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Saponins and a lignan derivative of *Terminalia tropophylla* from the Madagascar Dry Forest†

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Abstract

A study of an EtOH extract obtained from the roots of the Madagascan plant *Terminalia tropophylla* H. Perrier (Combretaceae) led to the isolation of the new oleanane-type triterpenoid saponin **1**, the new lignan derivative **2**, and the two known saponins arjunglucoside I (**3**) and sericoside (**4**). The structures of the new compounds **1** (terminaliaside A) and **2** (4'-O-cinnamoyl cleomiscosin A) were elucidated using 1D and 2D NMR experiments and mass spectrometry. Compound **1** showed antiproliferative activity against the A2780 human ovarian cancer cell line with an IC₅₀ value of 1.2 μM.

Keywords

Terminalia tropophylla; Combretaceae; saponins; lignan; NMR; antiproliferative activity

1. Introduction

In our continuing search for bioactive molecules from the Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, we obtained an extract of the roots of *Terminalia tropophylla* (Perrier) (Combretaceae). This extract, designated MG 4035, showed activity against the A2780 ovarian cancer cell line, with an IC₅₀ value 11 μg/mL. The extract was selected for bioassay-guided fractionation based on the above activity.

Previous phytochemical studies have revealed the genus *Terminalia* to be a rich source of secondary metabolites, such as lignans (Valsaraj et al., 1997), flavonoids (Srivastava et al., 1999), terpenoids (Conrad et al., 1998, 2001), and tannins (Kandil and Nassar, 1998; Conrad et al., 2001). Some of these metabolites have shown a wide range of biological activities,

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including antimalarial (Valsaraj et al., 1997), antifungal (Valsaraj et al., 1997; Conrad et al., 1998, 2001), antibacterial (Conrad et al. 1998, 2001; Eldeen et al., 2006), and cytotoxic (Kandil and Nassar, 1998; Conrad et al., 2001) activities. The anthelmintic and haemolytic properties of terpene esters from *T. macroptera* have been studied (Conrad et al., 1998). Tannins from the same plant showed bacteriostatic activity, and the hydrolysable tannin isoterchebulin showed moderate nematocidal and piscicidal activity (Conrad et al., 2001). The antioxidant effects of an aqueous extract of *T. chebula* have also been investigated (Lee et al., 2005).

In this paper, we report the isolation, structure elucidation, and antiproliferative activities against A2780 cells of three triterpenoid saponins (**1**, **3** and **4**) and one lignan derivative (**2**) obtained from the roots of *Terminalia tropophylla*.

2. Results and Discussion

Liquid-liquid partitioning of a portion of an EtOH extract (2.2 g) of the roots of *Terminalia tropophylla* into hexane, CH₂Cl₂ and aqueous MeOH fractions indicated that the aqueous MeOH fraction (1.7 g) was the most active fraction, with an IC₅₀ value of 10 µg/mL against A2780. Purification of this fraction using a C₁₈ open column, followed by preparative HPLC on a C₁₈ column led to the isolation of compounds **1**, **3**, and **4**. Compound **2** was purified from the CH₂Cl₂ fraction after repeated chromatography on silica gel.

