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ISOLATION OF A STILBENE GLYCOSIDE AND OTHER CONSTITUENTS OF *TERMINALIA SERICEAE*

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Abstract

The ethanol extract of the root bark of *Terminalia sericea* yielded an unreported stilbene glycoside, 3'5'-dihydroxy-4-(2-hydroxy-ethoxy) resveratrol-3-O-β-rutinoside (**1)** together with known compounds resveratrol-3-β-rutinoside glycoside (**2**), 3',4,5'-Trihydroxystilbene (resveratrol) (**3**), triterpenoic acid arjungenin and a mixture of β-sitosterol and stigmasterol. Structure determination of the isolated compounds was achieved on the basis of spectroscopic measurements.

Key words: *Terminalia sericea*, stilbenes, combretastatin, resveratrol, glycosides.

Introduction

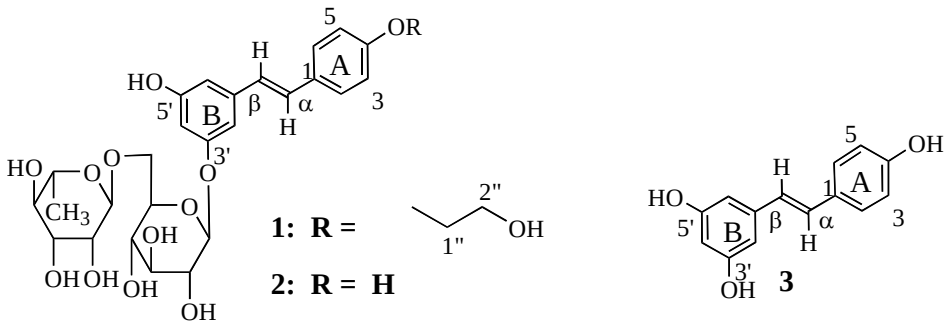
Terminalia sericea Burch. Ex. DC (Combretaceae) is widely distributed in the tropical and warm temperate regions, especially in Africa (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The plant has wide spread traditional uses in eastern and southern African countries (Arnold and Gulumian, 1984; Kokwaro, 1976; Msonthi and Magombo, 1983). Among some of its proven biological activities, include inhibition of topoisomerase II (Wall et al., 1996), antibacterial, and antifungal activity (Eloff, 1999; Fyhrquist et al., 2002; 2004). However, there is scanty information on compounds that are responsible for the exhibited biological activities of the plant. The stilbene glycoside, resveratrol-3-*O*-β-rutinoside (**2**) (Bombardelli et al., 1975), and pentacyclic triterpenes of the oleanane skeleton, together with their glucosides (Bombardelli et al., 1974; 1975) have been isolated. This includes also a triterpene, sericoside (Maeda and Fukuda, 1996). Skin lightening preparations containing sericoside have been patented in Japan (Maeda and Fukuda, 1996). A β-D-glucoside (**4**) of resveratrol was isolated from wine, a compound that is considered to be responsible for the protective ability of wines against coronary heart disease (Jeandet et al., 1991).

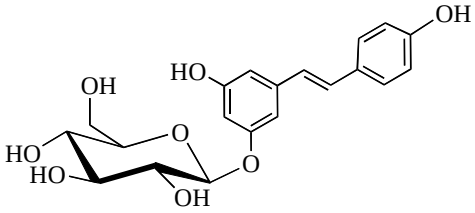
We hereby report the isolation of a an unreported stilbene glycoside **1**, resveratrol-3-*O*-β-rutinoside (**2**), resveratrol (**3**) and other known compounds such as sericic acid, stigmasterol, β-sitosterol and triterpenoid acid were also isolated from the ethanol extract of the root bark of *T. sericeae* occurring in Tanzania.

Materials and Methods

Materials

Petroleum ether, dichloromethane, ethyl acetate (EtOAc), methanol (MeOH), chloroform ($CHCl_3$), and ethanol were purchased from Fisher Scientific UK Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK). Precoated silica gel plates (60 F_{254}, 250 µm) were purchased from Merck and sephadex LH-20 from Pharmacia.





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Collection of Plant materials

The root barks of *T. sericea* were collected from Iringa region, and identified by Mr. Frank Mbago. Voucher specimen no. IMPP 001-0144 is kept in the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Sciences, University of Dar es Salaam.

