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A new stilbene glycoside from *Elephantorrhiza goetzei*

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Abstract

A new stilbene glycoside, 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**1**) was isolated from the root bark of *Elephantorrhiza goetzei*, along with known compounds, namely gallic acid, (*E*)-resveratrol, (±)-catechin, (±)-gallocatechin and oleanene triterpenoids. The combined ethyl acetate-methanol extract exhibited high lethality against brine shrimp larvae (LC_{50} 10.8 ppm) compared to the isolates, some of which were not active.

Keywords: *Elephantorrhiza goetzei*; Stilbenes; Flavonoids; Phenolics; Triterpenoids; 5-Methoxy-(*E*)-resveratrol 3-*O*-rutinoside

1. Introduction

The genus *Elephantorrhiza* consists of shrubs, small trees or low bushes springing from underground rhizomes [1]. In Southern Africa four species of *Elephantorrhiza*, namely *E. burkei*, *E. elephantina*, *E. goetzei* and *E. suffruticosa* are known [2]. *E. goetzei* Harms (Fabaceae) is a tree that grows to over 8 m, found in North Eastern Botswana and is used by the local communities as a remedy for sores of the penis and vulva, irregular menstruation and for cleansing the womb after abortion [2,3]. *E. goetzei* root previously investigated was found to contain flavan-3-ols and the extractives showed activity against gram-positive and gram-negative bacteria and

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fungi [4]. In this paper, we report the isolation of stilbenes, flavan-3-ols, triterpenoids and gallic acid.

2. Experimental

2.1. General methods

Melting point: Stuart Scientific (SMP1) melting point apparatus; Specific rotation $[\alpha]_D$: Polatronic-D (Schimdt + Haensch) polarimeter; UV: Shimadzu UV-2101PC spectrophotometer; IR: Perkin-Elmer 2000 FT-IR spectrometer. The one-dimensional [^{1}H (300 MHz), ^{13}C (75.4 MHz), DEPT] and two-dimensional (COSY, HMQC, HMBC) spectra acquired on Bruker Avance DPX 300 spectrometer and referenced to residual solvent signal. MS: HRMS was done on autospec TOF spectrometer. EI and ESIMS on Finnigan MAT SSQ 700 single quadrupole instrument. Column chromatography-silica gel 60 particle size 0.040–0.063 mm for column chromatography (Merck); vacuum liquid chromatography (VLC)-silica gel HF_{254} 5–15 μm mesh (Merck); Sephadex LH-20 (Sigma); preparative TLC-silica gel 60 PF_{254} for preparative layer chromatography (Merck); analytical TLC-TLC silica gel 60-F_{254} pre-coated alumina sheets (Merck) and visualized using UV (254 and 366 nm) and vanillin-sulfuric acid spray.

2.2. Plant material

E. goetzei roots were collected from Mapoka, North East District, Botswana, in August 1997 and identified by Dr L.M. Turton. A voucher specimen (No. 3393) was deposited at the University of Botswana Herbarium.

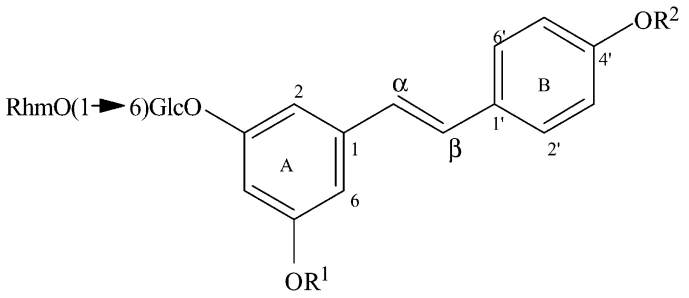
2.3. Extraction and isolation

Air-dried and powdered root bark (2.0 kg) was extracted sequentially to exhaustion with CH_2Cl_2, EtOAc and MeOH. The solvents were removed to give the crude extracts (5, 10 and 25 g), respectively. The EtOAc and MeOH extracts, giving relatively similar TLC profiles ($CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2), were combined (35 g) and part of this (15 g) was subjected to VLC using CH_2Cl_2 with increasing amounts of MeOH stepwise to give fractions A (CH_2Cl_2/MeOH 1:1, 3 g) and B (MeOH, 4 g). Fraction A was further separated by gel filtration on Sephadex LH-20 with $CHCl_3$/MeOH 1:1 and the concentrated eluate was resolved by PTLC with ($CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2), to give (*E*)-resveratrol (80 mg) [5] and gallic acid (110 mg). Fraction B was further separated by gel filtration on Sephadex LH-20 with MeOH to give fractions B_1 and B_2. Fraction B_1 was resolved by PTLC ($CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2) to give (±)-catechin (70 mg) [6,7], (±)-gallocatechin (85 mg) [6,8] and the triterpenoids sericoside [9] (100 mg), bellericoside [10] (20 mg) and arjungenin [9] (40 mg). Fraction B_2 was further purified on gradient Si-gel CC and PTLC by multiple development

($CHCl_3$/MeOH/H_2O/HOAc 70:26:2:2) to give 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**1**) (90 mg) and (*E*)-resveratrol 3-*O*-rutinoside [11] (400 mg).

