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A New Triterpenoid from *Terminalia glaucescens* (Planch. ex Benth.)

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Abstract: Phytochemical investigation of the root extract of T*erminalia glaucescens* afforded three known compounds, ellagic acid **1,** arjungenin **2**, hypatic acid **3** and and a new triterpenoid named glaucescic acid, 2α,3α,6α,23-tetrahydroxyolean-12-en-28-oic acid **4**. The structure and relative configuration of this new compound was elucidated on the basis of spectroscopic data, especially 2D NMR techniques.

Keywords: *Terminalia glaucescens*; *Combretaceae*; 2,3,8-tri-*O*-methylellagic acid; 2α,3α,6α,23-tetrahydroxyolean-12-en-28-oic acid.

1. Introduction

The genus *Terminalia* (Combretaceae), consisting of over 100 species of trees and shrubs, is widely distributed in the savanna woodland of Asia and Africa [1]. *Terminalia glaucescens* (Planckon) is a deciduous, multipurpose perennial tree that grows across Africa especially South-West Nigeria. The leaves extract of this plant is used in medicinal preparations for the treatment of AIDS, amenorrhoea, scrofulous infections, syphilis, sores and nervous diseases [2-3]. Ethanol extract of the leaves is also used as antiplasmodial, antiparasitic, antiviral and antimicrobial [3-8]. *Terminalia* plants are known to contain several acidic triterpenes, some of which showed analgesic, immunosuppresant, hepatoprotective and antimibrobial activities. β- amyrin, glaucinoic acid, arjunic acid, sericoside, ursane and lupane-type pentacyclic terpenoids such as kanetic acid were among the isolated compounds from this genus [8-14].

In this paper, we present the isolation and structure elucidation of a new triterpenoid, named glaucescic acid (2α,3α,6α,23-tetrahydroxyolean-2-en-28-oic acid) **4**, two known triterpenoids; arjungenin **2** and hypatic acid **3** and an ellagic acid, 2,3,8-tri-*O*-methylellagic acid **1**. However, compounds **1** and **3** are being reported for the first time from the roots of *Terminalia glaucescens.*

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2. Materials and Methods

2.1. Plant material

The roots of *Terminalia glaucescens* were collected from a farmland in Igbo-Otin, Otan Ayegbaju, Osun state, Nigeria and identified by Professor Faluyi of Department of Botany, Obafemi Awolowo University, Ile-Ife. The sample was authenticated at Forestry Reseach Institute of Nigeria (FRIN), Ibadan, Nigeria by Mr O. A. Micheal where a voucher specimen (FHI 108334) was deposited.

2.2. Extraction and isolation

The dried, ground roots of *T. glaucescens* (3.5 kg) were exhaustively extracted using soxhlet apparatus with n-Hx, EtOAc and MeOH successively. The extracts were concentrated in vacuo to give yellow solid (24.8 g), yellow solid (28.8 g) and brown solid (354 g) respectively for n-Hx, EtOAc and MeOH. The MeOH extract (100 g) was further partitioned between H_2O, MeOH and $CHCl_3$ (1: 1: 1) to give the $CHCl_3$ extract. The $CHCl_3$ extract (5 g) was chromatographed on a silica gel column (70-230 mesh) eluting with increasing gradient of n-Hx - EtOAc and EtOAc - MeOH to give seventy 100 mL fractions. Similar fractions as determined by TLC were pooled together giving 10 combined fractions. Fraction 3 eluted with EtOAc /n-Hx (1:1) afforded **1** (20 mg) purified by recystallization in Hx. Fraction 7 eluted with 100% EtOAc was subjected to further purification by preparative RP HPLC (C-18 column, 5 µm particle size, flow rate of 40.0 mL min^{-1}) with an isocratic elution of 70% MeOH/ H_2O over 20 min to give **2** (12 mg, t_R = 3.1 min); **3** (7 mg, t_R =4.1 min) and **4** (20 mg, t_R = 5.2 min).

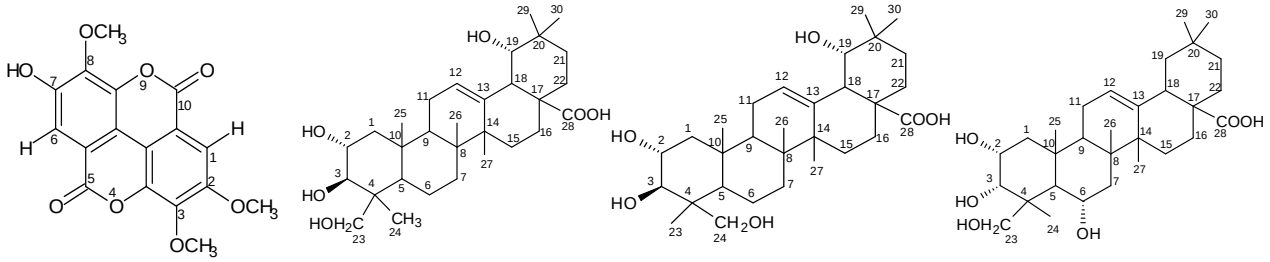

Figure 1. The structures of compounds **1**-**4**.

