Some Chemical Constituents of *Terminalia glaucescens* and their Enzymes Inhibition Activity

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Z. Naturforsch. **60b,** 347 – 350 (2005); received September 9, 2004

A new triterpenoid, glaucinoic acid (2α, 3β, 19α, 24-tetrahydroxyolean-12-en-30-oic acid) (**1**) along with several known compounds, arjunic acid (**2**), arjungenin (**3**), sericoside (**4**), and friedelin (**5**) were isolated from the stem barks of *Terminalia glaucescens*. These compounds showed β-glucuronidase inhibitory activity. The structures were identified on the basis of spectroscopic techniques.

Key words: Terminalia glaucescens, Combretaceae, Glaucinoic Acid, β-Glucuronidase Inhibition

Introduction

The genus *Terminalia* (Combretaceae) comprises 135 species distributed in the tropical regions of the world [1]. Various species of the genus are used for cardiac effects, anti-atherogenic, hypolipidemic actions, hepatoprotection, and as antimicrobials [2]. *T. glaucescens* is prescribed as an anti-dysenteric and anti-microbial agent, and useful in the last stages of AIDS [3]. The extract of the plant showed a wide spectrum of antibacterial activity against periodontopathic bacteria [4]. The ethanolic decoction of this plant exhibited antiplasmodial activity [5], cytotoxic effects [6] and aldose reductase inhibition [7]. It is an important drug in folk medicine [8]. The *Terminalia* species are known to contain several triterpenes, some of which showed antifungal as well as antiviral activities [2]. The current phytochemical investigation on *T. glaucescens* led to the isolation of a new glaucinoic acid (**1**) along with several known compounds. Some of them showed inhibitory activity against β-glucuronidase.

Results and Discussion

Compound **1** was obtained as an amorphous powder from the chloroform extract of *T. glaucescens*. The IR spectrum of compound **1** displayed the absorption bands at 1692 and 3408 cm^{-1}, indicating the presence of carbonyl and hydroxyl groups, respectively. The EI MS of compound **1** showed the highest ion at *m/z* 486 [M$^+$-18] indicating the loss of a water molecule. The molecular ion was deduced from the [M + H]$^+$ (*m/z* = 505.3529) in the HRFAB MS, in agreement with the formula $C_{30}H_{48}O_6$ (calcd. 505.3536): This showed seven degrees of unsaturation which accounted for five rings, one olefinic bond and a carbonyl carbon functionality. The base peak at *m/z* 264.3054 (D/E ring, [$C_{16}H_{24}O_3$]) may arise by the retro Diels-Alder cleavage of ring C, which indicated the presence of a Δ^{12}-β-amyrin skeleton with one-hydroxyl and carboxyl groups on rings D and E, respectively [9].

The ^{1}H NMR spectrum of compound **1** exhibited signals for six methyl protons at $\delta = 1.25$ (CH_3-27), 1.22 (CH_3-23), 1.07 (CH_3-28), 0.99 (CH_3-29), 0.98 (CH_3-25) and 0.78 (CH_3-26). A characteristic downfield triplet resonating at $\delta = 5.32$ ($J = 3.2$ Hz) was assigned to the olefinic C-12 proton. This supported a pentacyclic triterpenoidal skeleton. A set of AB doublets, appeared at $\delta = 3.45$ and 4.10 ($J = 11.1$ Hz), were due to the C-24 hydroxymethylene protons. A downfield proton resonating as a doublet of double doublet at $\delta = 3.81$ ($J = 13.9$ Hz, $J = 9.2$ Hz, $J = 4.4$ Hz) was assigned to the C-2 (axial) proton. A dou-

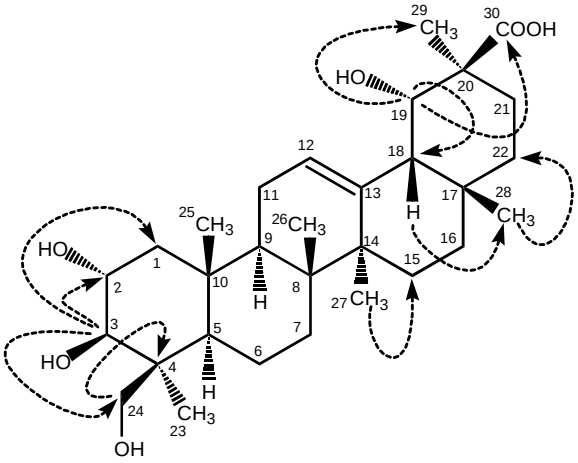


Fig. 1. Key HMBC correlations in compound **1**.