2.4. Methylation of (E)-resveratrol 3-O-rutinoside

Diazomethane in ether [12,13] (100 ml) was added dropwise with swirling to an ice-cooled solution of (*E*)-resveratrol 3-*O*-rutinoside (200 mg). After 6 h, at complete reaction of the starting product (TLC monitored), the mixture was concentrated and resolved by PTLC to afford 4′,5-dimethoxy-(*E*)-resveratrol 3-*O*-rutinoside (150 mg) [14], 5-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**1**) (43 mg) and 4′-methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**) (9 mg).



1 $R^1 = CH_3$, $R^2 = H$

2 $R^1 = H$, $R^2 = CH_3$

(*E*)-*Resveratrol 3-O-rutinoside*. White crystals (MeOH), mp 188–190°C (uncorrected); R_f 0.30, $CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2; $[\alpha]_D$ −30.2 (*c* 0.1, MeOH); UV max (MeOH): 317 (log ε 4.41), 305 (4.42); +NaOMe 336, 340 (sh); +NaOAc 317, 306 nm; IR bands (KBr): 3353, 2920, 1599 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): H-2 (6.65, *br s*), H-4 (6.38, *br s*), H-6 (6.65, *br s*), H-α (6.87, *d*, *J* 16.3 Hz), H-β (7.02, *d*, *J* 16.3 Hz), H-2′,6′ (7.42, *d*, *J* 8.3 Hz), H-3′,5′ (6.78, *d*, *J* 8.3 Hz), glucosyl H-1 (4.84, *d*, *J* 7.2 Hz), H-2 (3.30–3.33), H-3 (3.30–3.33), H-4 (3.40–3.45), H-5 (3.40–3.45), H-6 (3.86, 3.40–3.45), rhamnosyl H-1 (4.60, *br s*), H-2 (3.40–3.45), H-3 (3.68 *m*), H-4 (3.30–3.33), H-5 (3.40–3.45), H-6 (1.12, *d*, *J* 6.0 Hz); ^{13}C-NMR (75.4 MHz, DMSO-d_6): C-1 (140.2), C-2 (107.6), C-3 (159.7), C-4 (103.7), C-5 (159.2), C-6 (106.3), C-α (126.0), C-β (129.4), C-1′ (128.8), C-2′,6′ (128.9), C-3′,5′ (116.5), C-4′ (158.2), glucosyl C-1 (101.5), C-2 (74.1), C-3 (77.3), C-4 (70.4), C-5 (76.2), C-6 (67.1), rhamnosyl C-1 (101.5), C-2 (71.6), C-3 (71.2), C-4 (73.0), C-5 (69.2), C-6 (18.7); HRTOFMS *m/z*: 536.1631 (calcd. for $C_{26}H_{32}O_{12}$, 536.1682).

5,4′-Dimethoxy-(E)-resveratrol 3-O-rutinoside. White crystals (MeOH), mp 126–129°C (uncorrected); R_f 0.35, $CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2; $[\alpha]_D$

−14.3 (*c* 0.3, MeOH); UV max (MeOH): 316 (log ε 4.58) 305 (4.58); +NaOMe 316, 305 nm; IR bands (KBr): 3422, 2922, 1594 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD): H-2 (6.84, *br s*), H-4 (6.60, *br s*), H-6 (6.78, *br s*), H-α (6.95, *d*, *J* 16.4 Hz), H-β (7.07, *d*, *J* 16.4 Hz), H-2′,6′ (7.47, *d*, *J* 8.6 Hz), H-3′,5′ (6.93, *d*, *J* 8.6 HZ), 4′-MeO (3.79, *s*), 5-MeO (3.82, *s*), glucosyl H-1 (4.90, *d*, *J* 7.2 Hz), H-2 (3.30–3.33), H-3 (3.30–3.33), H-4 (3.40–3.45), H-5 (3.40–3.45), H-6 (3.86, 3.40–3.45), rhamnosyl H-1 (4.73, *br s*), H-2 (3.40–3.45), H-3 (3.68 *m*), H-4 (3.30–3.33), H-5 (3.40–3.45), H-6 (1.12, *d*, *J* 6.2 Hz); ^{13}C-NMR (75.4 MHz, CD_3OD): C-1 (141.1), C-2 (108.6), C-3 (162.2), C-4 (103.2), C-5 (160.8), C-6 (106.5), C-α (127.2), C-β (129.9), C-1′ (131.2), C-2′,6′ (128.9), C-3′,5′ (115.1), C-4′ (160.2), 4′-MeO (55.7), 5-MeO (55.9), glucosyl C-1 (102.3), C-2 (74.8), C-3 (77.8), C-4 (71.3), C-5 (76.7), C-6 (67.5), rhamnosyl C-1 (102.0), C-2 (72.3), C-3 (72.0), C-4 (74.0), C-5 (69.7), C-6 (17.9); HRTOFMS *m/z*: 564.2206 (calcd. for $C_{28}H_{36}O_{12}$, 564.2207); EIMS *m/z*: 564 (70), 418 (15), 270 (65) 228 (75).

