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DESMIFLAVANOSIDE, A NEW BIOACTIVE FLAVONOID GLYCOSIDE ISOLATED FROM *Desmidorchis flava*

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*A new flavonoid glycoside, named desmiflavanoside (**1**), was isolated from the MeOH extract of* Desmidorchis flava *(N.E.Br.) Meve & Liede, and its structure was elucidated based on 1D and 2D NMR spectroscopic techniques as well as mass spectrometry. Preliminary studies demonstrated that although compound **1** is moderately cytotoxic at lower concentrations, its activity plateaus at higher concentrations. Notably, compound **1** demonstrated a significant dose-dependent response to lipid peroxidation and that its antilipid peroxidation was similar (89.6 ± 0.81%) to the known standard (BHT; 91.3 ± 0.49%;* $p < 0.051$*). On the other hand, the flavone glycoside **1** did not show inhibitory effects against urease and acetylcholinesterase enzymes.*

Keywords: *Desmidorchis flava*, desmiflavanoside, antilipid peroxidation, anti-proliferative effect.

Previously, our group reported on the structural elucidation and biological effects of nine new pregnane glycosides isolated from the sap of *Desmidorchis flava* (N.E.Br.) Meve & Liede [1–4]. It was reasoned that in order to isolate further natural products from *D. flava*, the whole plant needed to be extracted and not just individual parts. Thus a crude extract of whole plant of *D. flava* was phytochemically investigated, which resulted in the isolation and characterization of one new flavone named desmiflavanoside (**1**). Subsequently compound **1** was further evaluated for its anticancer, antilipid peroxidation, as well as for its urease and acetyl cholinesterase enzyme inhibitory effects.

The molecular formula of compound **1** (Fig. 1) was confirmed as $C_{27}H_{30}O_{15}$ on the basis of its Q-TOF LC/MS and NMR data. Preliminary inspection of the ^{1}H NMR spectrum illustrated three 1-proton singlets at δ 6.61, 6.27, and 6.02 (each 1H, s) and their corresponding ^{13}C NMR signals at δ 103.5, 94.5, and 99.9, respectively. These three proton signals were assigned as H-3, H-8, and H-6, respectively, in compound **1** and are based on HMBC correlations of H-3 to C-2, C-4, C-10; H-8 to C-6, C-7, C-9, C-10; H-6 to C-5, C-7, C-8, and C-10. Additionally, a 1-proton singlet at δ 12.80, which was not linked to any carbon signal in the HMBC and HSQC spectra, was assigned to the chelated OH at C-5 and which was further confirmed from an IR absorption for the hydroxyl group at 3360 cm^{-1}. Further analysis of the ^{1}H NMR spectrum illustrated three further 1-proton aromatic signals assigned to H-2′ [δ_H 7.41 (1H, d, J = 2.0 Hz); δ_C 113.3 by HSQC], H-6′ [δ 7.40 (1H, dd, J = 7.8, 2.0 Hz); δ 117.9 by HSQC] and H-5′ [δ 7.15 (1H, d, J = 7.8 Hz); δ_C 115.8 by HSQC]. Additionally, these three proton signals were confirmed based on the following HMBC correlations, viz. H-2′ to C-1′, C-3′, C-4′, C-6′; H-6′ to C-1′, C-2′, C-4′, C-5′; H-5′ to C-1′, C-3′, C-4′, and C-6′. Significantly, a strong ^{13}C NMR signal at δ 181.0 was assigned to C-4, which confirms the flavone skeleton [5, 6].

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TABLE 1. Cytotoxic Activity of Compound **1**

Concentration, μM	% Cell viability ± SD	Concentration, μM	% Cell viability ± SD
0	100.05 ± 11.14	25	66.62 ± 6.75
10	75.14 ± 5.7	50	67.88 ± 8.22

TABLE 2. Anti-Lipid Peroxidation of the Compound **1**

Concentration, μM	% Inhibition ± SD	Concentration, μM	% Inhibition ± SD
5	58.2 ± 0.15	30	88.5 ± 2.36
10	68.5 ± 1.21	50	89.6 ± 2.54
20	86.7 ± 1.25		

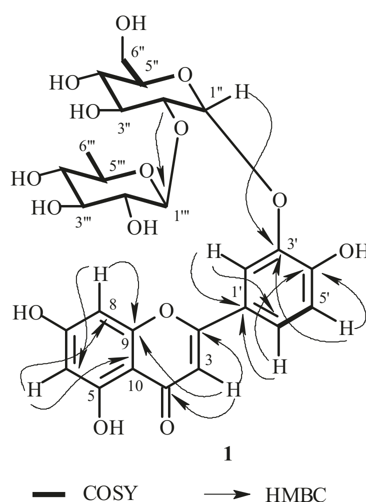

Fig. 1. Key COSY and HMBC correlations of **1**.