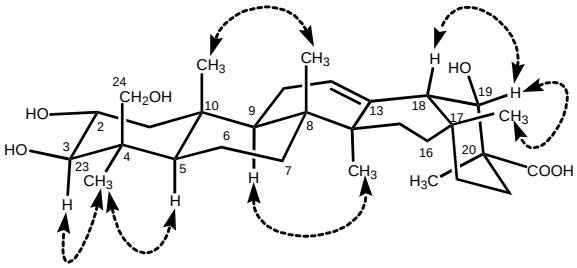
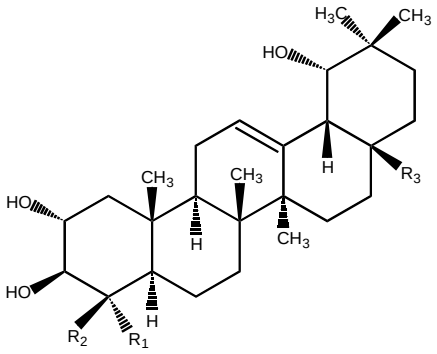
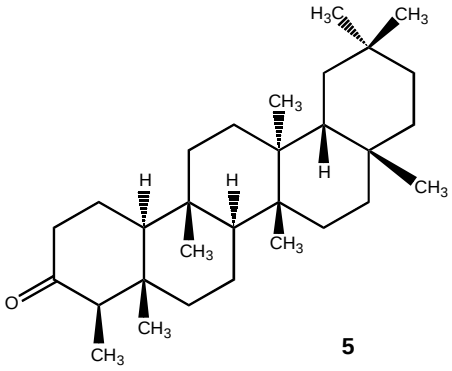


Fig. 2. Selected NOESY correlations in compound **1**.



	R_1	R_2	R_3
2	CH_3	CH_3	COOH
3	CH_2OH	CH_3	COOH
4	CH_3	CH_2OH	-gluco



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blet at $\delta = 3.07$ ($J = 9.2$ Hz) was assigned to the C-3 (axial) proton geminal to the hydroxyl group. A doublet at $\delta = 3.27$ ($J = 4.0$ Hz) was assigned to the H-19 equatorial proton. A broad singlet at $\delta = 2.92$ ($W_{1/2} = 4.4$ Hz) was due to the C-18 (axial) proton [10].

The broad-band decoupled ^{13}C NMR spectrum of compound **1** showed the signals for thirty carbons including six methyl, seven methine, nine methylene and eight quaternary carbons. The ^{13}C NMR spectrum also supported the presence of CH_2OH, COOH, three CHOH and one olefinic bond in the molecule. The overall NMR data was in good agreements with a Δ^{12}-β-amyrin skeleton [11].

The HMBC spectrum of compound **1** showed correlations between various protons and carbons. The C-3 methine proton ($\delta = 3.07$) showed $^3J_{CH}$ correlations with C-2 ($\delta = 68.3$), C-4 ($\delta = 42.7$) and C-1 ($\delta = 45.4$). The C-2 methine proton ($\delta = 3.81$) showed correlations with the C-3 ($\delta = 84.7$) and C-1. Similarly, the C-1 methylene protons ($\delta = 1.96, 1.55$) showed correlations with the C-2 and C-3. These interactions supported the partial structure of rings A and B. Similarly, the C-25 methyl protons ($\delta = 0.98$) exhibited connectivity with the C-10 ($\delta = 37.7$) and C-5 ($\delta = 55.5$). The H-12 ($\delta = 5.32$) showed long-range couplings with the C-13 ($\delta = 142.7$), C-18 ($\delta = 43.4$) and C-19 ($\delta = 81.2$), which supported the position of a double bond between the C-12 and C-13. The C-18 proton ($\delta = 2.92$) exhibited correlations with the C-19 ($\delta = 81.2$), C-17 ($\delta = 32.4$) and C-30 ($\delta = 182.3$) atoms. A proton resonated at $\delta = 3.27$ (C-19, CHOH) showed long-range connectivities with the C-20 ($\delta = 44.8$) and C-30 ($\delta = 182.3$). These assignments were further supported the presence of a carboxylic acid at C-20. Further in the HMBC spectrum of compound **1**, the signal of the C-29 methyl protons ($\delta = 0.99$) showed strong correlations with the carboxyl carbon at $\delta = 182.3$ and that of the quaternary carbon atom at $\delta = 44.8$ (C-20), indicating that the carboxyl group was attached to the C-20.