5-Methoxy-(E)-resveratrol 3-O-rutinoside (**1**). White crystals (MeOH), mp 160–162°C (uncorrected); R_f 0.33, $CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2; $[\alpha]_D$ 20.3 (*c* 0.2, MeOH); UV max (MeOH) 317 (log ε 4.15), 304 (4.29); +NaOMe 345, 337 (sh) nm; IR bands (KBr): 3413, 2921, 1590 cm^{-1}; HRTOFMS *m/z*: 550.2053 (calcd. for $C_{27}H_{34}O_{12}$, 550.2050); ^{1}H and ^{13}C-NMR: see Table 1.

4′-Methoxy-(E)-resveratrol 3-O-rutinoside (**2**). White crystals (MeOH), mp 166–169°C (uncorrected); R_f 0.33, $CHCl_3$/MeOH/HOAc/H_2O 70:26:2:2; $[\alpha]_D$ −27.1 (*c* 0.3, MeOH); UV max (MeOH) 317 (log ε 4.21), 305 (4.30); +NaOMe 340, 332 (sh) nm; IR bands (KBr): 3414, 2921, 1591 cm^{-1}; HRTOFMS *m/z*: 550.2053 (calcd. for $C_{27}H_{34}O_{12}$, 550.2051); ^{1}H and ^{13}C-NMR: see Table 1.

2.5. Studied activity

Cytotoxicity by brine shrimp (*Artemia salina*) lethality according to Meyer et al. [15] on the combined ethyl acetate-methanol extract and isolated compounds.

3. Results and discussion

HRTOFMS of **1** gave molecular ion peak *m/z* $[M]^+$ 550.2053, consistent with the molecular formula $C_{27}H_{34}O_{12}$. ^{1}H and ^{13}C-NMR spectra (Table 1) indicated **1** had an (*E*)-resveratrol aglycone moiety by showing signals consistent with those published for (*E*)-resveratrol glucoside [5,11]. The assignments in Table 1 were confirmed by HMQC, HMBC and COSY spectra. The ^{1}H-NMR spectrum showed signals for two *trans* olefinic protons at δ 6.89 (H-α, *d*, *J* = 16.0 Hz) and δ 7.05 (H-β, *d*, *J* = 16.0 Hz). The ^{13}C-NMR of **1** gave 27 carbon signals and the DEPT spectrum together with HMQC data confirmed that 22 of these were protonated. DEPT spectrum also showed two methyl, one methylene and 19 methine carbons. In the HMBC spectrum, the H-α proton showed a cross-peak to C-2 (δ 108.5 ppm) and C-6 (δ 106.4 ppm) carbon signals of the A ring. The H-β proton signal in turn

