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TWO TRITERPENE ESTERS FROM *TERMINALIA MACROPTERA* BARK

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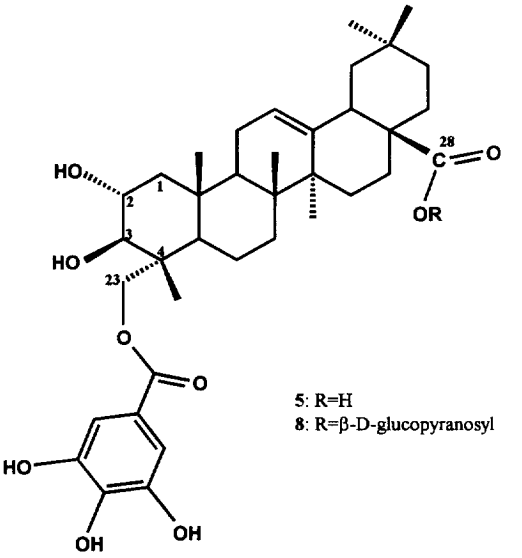
Key Word Index—*Terminalia macroptera*; Combretaceae; 23-galloylarjunolic acid; 23-galloylarjunolic acid 28-O-β-D-glucopyranosyl ester; triterpene; triterpene glucoside; antimicrobial activity; anthelminthic activity.

Abstract—Investigation of the bark from *Terminalia macroptera* led to the isolation of two novel esterified triterpenes, identified as 23-galloylarjunolic acid and its β-D-glucopyranosyl ester. In addition terminolic acid, arjunic acid, arjungenin, arjunglucoside **1**, sericic acid and sericoside were isolated and tested in a number of bioassays (antibacterial, antifungal and anthelminthic activity as well as haemolytic property). © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Terminalia macroptera L. is a tree, up to 13 m high, which occurs widely in Africa. In traditional african folk medicine it is used for the treatment of various diseases and ailments [1]. Four C- glycosyl flavones, orientine, isorientine, vitexine and isovitexine have been isolated from the flowers [2, 3]. However, to our knowledge, the bark has not been investigated until now. In this paper we report on the isolation and structure elucidation of a novel type of esterified triterpene, 23-galloylarjunolic acid (**5**), and its β-D-glucopyranosyl ester, 23-galloylarjunolic acid 28-O-β-D-glucopyranosyl ester (**8**) from the bark of *T. macroptera*. In addition, terminolic acid, arjunic acid, arjungenin, arjunglucoside 1, sericic acid and sericoside were isolated and identified. We report also on the antibacterial, antifungal, haemolytic and anthelminthic properties of the isolated compounds.



5: R=H
8: R=β-D-glucopyranosyl

RESULTS AND DISCUSSION

Four triterpenes (**1–4**) and 23-galloylarjunolic acid (**5**) were isolated from the ethyl acetate extract of the bark. The triterpene glucosides (**6–8**) were obtained from the chloroform-soluble fraction of the methanol extract. Triterpenes (**1–4, 6,7**) were identified by comparison of their spectral data with those reported in the literature for arjungenin (**1**), sericic acid (**2**), arjunic acid (**3**), terminolic acid (**4**), arjunglucoside 1 (**6**) and sericoside (**7**) [4–9].

The M_rs of **5** and **8** were determined by their positive and negative ESI-mass spectra. The negative ESI-mass spectra of **5** and **8** showed their $[M-H]^-$ ions at *m/z* 639.4 and 801.6, respectively. The positive ESI-mass spectra exhibited their $[M+Na]^+$ ions at *m/z* 663.1 and 825.3 and their $[M+K]^+$ ions at *m/z* 679.3 and 841.2, respectively.

The ^{13}C- and DEPT NMR spectra of **5** displayed six quarternary carbons, nine methylene, three methine

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and six methyl groups in the region 10–50 ppm, one primary and two secondary alcoholic groups in the region 60–80 ppm, a trisubstituted double bond and a carboxyl carbon. Thus the skeleton of the aglycone of **5** proved to be that of a typical Δ^{12}-oleane. Furthermore, the ^{13}C NMR spectrum of **5** exhibited an additional ester carbon and four carbons of a tetrasubstituted aromatic residue with two protons appearing in the ^{1}H NMR spectrum as a singlet at δ 7.88. HMBC correlations of the these protons to all aromatic carbons, inclusive to the additional ester carbon and comparison of the ^{13}C NMR data with those reported in literature for galloyl esters proved the presence of a galloyl unit in compound **5** [10].