3. Results and Discussion

Compound **4** was obtained as white solid which was recrystallised in methanol to give white powdery solid [decomp. 283-285 °C; $[\alpha]^D$: +31.5 (*c* 0.02, CH_3OH)] . The negative ion ESIMS of **4** showed the molecular ion at *m/z* 503.3 $[M-H]^-$, corresponding to the formula $C_{30}H_{48}O_6$, and indicated 7 degrees of unsaturation. The IR spectrum displayed the absorptions for OH (3454 cm^{-1}), C = C (1637 cm^{-1}) and C = O (1691 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1) of **4** revealed 30 carbon signals, which were deduced by DEPT NMR as six methyl groups, eight methylene groups, three methines, six quaternary carbons, one oxymethylene, three secondary alcohols, one carboxylic acid and two olefinic carbons. The detailed analysis of the ^1H NMR spectrum showed the presence of oleanane-type triterpenoid skeleton. The tertiary methyl groups appeared at δ 1.07, 1.10, 1.39, 1.15, 0.95 and 0.91 (3H each, s, CH_3-24, CH_3-25, CH_3-26, CH_3-27, CH_3-29 and CH_3-30 respectively) while the oxymethine protons, (H-2, H-3 and H-6) were seen at δ 3.73, 3.31 and 4.39. Two protons of a primary alcohol (δ 3.59 and 3.44) (1H each, d, J=11.1 Hz, H-23a and H-23b) were also observed in the proton spectrum. The equatorial disposition of H-2 and H-3 was deduced from W1/2= 8.0 and 7.8 Hz

respectively and also by the interaction between H-2//H-3 and H-6 in the NOESY spectrum. In the HMBC spectrum, the oxymethine proton at δ 3.31(H-3) showed $^{2}J_{CH}$ and $^{3}J_{CH}$ interactions with C-2 (δ 69.9), C-4 (δ 45.0), C-23 (δ 66.2) and C-24 (δ 15.3) while the alcoholic methine carbon at δ 78.5 (C-3) showed $^{2}J_{CH}$ and $^{3}J_{CH}$ interactions with H-1 (δ 0.87, 1.92); H-2 (δ 3.73), H-5 (δ 1.29), H-23a,b (δ 3.44, 3.59) and H-24 (δ 1.07).

Table 1. $^{1}H^{a}$ NMR, $^{13}C^{b}$ NMR and HMBC Assignment of Compound 4 in DMSO

Position	δH (mult.)	J (Hz)	δC	HMBC (δH/C)
1a	0.87, br t	12	50.3	C-2, 3, 9, 10, 25
b	1.92, m			
2	3.73, ddd	5,10,12	69.9	C-3
3	3.31, d	10	78.5	C- 2, 4, 23, 24
4	_		45.0	_
5	1.29		49.1	C- 4, 6, 10, 24, 25
6	4.39 br s		68.7	C-10
7a	1.50, m		41.3	C-6, 8, 9
b	1.78, br t	12		
8	_		40.0	_
9	1.30		49.3	_
10	_		38.8	_
11a	1.89, m		24.7	C- 12,13
b	2.10, m			
12	5.30, t		123.9	C- 9, 11, 18
13	_		144.9	_
14	_		43.7	_
15a	1.80		29.0	C-8
b	1.62			
16a	1.58		24.3	_
b	1.90			
17	_		47.9	_
18	2.90, dd		43.0	_
19a	1.13, m		47.4	C-12, 13,17, 28
b	1.75,m			
20	_		31.8	_
21a	1.21, m		35.1	_
b	1. 36			
22a	1.55		34.0	C-16, 18, 20,28
b	1.75			
23a	3.59		66.2	C- 3, 4, 5,24
b	3.44			
24	1.07 (s)		15.3	C- 3, 4, 5, 23
25	1.10 (s)		19.2	C- 9,10
26	1.39(s)		19.6	C-8, 9, 14
27	1.15 (s)		26.6	C-8, 13,14,15
28	_		182.0	_
29	0.95 (s)		24.1	C- 19,20,30
30	0.91 (s)		33.7	C- 19, 20, 21, 29

[a]500 MHz; [b]125 MHz

The position of the C-23 hydroxyl group was established from the HMBC data, in which the H-23b proton at δ 3.44 showed long range correlations with C-3 (δ 78.5), C-4 (δ 45.0), C-3 (δ 78.5), C-5 (δ 49.1) and C-24 (δ 15.3); while the C-24 methyl protons at δ 1.07 showed similar correlations with C-3 (δ 78.5), C-4 (δ 45.0), C-3 (δ 78.5), C-5 (δ 49.1) and C-23 (δ 66.2).The presence of the double bond at C-12 was confirmed by the chemical shifts of C-12 (δ 123.9) and C-13 (δ 144.9), characteristic of a

Δ^{12} moiety [16]. These correlations were in agreement with the proposed structure for **4**. The ^{1}H and ^{13}C-NMR chemical shift assignments of compound **4** were based on the ^{1}H-^{1}H – COSY, HMQC, HMBC and NOESY spectra. The structure was therefore established to be 2α,3α,6α,23-tetrahydroxyolean-12-en-28-oic acid and named glaucescic acid. The two other triterpenoids isolated were identified as 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oic acid (arjungenin) **2** and 2α,3β,19α,24-tetrahydroxyolean-12-en-28-oic acid (hypatic acid) **3** by comparison with spectroscopic data in the literature [15-16].