Regarding the two sugar units of **1**, the ^{1}H NMR spectrum displayed two anomeric 1-proton signals at δ 5.19 (1H, d, J = 7.8 Hz) and 5.14 (1H, br.s), which was further supported by two anomeric signals in the ^{13}C NMR spectrum (δ 97.8 and 100.5). Additionally, the ^{13}C NMR spectrum displayed 10 further signals for two sugar units in the range of 77.3–18.0 ppm. Furthermore, signals for the typical rhamnose methyl [δ_H 1.08 (3H, d, J = 6.0 Hz); δ_C 18.0], glucose C-6 methylene [δ_H 3.64 (1H, m), 3.45 (1H, m); δ_C 60.5], the two above-mentioned anomeric signals, key COSY, HSQC, and HMBC correlations (Fig. 1), and an intensive comparison of NMR values with those reported in the literature [5, 6] confirmed that the disaccharide moiety is neohesperidoside. Finally, attachment of the neohesperidoside units at C-3′ was confirmed via HMBC correlations of H-1″ to C-3′, and consequent key COSY and HMBC correlations (Fig. 1) confirmed the structure of compound **1** as has been assigned.

The anti-proliferative effect of compound **1** was assessed against the triple negative human breast adenocarcinoma cell line MDA-MB-231. The culture of the breast cancer cells was treated with varying concentrations (10, 25, and 50 μM) of compound **1** for 24 h and the level of cytotoxicity was measured by performing an MTT assay. The results, as shown in Table 1, demonstrate that a low concentration of 10 μM of **1** is able to induce a 25% inhibition of cell proliferation. As the treatment dose is increased to 25 μM, cell viability is reduced by 33.4%. However, no further decline in cell growth is observed by increasing the concentration of compound **1** to 50 μM. This indicates that compound **1** is moderately cytotoxic at lower concentrations but its activity plateaus at higher concentrations.

The results illustrated (Table 2) that different concentrations of compound **1** express a significant dose-dependent response upon the degree of lipid peroxidation. At the lowest concentrations (5 μg/mL), compound **1** exhibited 58.2 ± 0.38%

(Student′s t test; $p < 0.069$) while at the maximum concentration (50 μg/mL), the anti-lipid peroxidation was almost similar (89.6 ± 2.54%) to the known standard (BHT; 91.3 ± 0.49%; $p < 0.051$). The IC_{50} value was determined to be 56.41 ± 0.12 μg/mL. On the other hand, and somewhat disappointingly, flavone glycoside **1** did not show inhibitory effects against urease and acetyl cholinesterase enzymes.

EXPERIMENTAL

General Methods. Multinuclear and multidimensional NMR spectra were recorded on a Bruker NMR spectrometer operating at 600 MHz (150 MHz for ^{13}C) with cryoprobe prodigy. The chemical shift values are reported in ppm (δ) units, and the coupling constants (J) are given in Hz. Optical rotations were measured on a KRUSS P P3000 polarimeter (A. Kruss Optronic, Germany). IR spectra were recorded on a Bruker, ATR-Tensor 37 spectrophotometer. ESI-MS spectra were recorded on a Waters Quattro Premier XE mass spectrometer (Waters, Milford, MA). For TLC, precoated aluminum sheets (silica gel 60F-254, E. Merck) were used. Visualizations of the TLC plates were achieved under UV light at 254 and 366 nm and also by spraying with the ceric sulfate reagent.

Plant Material. The fresh whole plant of *D. flava* (10 kg) was purchased from the local market of Nizwa Souq, Oman and was identified by the plant taxonomist Dr. Syed Abudallh Gilani (Department of Biological Sciences and Chemistry, University of Nizwa, Oman). The voucher specimen (BSHR-01/2014) was kept in the Herbarium of the University of Nizwa.

Extraction and Isolation. The air-dried whole plant powder (3.5 kg) of *D. flava* was exhaustively extracted with MeOH (7.5 L) for 3 weeks. The resulting MeOH extract (245 g) was suspended in H_2O and successively partioned to provide the following major fractions, viz. *n*-hexane (7.9 g), CH_2Cl_2 (102 g), AcOEt (3.7 g), *n*-BuOH (44.3 g), and aqueous (80.5) fractions. The CH_2Cl_2 fraction (102 g) was subjected to column chromatography and eluted with an eluent of increasing polarity, viz. *n*-hexane–EtOAc, EtOAc, EtOAc–MeOH, and MeOH, to provide 14 fractions (DF_1–DF_{14}). Fraction (DF_{11}; 1.5 g), eluted with 70–90% EtOAc–*n*-hexane, was subjected to further careful column chromatography using MeOH–EtOAc (1:9) as eluent to provide the new compound (**1**, 17 mg).