Compound **1** was obtained as a white solid. Its HRFABMS (positive-ion mode) exhibited a quasimolecular ion peak at *m/z* 1079.5376, consistent with a molecular composition of C₅₃H₈₄O₂₁Na. The aglycone of **1** was identified as an oleanane triterpenoid by analysis of its ¹H and ¹³C NMR spectra (Table 1) and from observation of connectivities in the ¹H-¹H COSY, TOCSY, ROESY, HSQC, and HMBC NMR spectra. The NMR spectra indicated the presence of one trisubstituted double bond (12-position), one ketone (16-position), three oxygenated methines (3-, 21-, and 22-positions), and one oxygenated methylene (28-position) in the aglycone of **1**. The signals for H₃-23 (δ_H 1.06, s) and H₃-24 (δ_H 0.86, s) showed ³J HMBC correlations to C-3 (δ_C 90.8) (Figure 1). Of the seven methyl groups in the aglycone, only H₃-27 (δ_H 1.22, s) exhibited a ³J HMBC correlation to C-13 (δ_C 140.6), which confirmed the location of the double bond at the 12-position. H₂-28 (δ_H 4.18, d, *J* = 9.6 Hz; 4.01, d, *J* = 9.6 Hz) exhibited ³J HMBC correlations to C-16 (δ_C 216.8) and C-22 (δ_C 77.1), while H₃-29 (δ_H 0.95, s) and H₃-30 (δ_H 0.88, s) correlated to C-21 (δ_C 77.7). ROESY correlations between H₃-23 and H-3 (δ_H 3.24), and H₃-24 and H₃-25 (δ_H 1.01, s) revealed that H-3 has an α-axial orientation. H-21 (δ_H 3.66, d, *J* = 9.3 Hz) was also determined as α-axial due to its coupling constant, which was confirmed by a ROESY correlation between H₃-29 and H-21 (Figure 2). In turn, H-22 (δ_H 3.56, dd, *J* = 9.3, 2.8 Hz) of **1** was assigned as β-axial due to its coupling constant and ROESY correlations to H-18 (δ_H 2.79, dd, *J* = 13.8, 3.0 Hz) and H₃-30. Both H-18 and H-22 showed ROESY correlations to H₂-28, which further confirmed the relative configurations of the E ring of the aglycone. The aglycone of compound **1** has not been reported before hence the ¹³C NMR chemical shifts of the aglycone were compared with those of similar known aglycons. The ¹³C NMR chemical shifts of the aglycone (A-B-C-D-E: chair-chair-boat-boat-chair) of **1** matched those of the carbons in rings A, B, C, and E of marsglobiferin (Qiu et al., 1993), and those of the carbons in ring D of 3-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-3β,22α-dihydroxyolean-12-en-16-one (De Leo et al., 2007).

Analysis of the ¹H, ¹³C NMR, and HSQC spectra of **1** indicated the presence of three sugar units and an additional α,β-unsaturated ester unit. The anomeric proton of one sugar, defined as the first sugar here, showed a ³J HMBC correlation to C-3 (δ_C 90.8). The spin system from the anomeric proton (H-1', δ_H 4.43, d, *J* = 8.0 Hz) to the other protons of this sugar was clearly exhibited in a 1D TOCSY spectrum [H-1' (selected): δ_H 4.43, d, *J* = 8.0 Hz; H-2': δ_H 3.33, t,