The positions of the alcoholic functions and the galloyl unit were determined by ^{1}H NMR,^{1}H- ^{1}H-DQF COSY-, and HMBC NMR as follows: the secondary alcoholic proton H-3 at δ 3.97 (*d*, $J=8.86$ Hz) showed only one coupling to the secondary alcoholic proton H-2 at δ 4.19 in the ^{1}H-^{1}H- DQF COSY spectrum, whereas H-2 exhibited two more couplings to the methylene protons H-1α/β. The HMBC- spectrum of **5** clearly indicated correlations of the protons of the primary alcoholic function (C-23) to C-3, C-4 and C-5 of the aglycone and to the ester carbon C-7 of the galloyl unit. The structure of **5** was confirmed by a NOESY experiment to be 2α,3β-dihydroxy-23-galloyloxy-olean-12-en-28-oic acid (23-galloylarjunolic acid). The triterpene part of **5** was identified as arjunolic acid by comparison of its NMR data with reported data [11].

The ^{13}C- and ^{1}H-NMR data of compound **8** were closely related to the spectral data of **5** up to a sugar moiety in **8**. The ^{1}H NMR spectrum of **8** showed one anomeric proton signal at δ 6.28 as a doublet ($J=8.18$ Hz) indicating the β-linkage with the aglycone. The sugar moiety was determined to be β-D-glucopyranosyl by ^{1}H NMR, ^{1}H-^{1}H-TOCSY NMR and comparison of the ^{13}C data with literature values [8, 9]. The upfield shift of the ^{13}C signal of C-28 and the HMBC correlation of the anomeric proton to the carboxyl carbon C-28 indicated the C-28 ester linkage between the 23-galloyl-triterpene aglycone and the β-D-glucopyranosyl unit. Thus the structure of **8** was proved to be β-D-glucopyranosyl-2α,3β-dihydroxy-23-galloyloxy-olean-12-en-28-oate (23-galloylarjunolic acid 28-O-β-D-glucopyranosyl ester).

All isolated compounds **1-8** were tested for antifungal, antibacterial and haemolytic properties by direct bioautographic TLC assay as described in the experimental section. The results are summarized in Table 2. The assay for anthelminthic activity was carried out according to K. G. Simpkin and G. C. Coles [12]. The results are shown in Table 2.

EXPERIMENTAL

General.

IR: KBr disc and $CHCl_3$ film; 1D- and 2D homo- and heteronuclear NMR (VARIAN UNITY INOVA-500): 500 MHz and 75.42 MHz (^{13}C) in pyridine-d_5; ESI-MS (Finnigan PSQ 700): direct flow injection of sample dissolved in MeOH (5 μl min^{-1}) for negative ionization (3.5 kV) and positive ionization (4.5 kV); EI MS (Varian–MAT 311A): 70 eV; TLC: silica gel 60 F_{254} plates (Merck) with $CHCl_3$-MeOH (9:1 and 4:1) as solvent system. Active compounds on TLC plates were visualized by direct bioautographic TLC assay after spraying with bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*)- and a fungus (*Cladosporium cucumerinum*) [13, 14].

Plant material.

The plant was collected at Wakwa, Cameroon in 1995 and identified by Dr S. Yonkeu, Chief of the herbar at the Institute de Recherche Zoo Veterinaire, Wakwa, Cameroon.

Extraction.

The air dried powdered bark (2.5 kg) of *Terminalia macroptera* was successively extracted with petrol (30–60°) (7 g), EtOAc (53.8 g) and MeOH (702 g). A portion (300 g) of the MeOH extract was dissolved in H_2O and extracted three times with *n*-BuOH. The combined organic layers and the aq layer were each evaporated to dryness at 35°. The *n*-BuOH extract (52 g) was partitioned between $CHCl_3$, MeOH and H_2O (1.5:1:1). The aq layer was extracted six times with the organic phase. The combined $CHCl_3$ layers and the aq phase were each concentrated under reduced pressure.

Isolation and purification of compounds **1–5**.

Four aliquots (each 10 g) of the EtOAc extract were chromatographed on a Sephadex LH-20 column with MeOH as eluant to give seven fractions A1–A7 with different biological activities in the bioautographic TLC assays with *B. subtilis*, *P. fluorescens* and *C. cucumerinum* in test organisms. An aliquot (1 g) of the most active fraction, A2 (11.2 g), was subjected to Sephadex LH-20 column (MeOH) once more and gave ten fractions B1-B10.

Fraction B5, B6 and B8 were further purified by HPLC. HPLC of fraction B6 (150 mg) on RP18 LiChrospher 100 (250 mm × 16 mm; 46% EtOH/0.05% TFA; 5 ml min^{-1}; UV detection $\lambda=220$ nm) gave arjungenin (**1**, 41.4 mg), sericic acid (**2**, 25.8 mg) and terminolic acid (**4**, 7 mg). Fraction B5 (78.5 mg) (80% MeOH/0.05% TFA) gave arjunic acid (**3**, 14 mg) and fraction B8 (78 mg) (60% MeOH/0.05% TFA) 23-galloylarjunolic acid (**5**, 18.5 mg).