Ellagic acid **1**, was obtained as white crystals. The molecular formula, $C_{17}H_{12}O_8$ and structure were determined by a combination of high resolution ESIMS, ^{1}H and ^{13}C NMR spectra. The spectra of compound **1** were compared with the existing literature and it was concluded to be ellagic acid [9-13].

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] R. Mangoyi, W. Mafukidze, K. Marobela and S. Mukanganyama (2012). Antifungal activities and preliminary phytochemical investigation of *Combretum* species from Zimbabwe, *J. Microbial Biochem Technol*. **4**, 037-044.

[2] J. Koudou, G. Roblot and R. Wylde (1995). Tannins constituents of *Terminalia glaucescens*, *Planta med*, **61**, 490-491.

[3] T. Okpekon, S. Yolou, C. Gleye, F. Roblot, P. Loiseau, C. Bories, P. Grellier, F. Frappier, A. Laurens and R. Hocquemiller (2004). Antiparasitic activities of medicinal plants used in Ivory Coast, *J. Ethnophamcol*. **90**, 91-97.

[4] R. Nema, P. jain, S. Khare, A. Pradhan, A. Gupta and D. Singh (2012). Antibacterial and antifungal activity of *Terminalia Arjuna* leaves extract with special reference to flavonoids, *Bas. Res. J. Med. Clin Sci.* **1**, 63-65.

[5] G. Kumar, R. K. Pundir and S. Rana (2013). Evaluation of antimicrobial activity and phytochemical analysis of antidiabetic plant *Terminalia chebula*, *Int. J. Trad. Nat. M ed.* **2**, 9-20.

[6] N. F. Mushi, Z. H. Mbwambo, E. Innocent and S. Tewtrakul (2012). Antibacterial, anti HIV-1 protease and cytotoxic activities of aqueous ethanolic extracts from *Combretum adenogonium* Steud. Ex A. Rich (Combretaceae), *BMC Complement Altern Med*. **12**, 163.

[7] S. Agrawal, G. T. Kulkarni and V. N. Sharma (2012). Antimicrobial and Anti-inflammatory activities of bark of four plant species from Indian origin, *Pharm. Sci*. **3**, WMC002010.

[8] A. Mann, K. Ibrahim, A. O. Oyewale, J. O. Amupitan, M. O. Fatope and J. I. Okogun (2012). Isolation and elucidation of three triterpenoids and its antimycobacterial activity of *Terminalia Avicennioides*, *Am. J. Org. Chem*. **2**, 14-20.

[9] D. D. Khac, S. Tran-Van, A. M. Campos, J. Lallemand and M. Fetizon (1990). Ellagic compounds from *Diplopanax stachyanthus*, *Phytochem*. **29**, 251-256.

[10] W. E. Hillis and Y. Yazaki (1973). Properties of some methylellagic acids and their glycosides, *Phytochemistry*. **12**, 2963-2968.

[11] M. J. Faruk, N. Nahur, M. A. Aziz, N. Mosihuzzaman and M. A. Rashid (2002). Two new ellagic acids from *Lagerstroemia speciosa* Linn. plant, *J. Bangladesh Chem Soc.* **15**, 73-78.

[12] T. Hayashi, H. Murayana, R. Kasai, K. Hattori, S. Takasuga, O. Hazeki, K. Yamasaki and T. Tanaka (2002). Ellagitannins from *Lagerstroemia speciosa* as activators of glucose transport in fat cells, *Planta Med*. **68**, 173-175.

[13] G. I. Ndukwe and Y. Zhao (2007). Pharmacological activity of 2,3,8-tri–O-methylellagic acid isolated from the stem bank of *Irvingia gabonensis*, *Afr. J. Biotec.* **6**, 1910-1912.

[14] A. T. Tchinda, A. Tsopmo, M. Tene, P. Kamning, D. Ngnokam, P. Tane, J. F Ayafor, J. D. Connolly and L. J. Farrugia (2003). Diterpenoids from *Neoboutonia glabrescens*. *Phytochemistry*. **64,** 575- 581.
[15] S. B. Mahato and A. P Kundu (1994). ^{13}C NMR Spectra of pentacyclic triterpenoids - A Compilation and some salient features, *Phytochemistry.* **37,** 1517-1575.
[16] D. V Singh, M. M Gupta, A. K Tripathi, V. Prajapati and S. Kumar (2004). Arjunetin from *Terminalia arjuna* as an insect feeding-deterrent and growth inhibitor, *Phytother. Res*. **18**, 131-134.