$J = 9.6$ Hz; H-3': δ_H 5.02, t, $J = 9.6$ Hz; H-4': δ_H 3.46, t, $J = 9.6$ Hz; H-5': δ_H 3.20, m; H-6': δ_H 3.65 and 3.85, m]. Based on the coupling constants between the protons in the spin system and ROESY correlations between H-1', H-3' and H-5', the first sugar unit was identified as a β -glucopyranoside with a functional group at the 3'-position, since H-3' showed a 3J HMBC correlation to the carbonyl carbon (C-A1, δ_C 169.4) of an α,β -unsaturated ester unit. Both H₃-A4 (δ_H 1.98, dq, $J = 7.2, 1.4$ Hz) and H₃-A5 (δ_H 1.92, br s) showed HMBC correlations to C-A2 (δ_C 129.5) and C-A3 (δ_C 138.3), and H₃-A5 also correlated to C-A1. The ROESY correlation between H-A3 (δ_H 6.09, qq, $J = 7.2, 1.7$ Hz) and H₃-A5 confirmed that the substituent at the 3'-position was an angeloyl group. The ^{13}C NMR chemical shifts of C-1' ~C-6' matched well those of the 3''-O-acetylglucosyl group of 3''-O-acetylsaikosaponin d (Ebata et al., 1996), and the ^{13}C NMR chemical shifts of the angeloyl carbons matched those of methyl angelate (Zhong et al., 1999). The H-1'' proton of the second sugar (δ_H 4.32, d, $J = 7.7$ Hz) showed a 3J HMBC correlation to C-28 (δ_C 75.1), establishing the connectivity from the anomeric position of the second sugar unit to the 28-position of the aglycone. The spin system from the anomeric proton to the other protons of the second sugar was also clearly exhibited in a 1D TOCSY spectrum [H-1'' (selected): δ_H 4.32, d, $J = 7.7$ Hz; H-2'': δ_H 3.43, t, $J = 9.6$ Hz; H-3'': δ_H 3.51, t, $J = 9.6$ Hz; H-4'': δ_H 3.29, t, $J = 9.6$ Hz; H-5'': δ_H 3.22, m; H-6'': δ_H 3.65 and 3.85, m] in which all the protons had similar coupling constants to those of the corresponding protons in the first sugar. The second sugar unit was thus also identified as a glucoside. The presence of a substituent at the 2''-position was indicated by a 3J HMBC correlation from the H-1''' proton of the third sugar unit (δ_H 4.62, d, $J = 8.0$ Hz) to C-2'' (δ_C 81.5), thus establishing the connectivity from C-1''' (δ_C 104.8) to C-2'' via an oxygen bridge. The third sugar unit was identified as a glucoside as by its 1D TOCSY data [1D TOCSY: H-1''' (selected): δ_H 4.62, d, $J = 8.0$ Hz; H-2''': δ_H 3.16, t, $J = 9.6$ Hz; H-3''': δ_H 3.20, t, $J = 9.6$ Hz; H-4''': δ_H 3.30, t, $J = 9.6$ Hz; H-5''': δ_H 3.20, m; H-6''': δ_H 3.65 and 3.85, m]. The ^{13}C NMR chemical shifts of the sophorose moiety at the 28-position matched those of justiciocide E (Kanchanapoom et al., 2005). Hence, the structure of compound **1** was determined as shown, and was named terminaliaside A ((β ,21 β ,22 α)-3-O-(3'-O-angeloylpyranoglucosyl)-21,22-dihydroxy-28-O-sophorosyl-16-oxoolean-12-ene).

Compound **2** was isolated as a colorless powder. Its positive ion HRFAB mass spectrum revealed a pseudo-molecular ion $[(M+H)^+]$ consistent with the molecular formula $C_{29}H_{25}O_9$, requiring eighteen double-bond equivalents. Its 1H NMR spectrum in DMSO- d_6 (Table 2) showed signals for thirteen protons between 6.30 and 8.00 ppm [δ_H 7.94, d, $J = 9.7$ Hz; 7.82, d, $J = 16.2$ Hz; 7.76, m (2H); 7.46, m (3H); 7.24, d, $J = 1.7$ Hz; 7.22, d, $J = 8.0$ Hz; 7.09, dd, $J = 8.0, 1.7$ Hz; 6.90, s; 6.85, d, $J = 16.2$ Hz; 6.33, d, $J = 9.7$ Hz;]. Signals for two oxygenated methines (δ_H 5.15, d, $J = 8.0$ Hz; 4.43, m), one oxygenated methylene (δ_H 3.40, m and 3.70, m), two methoxy groups (δ_H 3.75, s; 3.75, s), and one hydroxyl group (δ_H 5.33, dd, $J = 5.3, 4.7$ Hz) were also observed. The *cis*-coupling between H-3 (δ_H 6.33, d, $J = 9.7$ Hz) and H-4 (δ_H 7.94, d, $J = 9.7$ Hz) and the HMBC correlation (Figure 3) between H-4 (δ_H 7.94, d, $J = 9.7$ Hz) and C-5 indicated the existence of a 6,7,8-trisubstituted coumarin. Signals for a cinnamoyl group [H-7'': δ_H 7.82, d, $J = 16.2$ Hz; H-8'': δ_H 6.85, d, $J = 16.2$ Hz; mono-substituted benzene ring: δ_H 7.76, m (2H) and 7.46, m (3H)] were also observed in the 1H NMR spectrum of **2**. H-7' (δ_H 5.15, d, $J = 8.0$ Hz) in the 1,2,3-trioxygenated propenyl group exhibited 3J HMBC correlations to C-2' and C-6' of the 1,3,4-trisubstituted benzene ring (H-2': δ_H 7.24, d, $J = 1.7$ Hz; H-5': 7.22, d, $J = 8.0$ Hz; H-6': δ_H 7.09, dd, $J = 8.0, 1.7$ Hz) and C-7 of the coumarin moiety, while H-8' (δ_H 4.43, m) correlated to C-8 and C-1', which indicated the existence of a 2,3-dihydro-1,4-dioxine group. The positions of the two methoxy groups were determined by the NOESY correlations between H-2' (δ_H 7.24, d, $J = 1.7$ Hz;) and the methoxy at δ_H 3.75 ppm, and H-5 and the methoxy at δ_H 3.75 ppm (Figure 3). The 9'-OH (δ_H 5.33, dd, $J = 5.3, 4.7$ Hz) showed COSY correlation to H₂-9' (δ_H 3.40 & 3.70, m), therefore, the cinnamoyl group was deduced to be located at the 4'-position. The relative stereochemistries of H-7' and H-8' were determined as *trans* from the coupling constant between H-7' and H-8' ($J = 8.0$ Hz) and