Isolation and purification of compounds **6–8**.

The $CHCl_3$ extract (2 × 5.5 g) was chromatographed on a Sephadex LH-20 column with MeOH as eluant to afford seven fractions C1-C7. The fraction of highest

biological activity in the direct bioautographic TLC assay (C 2) was purified by MPLC on RP18 (2 × 2.5 g). Successive elution with 35%, 50%, 70% and 90% MeOH and 0.01% TFA gave nine fractions D1–D9. HPLC of fraction D5 (856 mg) on RP18 LiChrospher 100 (250 mm × 25 mm; gradient: 58% to 65% MeOH/0.01%TFA in 35 mins; 5 ml min^{-1}; UV detection $\lambda = 220$ nm) yielded three fractions E1–E3, which were finally purified by HPLC on RP18- or CN LiChrospher 100 (250 mm × 16 mm; 5 ml min^{-1}; UV detection $\lambda = 220$ nm). HPLC of fraction E1 (179.3 mg) gave arjunglucoside 1 (**6**, 107.6 mg) (RP18; 45% MeOH/0.01%TFA), fraction E2 (327.6 mg) yielded sericoside (**7**, 68 mg) (CN; 10% MeOH/0.01%TFA) and fraction E3 (74 mg) gave 23-galloylarjunolic acid 28-O- β-D- glucopyranosyl ester (**8**, 48 mg) (RP18; 48% MeOH/0.01%TFA).

Identification of known compounds.

Compounds **1–4**, **6** and **7** were identified by comparison of their ^{1}H- and ^{13}C NMR data and their EI- or ESI MS spectra with those reported in literature for arjungenin (**1**), sericic acid (**2**), arjunic acid (**3**), terminolic acid (**4**), arjunglucoside 1 (**6**) and sericoside (**7**) [4, 5, 6, 7, 8, 9].

23-*Galloylarjunolic acid* (**5**).

Yellowish powder, $[\alpha]^{25}$ (λ, nm): −6° (589), +4° (546), +14° (436), 0° (365) (c = 0.05, MeOH). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2924, 1695, 1683, 1202; UV: λ_{max}^{MeOH} nm (log ϵ): 218 (4.16), 278 (3.74); positive ESI MS *m/z*: 663.1 $[M+Na]^+$, 679.3 $[M+K]^+$; negative ESI MS *m/z*: 639.4 $[M-H]^-$; ^{1}H NMR (500 MHz, pyridine-d_5,): δ 7.88 (2H, *s*, H-2, H-6 of Ga), 5.42 (1H, *t*, *J* = 3.54 Hz, H-12), 4.65 (1H, *d*, *J* = 11.11 Hz, H-23), 4.52 (1H, *d*, *J* = 11.11 Hz, H-23), 4.19 (1H, *m*, H-2), 3.97 (1H, *d*, *J* = 8.86 Hz, H-3), 3.24 (1H, *dd*, *J* = 4.64, 14.16 Hz, H-18), 2.21 (1H, *dd*, *J* = 4.09, 12.57 Hz, H-1β), 1.15, 1.02, 0.98, 0.97, 0.91 (3H × 6, each *s*, *tert*-Me × 6); ^{13}C NMR: Table 1.

23-*Galloylarjunolic acid 28-O-β-D-glucopyranosyl ester* (**8**).