Extraction and isolation

Air-dried and powdered plant material (2 Kg) was cold extracted sequentially in petroleum ether (2.0 L; 40-60˚C), dichloromethane (2.0 L) and then ethanol (2.0 L). The amounts of both the petroleum ether and dichloromethane extracts were very small to the extent that these extracts could not be worked on further. The ethanol extract was then fractionated by vacuum liquid chromatography (VLC) eluting with a mixture of EtOAc/petroleum ether followed by MeOH/EtOAc to give 4 bulked fractions. Thin layer chromatography (TLC) was performed on pre-coated plates (silica gel 60 F_{254}, 250 μm on polyester backing, Merck) eluting with mixtures consisting of petroleum ether (b.p. 40-60˚C) and EtOAc; detection by UV and an anisaldehyde spray reagent (Jean, 1996). Column chromatography on silica gel 60 mesh (0.063-0.2 mm, Merck) eluting with mixtures of increasing polarity consisting of petroleum ether and EtOAc. Gel filtration was performed on Sephadex® LH-20 (Pharmacia) eluting with MeOH or a mixture of MeOH/$CHCl_3$. VLC was performed on silica gel 60 (Merck), eluting with petroleum ether containing increasing amounts of EtOAc. Recrystallisation was achieved from MeOH/$CHCl_3$, 9:1 v/v. Flash chromatography (FC) employed Merck silica gel 60 (0.063-0.2 mm, Merck) and petroleum ether (b.p. 40-60˚C)-EtOAc mixtures. Repeated column chromatography on silica gel of fraction 1 yielded compound **2** and **3**. Compound **1** was obtained from fraction 4 after repeated column chromatography followed by gel chromatography over Sephadex® LH-20 eluting with a mixture of methanol and water (1:1 v/v).

Spectra

^{1}H NMR spectra were recorded on a Bruker AM 400 spectrometer operating at 400 MHz. CD_3OD, $CDCl_3$ or d_6-DMSO were used as solvents. Chemical shifts are given in δ values relative to the internal standard TMS (δ = 0). Mass spectra were determined by direct inlet on a VG 7070 E instrument at 70 eV.

Results

The unreported stilbene glycoside, 3´5´-dihydroxy-4-(2 hydroxy-ethoxy) resveratrol-3-*O*-β-rutinoside **(1),** was isolated as a reddish gum.^{1}H-NMR ($CDCl_3$): δ 7.49 (1H, d, *J* = 8.6 Hz, H-2 or H-6), 6.99 (1H, d, *J* = 16.3 Hz,

H- α), 6.85 (1H, d, *J* = 16.3 Hz, H-β), 6.74 (1H, d, *J* = 2 Hz, H-2'), 6.66 (1H, d, *J* = 2 Hz, H-6′), 6.47 (1H, d, *J* = 2 Hz, H-4′), 4.90 (1H, d, H = 1′′′), 4.75 (1H, d, H = 1′′), 3.90 (1H, dd, *J* = 1.61 Hz, 3.4 Hz, H = 4′′), 3.71 (1H, m, H = 3′′′), 3.68 (2H, m, H-1′′′′), 3.58 (2H, m, H-2′′′′), 3.56 (1H, dd, *J* = 3.8 Hz, 5.4 Hz, H-3′′), 3.46 (1H, d, *J* = 5.7 Hz, H-2′′), 3.44 (1H, m, H-2′′′), 3.35 (1H, m, H-4′′′), 1.22 (d, d, *J* = 6.15 Hz, Me-6′′′). ^{13}C-NMR ($CDCl_3$): δ 159.32 (C-3′), 158.48 (C-5′), 157.41 (C-4), 140.39 (C-1′), 129.29 (C-β), 127.98 (C-2/6), 125.64 (C-C-a), 115.58 (C-3/5), 107.16 (C-C-2′), 106.76 (C-C-6′), 103.10 (C-C-4′), 101.33 (C-1′′′), 101.15 (C-1′′), 76.93 (C-3′′′), 75.85 (C-5′′), 73.94 (C-2′′), 73.08 (C-4′′′), 72.49 (C-1′′′′), 71.35 (C-3′′), 71.08 (C-4′′), 70.33 (C-2′′′), 68.82 (C-5′′′), 66.60 (C-6′′), 61.24 (C-2′′′′), 16.93 (C-6′′′). EIMS *m/z* (rel. int.): 580 $[M]^+$ ($C_{28}H_{36}O_{13}$), 564 (55), 537 (100), 391 (15), 375 (20), 229 (10). Compounds **2**, **3** and **4** were also isolated. Compound **2**, resveratrol-3-*O*-β-rutinoside, exhibited a weak antibacterial activity against *Escherichia coli* at 4 mg/ml. The compound was not tested on the other organisms due to its paucity.