the NOESY correlation between H-7' and H₂-9'. The structure of the new lignan was thus assigned as 4'-*O*-cinnamoyl cleomiscosin A (**2**). A comparison of the ¹³C NMR data of **2** with those of cleomiscosin A (Ahmad et al., 2004) showed only fair agreement, probably in part because of the different solvents used (DMSO-d₆ in the current work, and pyridine-d₅ in the literature work). The ¹³C NMR spectrum of **2** was thus calculated using the ChemDraw software (Table 2), and the calculated spectrum was in excellent agreement with the observed spectrum except for the shift of C-3', which was outside the standard deviation of the software (5 ppm).

Compounds **3** and **4** were determined as arjunglucoside I (Nandy et al., 1989) and sericoside (Zhou et al., 1992), respectively, by comparison of their ¹H and ¹³C NMR and mass spectroscopic data with literature data.

Compounds **1–4** were tested in the A2780 assay. Compound **1** was the most active compound with an IC₅₀ value of 1.2 μM, while compound **3** was weakly active with an IC₅₀ value of 16.5 μM. Compounds **2** and **4** were both inactive with IC₅₀ values >30 μM. It thus appears that the antiproliferative activity of olean-12-ene triterpene derivatives such as terminaliaside A (**1**) and gummiferaosides A-C (Cao et al., 2007a) is enhanced by substituents at the 3-, 16-, 21-, and 28-positions.

3. Conclusions

This work provides a further example of the importance of oleanane-type saponins as potential anticancer agents. Although saponins have not traditionally been regarded with favor by the pharmaceutical industry, the saponin OSW-1 is being actively investigated as a potential anticancer agent (Zhou et al. 2005), and several other saponins have shown useful activity in animal models (Bachran et al., 2008). Several of the saponins listed in this review are oleanane triterpene derivatives with sugar substitution at C-3 only (the soyasaponins), at C-3 and C-28 (gypsoside A), or at the C-3, C-21, and C-28 positions (avicin G). It is thus reasonable to infer that an oleanane-type saponin may eventually be approved for cancer chemotherapy.

4. Experimental

4.1 General

Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 for ¹H, ¹³C, HMQC, and HMBC and an INOVA 400 spectrometer for TOCSY, COSY, and ROESY. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive-ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative C₁₈ Varian Dynamax column (5 μm, 250 × 10 mm) and a preparative C₁₈ Varian Dynamax column (8 μm, 250 × 21.4 mm).