Yellowish powder, $[\alpha]^{25}$ (λ, nm): −22° (589), −16° (546), −14° (436), −36° (365) (c = 0.05, MeOH). IR: ν_{max}^{MeOH} cm^{-1}: 3417, 2944, 1694, 1614, 1349, 1235, 1032; UV: λ_{max}^{MeOH} nm (log ϵ): 220 (4.06), 278 (3.66); positive ESI MS *m/z*: 825.3 $[M+Na]^+$, 641.1 $[M-Glc+H]^+$, 444.3 $[M-Glc-CO_2-Ga+H]^+$; negative ESI MS *m/z*: 801.6 $[M-H]^-$; ^{1}H NMR (500 MHz, pyridine-d_5,): δ 7.85 (2H, *s*, H-2, H-6 of Ga), 5.38 (1H, *t*, *J* = 3.66 Hz, H-12), 4.67 (1H, *d*, *J* = 11.11 Hz, H-23), 4.52 (1H, *d*, *J* = 10.99 Hz, H-23), 4.19 (1H, *dt*, *J* = 4.39, 9.52, 11.23 Hz, H-2), 3.94 (1H, *d*, *J* = 9.28 Hz, H-3), 3.14 (1H, *dd*, *J* = 4.4, 13.68 Hz, H-18), 2.21 (1H, *dd*, *J* = 4.28, 12.58 Hz, H-1β), 1.19 (1H, *t*, *J* = 12.09 Hz, H-1α), 1.18, 1.07, 1.04, 1.01, 0.87, 0.85 (3H × 6, each *s*, *tert*-Me × 6), 6.28 (1H, *d*, *J* = 8.18 Hz, H-1 of Glc), 4.17 (1H, *t*, *J* = 8.67 Hz, H-2 of Glc), 4.27 (1H, *t*, *J* = 8.79 Hz, H-3 of Glc), 4.33 (1H, *t*, *J* = 9.28 Hz, H-4 of Glc), 4.02 (1H, *ddd*, *J* = 2.45, 4.4, 9.4 Hz, H-5 of Glc), 4.45 (1H, *dd*, *J* = 2.44, 11.97 Hz, H-6 of Glc), 4.37 (1H, *dd*, *J* = 4.63, 12.08 Hz, H-6 of Glc); ^{13}C NMR: Table 1.

Table 1. ^{13}C NMR data of 23-galloylarjunolic acid (**5**) and 23-galloylarjunolic acid 28-O-β-D-glucopyranosyl ester (**8**) in pyridine-d_5 (75.42 MHz).

C	**5**	**8**
1	47.7	47.8
2	68.3	68.3
3	77.6	77.6
4	43.2	43.2
5	48.7	48.9
6	18.6	18.8
7	32.9	32.9
8	39.7	39.9
9	48.5	48.5
10	38.1	38.2
11	23.8	23.9
12	122.1	122.2
13	144.8	144.2
14	42.1	42.1
15	28.0	28.0
16	23.6	23.4
17	46.6	47.0
18	42.0	41.8
19	46.3	46.2
20	30.8	30.7
21	34.1	34.0
22	33.1	32.5
23	66.7	67.0
24	13.9	14.0
25	17.4	17.3
26	17.1	17.6
27	25.8	25.9
28	180.1	176.5
29	33.2	33.1
30	23.7	23.6
Ga1*	121.4	121.5
Ga2/Ga6	110.0	110.0
Ga3/Ga5	147.6	147.5
Ga4	140.9	140.7
Ga7	167.0	167.1
Glc1†		95.7
Glc2		74.0
Glc3		78.8
Glc4		71.0
Glc5		79.3
Glc6		62.2

*Ga. Gallic acid; †Glc. Glucose

Antifungal, antibacterial and haemolytic activity.

The antibacterial activities of compounds **1–8** were examined by direct bioautographic TLC assay with *B. subtilis* and *P. fluorescens* as test organisms [13].

Table 2. Biological activities of compound **1–8**.

Test organism		MIC (μg)*							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Bacillus subtilis		5	5	2.5	5	5	≫40	≫40	2.5
Pseudomonas fluorescens		5	5	2.5	2.5	2.5	≫40	≫40	10
Cladosporium cucumerinum		10	5	10	20	20	≫40	≫40	≫40
Haemolytic activity		0.6	1.2	0.15	0.3	2.5	20	20	5
Caenorhabditis elegans	**1–5**: 50 μg/ml **6–8**: 100 μg/ml	—†	—†	—†	—†	+++†	—†	—†	+++†

*: Minimum inhibitory concentration in the direct bioautographic TLC assay.

†: +++, very active (no increase in number of worms, no movement); ++, active (no increase in number, worms move very slowly); +, active (increase in number less then 20%, worms move slowly); —, not active.

The antifungal properties of compounds **1–8** were observed in a similar direct bioautographic TLC assay using *C. cucumerinum* as test fungus [14]. Haemolytic activity was examined in a modified direct bioautographic TLC assay: Fresh pig's blood in PBS-buffer (PBS buffer-pig's blood-Nacitrate sol 150:9:1) was sprayed onto analytical TLC plates, prepared with serial dilutions of all isolated compounds. Agglutination zones showed the haemolytic activity, and the minimal amount in μg was thus established.

Assay for anthelmintic activity.

The nematode *Caenorhabditis elegans* was used as test organism. The test was carried out according to Simpkin and Coles [12]. A suspension of worms was treated with the isolated compounds and after a seven day incubation period assessed for the increase in number of worms and their movement. Compounds are considered very active (+++, no increase in number, no movement), active (++, no increase in number, worms move very slowly; +, increase in number less then 20%, worms move slowly) or not active (−) by comparing them with controls.

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