibition, and its structure was unambiguously deduced by a single-crystal X-ray diffraction study.

Duboscia macrocarpa Bocq. (Tiliaceae) is a tall tree that thrives in dense forests of Africa. The decoction from the seeds is used in African folk medicine to treat tuberculosis while the fruits are used to cure teeth problems.[1] Members of Tiliaceae are known for the presence of triterpenoids, saponins, flavonoids, and cadenolide glycosides.[2−4] There has been no report on the phytochemical or pharmacological potential of *Duboscia macrocarpa*. As part of a program to search for pharmacologically active natural products, we report here the isolation and structure determination of duboscic acid (**1**),[5] a triterpenoid with an unusual carbon skeleton from the trunk wood of *Duboscia macrocarpa*. The possible biogenetic basis

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of the unique structural features of duboscic acid, its single-crystal X-ray diffraction analysis, and α-glucosidase enzyme inhibitory activity are also described.

The plant material was collected from Evodoula (Cameroon) in May 2005 and identified by Prof. Sonke at the University of Yaounde-I. The voucher specimen (No. 95919) was deposited at the Cameroon National Herbarium. Air-dried and powdered trunk wood (10 kg) of *D. macrocarpa* was extracted thrice at 48 h intervals with $CH_3OH-CH_2Cl_2$ (1:1, 20 L) at room temperature. The solvent was evaporated under reduced pressure to obtain 99.7 g of the crude extract. This was then subjected to column chromatography (CC) over a silica gel and eluted with ethyl acetate/hexanes (1:19 to 4:1) to obtain four fractions (A_1-A_4). Fraction A_2 (12.1 g) from ethyl acetate/hexanes (3:7) was subjected to column chromatography over silica gel using ethyl acetate/hexanes (3:7−2:3) to obtain duboscic acid (**1**) (100.3 mg).

The molecular formula of duboscic acid was determined to be $C_{31}H_{48}O_7$ from the broad-band-decoupled ^{13}C NMR spectrum and HRTOF-ESI-MS, which exhibited an $[M + H]^+$ ion at *m/z* 533.3486 (calcd for $C_{31}H_{48}O_7$ + H, 533.3478). The DEPT spectrum was used to differentiate the ^{13}C NMR resonances as six methyl, ten methylene, six methine, and nine quaternary carbons. The ^1H and ^{13}C NMR spectra (Table 1) showed resonances for five tertiary methyls [$\delta_H/\delta c$ 1.26/24.5 (CH_3-23), 0.90/15.2 (CH_3-25), 1.09/20.2 (CH_3-26), 1.03/28.7 (CH_3-29), and 0.99/24.6 (CH_3-30)], three oxygenated methines [$\delta_H/\delta c$ 3.99 (br. s)/70.8 (CH-3), 3.52 (m)/79.2 (CH-12) and 3.45 (d, $J_{19.18}$ = 3.6 Hz)/74.2 (CH-19)], and a methoxy group [$\delta_H/\delta c$ 3.25 (s)/56.1]. The ^{13}C NMR spectrum showed resonances for two olefinic quaternary and two acidic carbonyl carbons [δc 126.0 (C-13) and 146.1 (C-14), 180.9 (C-24) and 181.2 (C-28), respectively]. The described spectral data suggested a pentacyclic triterpenoid skeleton for duboscic acid (**1**). The ^1H and ^{13}C NMR chemicals shifts (Table 1) were assigned using $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra (Figure 1).