4.2 Plant Material

Roots of *Terminalia tropophylla* H. Perrier (Combretaceae) were collected on October 8th, 2006 by Richard Randrianaivo et al. at an elevation of 52 m in the Lamiranta forest, Andavakoera, 15 km from National Route 6, Madagascar. The sample was collected from a dry degraded forest. The collection coordinates were 13°05'09"S 049°13'35"E, and the voucher number was RIR 1397. The sample was a large tree 25 m high, diameter at breast height 85 cm, with white flowers. It was abundant along a river. Voucher specimens have been deposited at herbaria of the Centre National d' Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN);

the Missouri Botanical Garden, St. Louis, Missouri (MO); and the Muséum National d'Histoires Naturelles, Paris, France (P).

4.3 Extraction and Isolation

Dried roots of *Terminalia tropophylla* (250 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 4035 (8.5 g), of which 2.8 g was made available to Virginia Polytechnic Institute and State University. Extract MG 4035 (2.2 g; IC₅₀ 11 µg/mL, A2780) was suspended in aqueous MeOH (MeOH-H₂O, 9:1, 100 mL) and extracted with n-hexane (3 × 100 mL portions). The aqueous layer was then diluted to 70% MeOH with H₂O and extracted with CH₂Cl₂ (3 × 100 mL portions). The aqueous MeOH extract (1.7 g) was active with IC₅₀ value of 10 µg/mL against A2780, while both the hexane and CH₂Cl₂ extracts were inactive. The aqueous MeOH extract was filtered through a C₁₈ open column and then subjected to HPLC separation on a C₁₈ column (28% MeCN/H₂O, 10 mL/min) to give 11 fractions. Fractions 6 and 10 yielded compounds **3** (*t*_R 14.2 min, 3 mg) and **4** (*t*_R 23.6 min, 5 mg). Compound **1** (*t*_R 18 min, 0.9 mg) was obtained by HPLC of fraction 11 using C₁₈ HPLC (33% MeCN/H₂O, 2 mL/min). Compound **2** (0.5 mg) was obtained from the CH₂Cl₂ extract after repeated chromatography on silica gel.

4.3.1 Terminaliaside A (1)—White solid; [α]_D²⁵ −6.1 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.2) nm; IR (film) ν_{max} 3366, 1698, 1025 cm^{−1}; ¹H NMR (500 MHz, CD₃OD), see Table 1; ¹³C NMR (125 MHz, CD₃OD), see Table 1; HRFABMS *m/z* 1079.5376 (calcd for C₅₃H₈₄O₂₁Na, 1079.5403).

4.3.2 4'-O-Cinnamoyl cleomiscosin A (2)—White solid; [α]_D²⁵ −77 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 211 (3.7), 284 (3.2), 352 (sh, 2.7) nm; ¹H NMR (500 MHz, CDCl₃ and DMSO-*d*₆), see Table 2; ¹³C NMR (125 MHz, DMSO-*d*₆), see Table 2; HRFABMS *m/z* 539.1322 (calcd for C₂₉H₂₄O₉Na, 539.1318).

4.4 Antiproliferative Bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported (Cao et al, 2007b). The A2780 cell line is a drug-sensitive ovarian cancer cell line (Louie et al., 1985).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.phytochem. 2009.XX.XXX.

Fig. 1.
Key HMBC correlations of compound 1

Fig. 2.
Key ROESY correlations of compound 1

Fig. 3.
Key HMBC (solid arrows) and NOESY (dashed lines) correlations of compound 2