Desmiflavanoside (1). Yellow solid. UV (MeOH, λ_{max}, nm) (log ε): 240, 260, 272. IR (solid, ν, cm^{-1}): 3360, 1610, 980; $[\alpha]_D^{25}$ –6.45° (c 0.93, MeOH). Q-TOF LC/MS *m/z* 595.1652 $[M + H]^+$ (calcd for $C_{27}H_{31}O_{15}$, 595.1667). ^1H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.80 (1H, s, 5-OH), 7.41 (1H, d, J = 2.0, H-2′), 7.40 (1H, dd, J = 7.8, 2.0, H-6′), 7.15 (1H, d, J = 7.8, H-5′), 6.61 (1H, s, H-3), 6.27 (1H, s, H-8), 6.02 (1H, s, H-6), 5.19 (1H, d, J = 7.8, H-1″), 5.14 (1H, br.s, H-1‴), 4.61 (1H, m, H-3″), 4.61 (1H, m, H-5″), 4.43 (1H, m, H-4″), 3.78 (1H, m, H-5‴), 3.70 (1H, m, H-4‴), 3.65 (1H, m, H-2‴), 3.64 (1H, m, H-6a″), 3.57 (1H, m, H-2″), 3.45 (1H, m, H-6b″), 3.16 (1H, m, H-3‴), 1.08 (3H, d, J = 6.0, H-6‴). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 181.0 (C-4), 162.5 (C-2), 161.3 (C-7), 161.3 (C-9), 157.6 (C-5), 148.1 (C-3′), 147.5 (C-4′), 124.5 (C-1′), 117.9 (C-6′), 115.8 (C-5′), 113.3 (C-2′), 103.5 (C-3), 100.5 (C-10), 100.5 (C-1‴), 99.9 (C-6), 97.8 (C-1″), 94.5 (C-8), 77.3 (C-2″), 77.0 (C-3″), 76.8 (C-5″), 72.0 (C-4″), 70.5 (C-2‴), 70.4 (C-4‴), 69.7 (C-3‴), 68.6 (C-5‴), 60.5 (C-6″), 18.0 (C-6‴).

Cell Line and Reagents. Breast cancer cell line MDA-MB-231 was maintained in DMEM (Sigma, St. Louis, MO, USA). The medium was supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured in a 5% CO_2-humidified atmosphere at 37°C. A stock solution of compound **1** was made in DMSO to a final concentration of 4 μM and was always made fresh just prior to the evaluations. A 5 mg/mL stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared in PBS.

MTT Assay. Cells were seeded at a density of 1×10^4 cells per well in a 96-well microtiter culture plate. After overnight incubation, normal growth medium was removed and replaced with either fresh medium (untreated control) or different concentrations of compound **1** in growth medium. After 24 h of incubation, MTT solution was added to each well (0.1 mg/mL in DMEM) and the plate was incubated for a further 4 h at 37°C. Upon termination, the supernatant was aspirated and the MTT formazan, formed by metabolically viable cells, was solubilized in DMSO (100 μL) by mixing for 5 min on a gyratory shaker. The absorbance was measured at 540 nm (reference wavelength 690 nm) on an xMark microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). Absorbance of control (without treatment) was considered as 100% cell survival. Each treatment had six replicate wells.

***In vitro* Anti-Lipid Peroxidation Activity of Compound 1**. Compound **1** was analyzed for its ability to inhibit the level of lipid peroxidation. This was performed by a modified method of thiobarbituric acid reactive substances (TBARS) mentioned by [7]. Using *in vitro* test conditions, the peroxidation of liposome (phosphatidylcholine, Sigma, Germany; 50 mg/mL) was initiated by adding iron chloride (0.001 mM; 200 μL), potassium chloride (300 mM; 200 μL), and the test

compound (50 μL). The peroxidation was initiated by ascorbic acid (0.001 M; 125 μL), after which the reaction mixture was incubated for 30 min at 37°C. Trichloroacetic acid (10% and 0.38%TBA) was added. The glass vials containing the reactants were kept at 95°C in a water bath to imitate the boiling status for 1 h. The resultant change in pink color was recorded on an ELISA at 535 nm absorbance. A control without compound **1** was used as negative control, while butyl hydroxy toluene (BHT) was used as positive control. The inhibition IP% = $(1 - A_t/A_o) \times 100$, where A_t and A_o are compound and control concentrations. The experiment was repeated three times.

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