Table 1. NMR Data of Duboscic Acid (**1**)[a]

position	δ_C mult.	δ_H[b] mult.
1	35.2, CH_2	1.61, 1.37
2	26.7, CH_2	2.18, 1.56
3	70.8, CH	3.99 br. s
4	48.2, C	–
5	49.5, CH	1.55
6	20.9, CH_2	1.80, 1.65
7	42.7, CH_2	2.05, 1.45
8	44.9, C	–
9	49.3, CH	1.85
10	39.7, C	–
11	31.7, CH_2	1.82, 1.50
12	79.2, CH	3.52, m
13	126.0, C	–
14	146.1, C	–
15	24.2, CH_2	2.25, 2.08
16	28.9, CH_2	1.78, 1.09
17	44.8, C	–
18	48.8, CH	2.59, d (3.6)
19	74.1, CH	3.45, d (3.6)
20	34.5, C	–
21	30.4, CH_2	1.85, 1.32
22	26.9, CH_2	2.30, 1.68
23	24.5, CH_3	1.26 s
24	180.9, C	–
25	15.2, CH_3	0.90 s
26	20.2, CH_3	1.09, s
27	39.1, CH_2	2.69, 2.15
28	181.2, C	–
29	28.7, CH_3	1.03, s
30	24.6, CH_3	0.99, s
OMe	56.2, CH_3	3.25, s

[a] ^1H and ^{13}C NMR data were recorded at 500 and 100 MHz, respectively, in $CDCl_3$ + CD_3OD. Chemical shifts (δ) are in ppm. [b] Multiplicity is not clear for some signals due to overlapping.

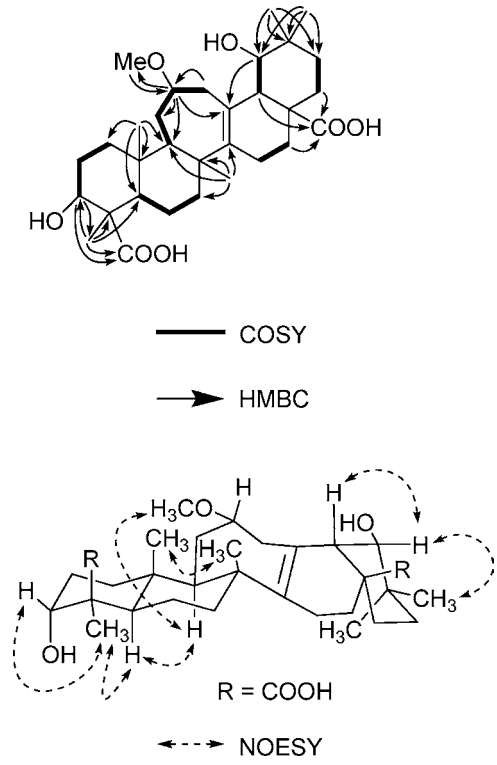

Figure 1. COSY, HMBC, and NOESY correlations of duboscic acid.

The seven-membered ring formation in duboscic acid (**1**) is unique due to C-27 methyl migration from C-14 to C-13 to form an unprecedented C-27/C-12 bond rather than a C-27/C-8 bond resulting from coupling of the C-27 methyl with C-8 as in the serratane skeleton[6−8] (Figure 2).

Conclusive evidence for the structure of duboscic acid (3α,19α-dihydroxy-12α-methoxydubos-13(14)-en-24,28-

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(5) Duboscic acid. White crystals from DMSO; mp: 289 °C; $[\alpha]_D^{28}$ −90.3 (*c* 0.025, MeOH); IR (KBr) υ_{max} 3340 (OH), 1689 (CO) cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRTOF-ESI-MS (positive ion mode): $[M + H]^+$ *m/z* 533.3486 (calcd for $C_{31}H_{48}O_7$ + H, 533.3478), $[M - OMe - H]^+$ *m/z* 501.3217 (calcd for $C_{30}H_{45}O_6$, 501.3216).