Table 1NMR Data of Compound 1^a

#	¹ H ^b	¹³ C ^c
1	1.00; 1.65	39.8
2	1.65	24.7
3	3.24	90.8
4		40.2
5	0.80 br d (11.0)	56.9
6	1.65	19.2
7	1.30; 1.70	33.6
8		41.5
9	1.62	47.9
10		37.7
11	1.95	27.0
12	5.52 br t (3.5)	126.8
13		140.6
14		47.7
15	2.90 d (14.1); 1.78 d (14.1)	47.9
16		216.8
17		59.0
18	2.79 dd (13.8, 3.0)	48.0
19	1.20	46.8
20		37.0
21	3.66 d (9.3)	77.7
22	3.56 dd (9.3, 2.8)	77.1
23	1.06 s	28.5
24	0.86 s	17.0
25	1.01 s	16.1
26	1.14 s	17.8
27	1.22 s	27.9
28	4.18 d (9.6); 4.01 d (9.6)	75.1
29	0.95 s	30.1
30	0.88 s	18.8
3'-Angeloyl		
1		169.4
2		129.5
3	6.09 qq (7.2, 1.7)	138.3
4	1.98 dq (7.2, 1.4)	16.0
5	1.92 br s	20.8
3-β-D-Glc		
1'	4.43 d (8.0)	106.6
2'	3.33 t (9.6)	74.0

#	$^1\text{H}^b$	$^{13}\text{C}^c$
3'	5.02 t (9.6)	78.8
4'	3.46 t (9.6)	69.9
5'	3.20 m	77.6
6'	3.65; 3.85 m	62.7
28- β -D-Glc		
1''	4.32 d (7.7)	103.0
2''	3.43 t (9.6)	81.5
3''	3.51 t (9.6)	77.9
4''	3.29 t (9.6)	71.5
5''	3.22 m	78.0
6''	3.65; 3.85 m	62.5
2''- β -D-Glc		
1'''	4.62 d (8.0)	104.8
2'''	3.16 t (9.6)	75.9
3'''	3.20 t (9.6)	78.3
4'''	3.30 t (9.6)	71.1
5'''	3.20 m	78.4
6'''	3.65; 3.85 m	62.5

^a In CD₃OD.

^b δ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses.

^c δ (ppm) 125 MHz

Table 2

NMR Data of Compound 2^a

#	¹ H ^a	¹ H ^b	¹³ C ^c	¹³ C ^d
1				
2			160.9	160.9
3	6.33 d (9.5)	6.33 d (9.7)	112.8	113.5
4	7.63 d (9.5)	7.94 d (9.7)	140.2	143.6
5	6.55 s	6.90 s	101.4	103.5
6			147.3	147.2
7			135.4	135.7
8			145.9	144.3
9			137.2	134.8
10			113.9	115.5
1'			134.25	136.4
2'	7.10 br s	7.24 d (1.7)	112.0	112.6
3'			151.6	157.2
4'			138.5	136.1
5'	7.18 d (8.0)	7.22 d (8.0)	123.7	122.7
6'	7.11 br d (8.0)	7.09 dd (8.0, 1.7)	120.8	120.9
7'	5.17 d (8.0)	5.15 d (8.0)	76.4	76.2
8'	4.12 br dd (8.0, 3.0)	4.43 m	78.1	78.3
9'	3.88 d (16.0) & 3.63	3.40 m & 3.70 m	60.3	61.2
9'-OH		5.33 dd (5.3, 4.7)		
1''			132.1	135.2
2'', 6''	7.42 m/7.61 m	7.46 m	129.7	126.4
3'', 5''	7.61 m/7.42 m	7.76 m	129.2	128.7
4''	7.42 m/7.61 m	7.46 m	131.7	128.0
7''	7.88 d (16.2)	7.82 d (16.2)	145.6	148.0
8''	6.67 d (16.2)	6.85 d (16.2)	117.1	115.6
9''			165.0	164.3
6-OMe	3.87	3.75	56.4	56.2
3'-OMe	3.90	3.75	56.4	55.9

^aIn CDCl₃; δ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses.^bin DMSO-*d*₆; δ (ppm) 500 MHz; multiplicities; *J* values (Hz) in parentheses.^cin DMSO-*d*₆; δ (ppm) 125 MHz.^dCalculated using ChemDraw Ultra 8.0 (CambridgeSoft Corporation, Cambridge, MA).