Table 1
^{1}H (300 MHz) and ^{13}C-NMR data for compounds **1** and **2** in CD_3OD[a]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		141.3 (*s*)		141.2 (*s*)
2	6.82, *br s*	108.5 (*d*)	6.83, *br s*	108.4 (*d*)
3		162.2 (*s*)		162.1 (*s*)
4	6.58, *br s*	103.0 (*d*)	6.59, *br s*	103.3 (*d*)
5		160.2 (*s*)		159.7 (*s*)
6	6.76, *br s*	106.4 (*d*)	6.77, *br s*	106.9 (*d*)
α	6.89, *d* (16.0)	126.4 (*d*)	6.94, *d* (16.4)	126.4 (*d*)
β	7.05, *d* (16.0)	130.2 (*d*)	7.04, *d* (16.4)	129.9 (*d*)
1′		130.1 (*s*)		130.3 (*s*)
2′,6′	7.39, *d* (8.4)	128.9 (*d*)	7.45, *d* (8.6)	128.9 (*d*)
3′,5′	6.86, *d* (8.4)	116.5 (*d*)	6.92, *d* (8.6)	116.7 (*d*)
4′		158.4 (*s*)		160.5 (*s*)
4′-OCH_3			3.79, *s*	55.7 (*q*)
5-OCH_3	3.82, *s*	55.9 (*q*)		
Glucose				
1	4.90 (7.2)	102.3 (*d*)	4.90 (7.3)	102.3 (*d*)
2	3.30–3.33	74.8 (*d*)	3.30–3.33	74.8 (*d*)
3	3.30–3.33	77.8 (*d*)	3.30–3.33	77.8 (*d*)
4	3.40–3.45	71.3 (*d*)	3.40–3.45	71.3 (*d*)
5	3.40–3.45	76.7 (*d*)	3.40–3.45	76.7 (*d*)
6	3.86, 3.40–3.45	67.5 (*t*)	3.86, 3.40–3.45	67.5 (*t*)
Rhamnose				
1	4.73, *br s*	102.0 (*d*)	4.73, *br s*	102.0 (*d*)
2	3.40–3.45	72.3 (*d*)	3.40–3.45	72.3 (*d*)
3	3.68, *m*	72.0 (*d*)	3.68, *m*	72.0 (*d*)
4	3.30–3.33	74.0 (*d*)	3.30–3.33	74.0 (*d*)
5	3.40–3.45	69.7 (*d*)	3.40–3.45	69.7 (*d*)
6	1.21, *d* (6.4)	17.8 (*q*)	1.21, *d* (6.3)	17.9 (*q*)

[a]Assignments were confirmed by HMQC, HMBC and DEPT experiments.

showed HMBC correlations to carbon signals C-2′,6′ (δ 128.9 ppm), thus confirming the positions of the A and B aromatic rings. The glucopyranosyl anomeric proton signal δ 4.90 (*d*, $J = 7.2$ Hz) showed HMBC correlation with C-3 (δ 162.2 ppm), confirming that the *O*-glucopyranose moiety which is attached to resveratrol A ring through the anomeric carbon. The rhamnopyranosyl anomeric proton signal δ 4.73 (*br s*) showed HMBC correlation with glucose moiety C-6 (δ 67.5 ppm), confirming that the *O*-rhamnopyranose moiety is 1 → 6 attached to glucopyranose. The carbon signal at δ 158.4 ppm was confirmed as the C-4′ signal by its three-bond connectivity to the two proton doublet at δ 7.39 (H-2′ and H-6′) which are protons of the phenolic ring B and the methoxyl protons δ 3.82 showed HMBC correlation with C-5 (δ 160.2). In contrast to the glycosylation of flavonoids which

causes an upfield shift of the *ipso* carbon, glycosylation in this stilbene has caused a downfield shift relative to the substituted C-5 MeO-bearing carbon [16,17]. The β-D-glucopyranosyl and α-L-rhamnopyranosyl moieties were evident from the 12 carbon signals from 101.5 to 18.7 ppm which were in agreement with literature values [16,17]. The doublet δ 4.90 (J = 7.2 Hz) for the anomeric proton signal gave confirmation of the β-anomeric configuration of glucopyranose and the broad singlet δ 4.73 for the anomeric proton signal confirmed the α-anomeric configuration of rhamnopyranose.

Methylation of (*E*)-resveratrol 3-*O*-rutinoside was necessary in order to assign the C-3, C-5 and C-4 carbons of the stilbenes isolated unambiguously with the help of the HMBC spectral expansions.

HRTOFMS of **2** gave molecular ion peak m/z $[M]^+$ 550.2053, consistent with the molecular formula $C_{27}H_{34}O_{12}$. The ^1H and ^{13}C-NMR spectra (Table 1) of compound **2** were similar to those of **1** except for the MeO group showing HMBC correlation with C-4′ (δ 160.5 ppm). Thus, compound **2** was identified as 4′-methoxy-(*E*)-resveratrol 3-*O*-rutinoside.

Both compounds **1** and **2** showed no lethality on brine shrimp larvae at concentrations of up to 1000 ppm, while a LD_{50} of 10.8 ppm was determined for the combined ethyl acetate-methanol extract (Table 2).

Table 2
Lethality of extractives from *Elephantorrhiza goetzei* root bark to brine shrimp larvae on exposure for 24 h

Extractive	LC_{50} (24) ppm
Combined EtOAc-MeOH extract	10.8
(±)-Catechin	25.3
(±)-Gallocatechin	20.3
(*E*)-Resveratrol	56.8
(*E*)-Resveratrol 3-*O*-rutinoside	398.7
5-Methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**1**)	$\gg 10^7$
4′-Methoxy-(*E*)-resveratrol 3-*O*-rutinoside (**2**)	$\gg 10^7$
4′,5-Dimethoxy-(*E*)-resveratrol 3-*O*-rutinoside	$\gg 10^7$
Sericoside	126.3
Bellericoside	213.2
Arjungenin	245.5

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