Discussion

Compound **1** was obtained as a red gum. Its structure was established on the basis of extensive spectroscopic analysis, in particular H/H-COSY, HMQC, HMBC and MS measurements, as well as from comparison of the observed spectral data with those reported in the literature for the main skeleton (Bombardelli et al., 1975., Breitmaier and Voelter, 1989., Dorman et al., 1970). Thus, the aromatic region of the ^{1}H and ^{13}C NMR spectra exhibited features which were virtually the same as those observed for the stilbene moiety in resveratrol-3-*O*-β-rutinoside (**2**) and this indicated the presence of a resveratrolyl unit in structure **1**, as was further also confirmed by the MS which consisted of a peak at *m/z* 228 due to the fragment ion corresponding to **3**. Furthermore, the ^{1}H NMR spectrum displayed two doublet at δ 4.94 and 4.75 which were assigned to protons at anomeric carbon atoms, thereby indicating the presence of a disaccharide unit that consisted of a rhamnoside and a glucoside unit, whereas the methyl carbon signal for the rhamnose unit was observed at δ 16.93 (Bombardelli et al., 1975., Breitmaier and Voelter, 1989., Dorman et al., 1970). The α-**L**-*O*-glycosidic linkage between the two sugar units was indicated from the observed *J* values as well as the ^{13}C NMR resonances of the anomeric carbon atoms, which appeared at δ 101.18 and 101.33, respectively (Kasai et al., 1977; 1979). The ^{1}H and ^{13}C NMR spectral features for the disaccharide unit were virtually the same as those observed in the spectra of **2** and this indicated that, like in **2**, glycoside **1** contained a rutinoside residue (Bombardelli et al., 1975., Breitmaier and Voelter, 1989., Dorman et al., 1970). The ^{13}C NMR spectrum with DEPT 135 measurements of compound **1** indicated signals due to 14 carbon atoms in the region δ 16-101. Of these signals, 12 were characteristic resonances observed for the rutinoside sugar residue in compound **2** as reported in the preceeding discussion, also corresponding to literature data for the rutinoside ^{13}C NMR resonances for compound **2** (Bombardelli et al., 1975., Breitmaier and Voelter, 1989. Dorman et al., 1970). The remaining two ^{13}C NMR signals at δ 61.24 and 72.49 were attributed to a dioxymethylene group. These spectral features, as well as the positions of the ^{13}C NMR resonances in the high field region being comparable to those observed for the resveratrolyl unit in compound **2** but having only slight differences led to the conclusion that compound **1** must be having structural features similar to those of **2**, the former compound however bearing an additional hydroxyethane group. Addition of a two carbon atom in compound **1** as compared to compound **2** was also indicated by the MS which exhibited a molecular ion peak at m/z 580, which is 44 amu higher than the M^+ peak for glycoside **2** (*m/z* 536). The additional 44 amu could be accounted for by considering the presence of an additional CH_2CH_2O unit in compound **1**.

As for compound **2**, the position of the rutinoside sugar residue in glycoside **1** was deduced based on the upfield shift of the ^{13}C NMR resonances for C-3' and C-5' in ring A as compared to the corresponding signals in the spectrum of **3**. The position of the rutinoside sugar residue was further corroborated by long range H/C interactions involving the anomeric proton at C-1" as revealed in the HMBC spectrum.

Establishment of the position of the hydroxyethane group at C-4 was facilitated by considering the slight upfield shift of the ^{13}C NMR resonance for C-4 in compound **3** as compared to the corresponding signals in the spectrum of glycoside **2**. Apparently, the substitution could also be at C-5' in ring B which makes structure **1** to be only tentative.

The MS of **1** consisted of the molecular ion peak at *m/z* 580 and a fragment ion peak at *m/z* 536 which corresponds to the M^+ peak for compound **2**, being formed through rearrangement of **1** followed by extrusion of

acetaldehyde. Cleavage of the sugar residue yielded a fragment ion at *m/z* 229 due to the resveratrolyl unit. The MS fragmentation pattern of compound **1** is consistent with the proposed structure.

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