The stereochemical assignments were based on NOESY spectrum of compound **1**. The NOESY spectrum showed a cross-peak between H-19 ($\delta = 3.27$)

and C-28 methyl protons ($\delta = 1.07$). Similarly, H-5 ($\delta = 1.01$) exhibited the cross-peak with C-23 methyl ($\delta = 1.22$). The key NOESY interactions are presented as in Fig. 2. The observed NOE between H-3 and H_3-23, and between H-18 and H_3-28, and also between H_3-28 and H-19, indicated the stereochemistry of H-18, H-19 and H_3-28 as equatorial, axial and axial, respectively, with reference to ring E. From the above spectral observations, the structure of compound **1** was deduced as 2α, 3β, 19α, 24-tetrahydroxyolean-12-en-30-oic acid.

The compounds **1**–**5** were tested for β-glucuronidase inhibition and showed good activity potential against the enzyme. β-Glucuronidase of intestinal bacterial in human and rats are related to colon cancer. In addition to this, β-glucuronidase of bacterial origin, which is present in biliary tract, is also associated with the gallstone formation. Pineda *et al.* demonstrated that liver damage cause an increase in the enzyme levels in blood. Liver cancer is also suspected to be related to the overexpression of this enzyme [12]. Compound **1** showed a significant β-glucuronidase inhibiting activity with IC_{50} value 80.1 μM. While compounds **2** ($IC_{50} = 500$ μM) and **4** ($IC_{50} = 200$ μM) have shown moderate inhibition against β-glucuronidase. No significant inhibation activity was observed in case of compounds **3** and **5**. Glucosaccharo-(1:4)-lactone was used as a standard inhibitor (positive control).

Experimental Section

General

UV and IR spectra were recorded on Hitachi UV 3200 and JASCO 302-A spectrophotometers, respectively. Optical rotations were measured on Schmidt Haensch Polartronic D. EI and HREI MS were measured on Varian MAT 311A and JEOL HX 110 mass spectrometers (*m/z*, rel. int. %). The NMR spectroscopic techniques (broad-band decoupled ^{13}C NMR, COSY, NOESY, NOE, HOHAHA, HMQC and HMBC) were recorded on Bruker AMX 400 and AMX 500 MHz NMR spectrometers. The chemical shifts are given in ppm (δ), relative to $SiMe_4$ as internal standard, and coupling constants are in Hz. Melting points were measured on the Yanaco melting point apparatus. Column chromatography (CC) was carried out on silica gel (70–230 mesh). Thin layer chromatography (TLC) was performed on precoated silica gel plates (DC-Alufolien 60 F_{254} of E. Merck) and spots were detected by using ceric sulfate spraying reagent.

β-Glucuronidase (E.C. 3.2.1.31) from *E. coli* and *p*-nitrophenyl-β-D-glucuronide were obtained from Sigma Chemical Co. Other reagents were also purchased from various commercial sources.

Collection and identification

The stem barks of *Terminalia glaucescens* (Planchon.) were collected at Mount Bankolo near Yaounde, Cameroon. A voucher specimen (# 9468 SRFCAM) was deposited at the National Herbarium (Yaounde, Cameroon).

Extraction and purification

Air-dried stem barks (7.5 kg) of *T. glaucescens* were cut into pieces, dried, pulverized and soaked in a mixture of MeOH-CH_2Cl_2 (1:1) at room temperature for 24 h then filtered and solvent was evaporated under reduced pressure. The methanolic extract (611.5 g) was suspended in distilled water and defatted with pet. ether and concentrated under reduced pressure to obtain a concentrated gum (18.3 g). Followed by extracted with chloroform (30.1 g), ethyl acetate (58.7 g) and butanol (32.8 g). The chloroform soluble fraction was chromatographed on a column of silica gel using pet. ether-ethyl acetate-methanol in increasing order of polarity to give six major fractions (Fc-1 to Fc-6). The major fraction Fc-2, which was eluted with pet. ether-ethyl acetate (5:5), was subjected to column chromatography over silica gel using various mixtures of pet. ether and ethyl acetate.

The fraction, which was eluted with pet. ether-ethyl acetate (2:8) was mixtures of three components obtained. Repeated preparative TLC over silica gel using pet. ether-ethyl acetate (2:8) as an eluent provided compounds **1** (8.53 mg, $1.13 \cdot 10^{-6}$), **2** (8.72 mg, $1.42 \cdot 10^{-3}$) and **3** (21.31 mg, $2.84 \cdot 10^{-6}$), respectively.