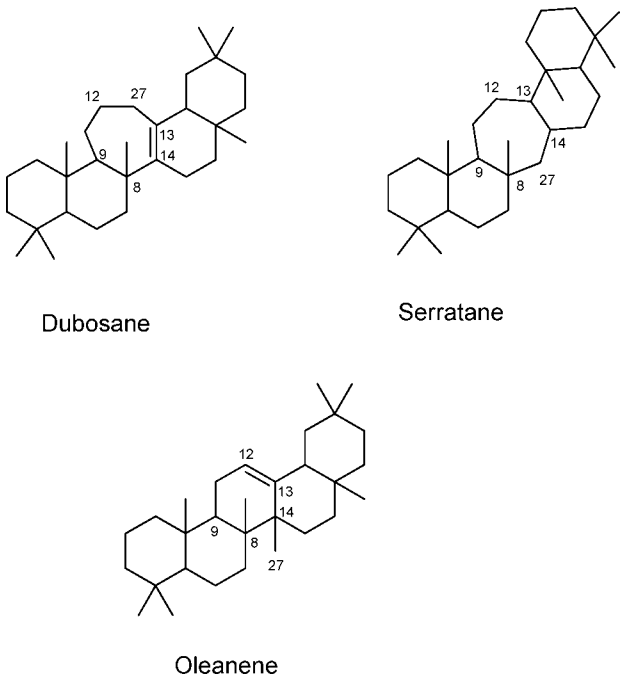


Figure 2. Dubosane, serratane, and oleanane skeletons.

dioic acid) was obtained from single-crystal X-ray diffraction analysis (Figure 3). The hydroxyl group at C-19 served as

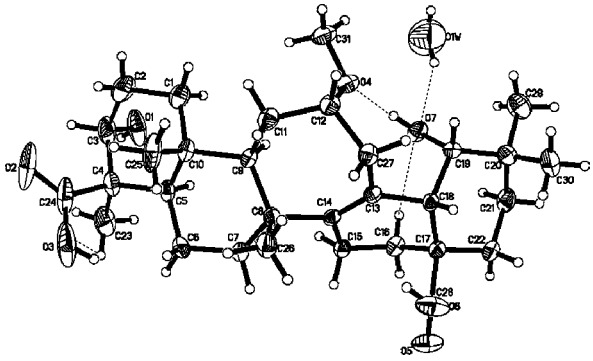
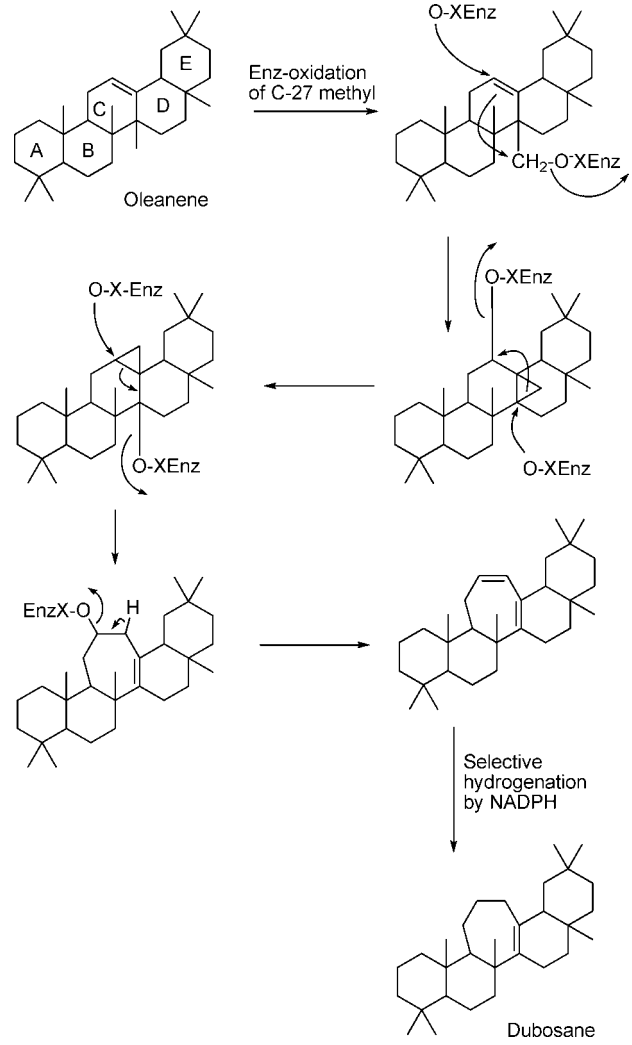


Figure 3. ORTEP drawing of duboscic acid (**1**) with atom labels and 30% probability displacement ellipsoids. The dashed lines indicate the intramolecular H-bonding.

an H-bond donor and acceptor. As shown in Figure 3, the O7−H7A...O4, C16−H16B...O7, and C23−H23B...O3 generated rings of graph-set motifs S (8), S (6), and S (5), respectively. A water molecule was detected as the solvent of crystallization forming an H-bond with O-7. The final *R* and *Rw* factors for data with $I > 2\sigma$ are 0.0698 and 0.0781, respectively.[9]

Duboscic acid (**1**) belongs to a new class of triterpenoids, for which the name "dubosane" is proposed. A plausible biogenetic route toward the novel sketeton of duboscic acid is shown in Scheme 1 and seems to be close to the formation

Scheme 1. Plausible Biogenetic Pathway towards Dubosane Skeleton



of homocardenolide.[10] The probable origin of the cycoheptane ring is through a formal ring expansion of the six-membered ring C of oleanene which is facilitated by homoallylic participation. Such a type of modification has been studied by Tanadier in homoallylic rearrangements of 19-substituted steroids[11] by using C-19 functionalized Δ^5 steroids as substrates. The first step involves the formation

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(10) Wolff, M. E.; Ho, W. *J. Org. Chem.* **1967**, *32*, 1839–1843.
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of a cyclopropane ring that undergoes a rearrangement sequence into a cycloheptene system (azulene).

Duboscic acid (**1**) was evaluated for α-glucosidase inhibition activity in a dose dependent fashion (Figure 4). It showed

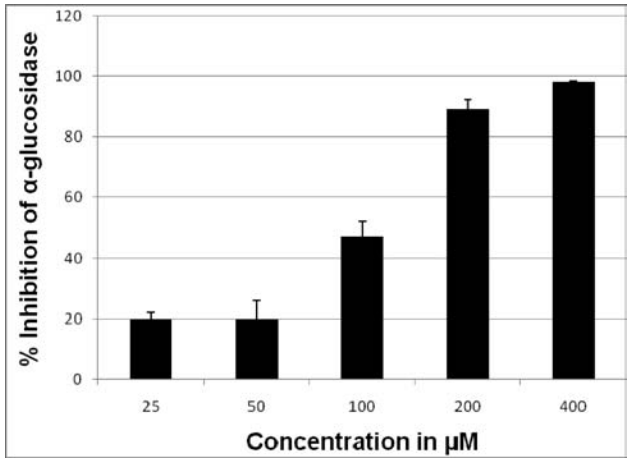

Figure 4. α-Glucosidase inhibitory activity of duboscic acid (**1**).

potent inhibition of the enzyme with an IC_{50} value of 100 ± 8.1 μM, as compared to deoxynojirimycin (IC_{50} = 425.6 ± 8.1 μM), one of the most potent α-glucosidase enzyme inhibitors, and acarbose (IC_{50} = 780 ± 0.26 μM), a clinically used drug. α-Glucosidase enzyme inhibitory activity makes duboscic acid (**1**) an interesting lead for future studies. The α-glucosidase inhibition assay was performed following the slightly modified method of Oki et al.[12] α-Glucosidase is an important membrane-bound enzyme that catalyzes the hydrolysis of disaccharides into monosaccharides, which are absorbed in the gut. Inhibition of α-glucosidase activity is an important intervention to delay the absorption of glucose and control postprandial hyperglycemia in diabetic patients.

α-Glucosidase (E.C.3.2.1.20) from *Sacchomyces sp*. was purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The inhibition was measured spectrophotometrically at pH 6.9 and at 37 °C by using 0.5 μM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 250 units/mL of enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) and acarbose (0.78 mM) were used as positive controls. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α-glucosidase, was monitored continuously with a spectrophotometer (Spectra Max, Molecular Devises CA, USA).

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Supporting Information Available: NMR spectra and CIF file of duboscic acid (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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