The pet. ether soluble fraction was subjected to column chromatography on silica gel (70–230 mesh size). Elution was made with pet. ether and compound **5** (1.27 g, $1.69 \cdot 10^{-4}$) was isolated. The ethyl acetate soluble fractions were subjected to column chromatography on column silica and eluted with increasing polarities of pet. ether-ethyl acetate-methanol mixtures to obtain further semi pure sub fractions, which were finally purified over flash silica gel column, eluted with chloroform-methanol, to obtain compound **4** (18.13 mg, $2.41 \cdot 10^{-9}$) (10% MeOH: $CHCl_3$). The known compounds **2**–**5** were characterized through comparison of physical and spectral data with that in the literature [13–16].

2α,3β,19α,24-Tetrahydroxyolean-12-en-30-oic acid (**1**): Amorphous powder (8.53 mg, $1.13 \cdot 10^{-6}$ % yield). – $[\alpha]_D^{25} +$ 19.3° (CH_2Cl_2-CH_3OH; $c = 0.06$). – IR $\nu_{max} = 3674$, 3408 (OH), 2935, 2875 (CH), 1652 (C=C), 1692 (C=O), 1456

(CH_2), 1392 (CH_3), 1049, 997 cm^{-1}. – UV λ_{max} (MeOH) (log ε) = 203 (3.2) nm. – MS (EI, 70 eV): *m/z* (%) = 486, 442, 424, 408, 264, 246, 240, 222, 201, 189, 57. – MS (HRFAB) $m/z = 505.3529$, $C_{30}H_{48}O_6$ (calcd. 505.3536). – ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) $\delta = 0.99$ (3H, s, C-29), 1.07 (3H, s, C-28), 1.25 (3H, s, C-27), 0.78 (3H, s, C-26), 0.98 (3H, s, C-25), 3.45 (1H, d, $J = 11.1$ Hz, C-24), 4.10 (1H, d, $J = 11.4$ Hz, C-24), 1.22 (3H, s, C-23), 3.27 (1H, d, $J_1 = 4.0$ Hz, H_{eq}-19), 2.92 (br s, $W_{1/2} = 4.4$ Hz, H-18), 5.32 (1H, t, $J = 3.5$ Hz, H-12), 3.07 (1H, d, $J = 9.2$ Hz, H_{ax}-3), 3.81 (1H, ddd, $J_1 = 13.9$ Hz, $J_2 = 9.2$ Hz, $J_3 = 4.4$ Hz, H_{ax}-2). – ^{13}C NMR (100 MHz, $CDCl_3$+CD_3OD) $\delta = 182.3$ (C-30), 142.7 (C-13), 123.8 (C-12), 84.7 (C-3), 81.2 (C-19), 68.3 (C-2), 65.1 (C-24), 55.5 (C-5), 47.6 (C-9), 45.4 (C-1), 44.8 (C-20), 43.4 (C-18), 42.7 (C-4), 41.2 (C-14), 39.2 (C-8), 37.7 (C-10), 34.4 (C-22), 32.8 (C-7), 32.4 (C-17), 29.4 (C-21), 27.7 (C-15), 27.6 (C-28), 27.1 (C-16), 24.3 (C-27), 24.1 (C-29), 23.6 (C-11), 22.6 (C-23), 18.2 (C-6), 16.5 (C-26), 16.4 (C-25).

Assay of β-glucuronidase inhibition

β-Glucuronidase activity was measured using *p*-nitrophenyl-β-D-glucuronide as substrate at 37 °C. The enzyme mixture (total volume of 0.250 ml) contained 185 μl of acetate buffer (pH 7.0), 10 μl enzyme, and 5 μl of inhibitor (or DMSO in control). The reaction was initiated by the addition of 50 μl of *p*-nitrophenyl-β-D-glucuronide, the absorbance was measured continuously at 405 nm for 30 min. Glucosaccharo-(1:4)-lactone was used as a standard inhibitor. The inhibitory activity (%) was calculated according to the following formula: [(E-S)/E·100] where "E" is the activity of enzyme without test material and "S" is the activity of enzyme with test material.

Acknowledgement

One of us (F. N. Ngounou), gratefully acknowledges the financial support of the Third World Academy of Sciences (Trieste, Italy).

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