(DMEM), supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 100 mg/L gentamycin in 5% CO_2 at 37 °C.

2. Chemicals

Randomly methylated β-CD (RAMEB) is a product of Wacker Chemie, Münich, Germany, all the other CDs were obtained from Cyclolab Ltd., Budapest, Hungary. The CDs were dissolved in phosphate buffered saline (PBS) at a concentration between 1–100 mM. The other reagents were purchased from Sigma-Aldrich (Budapest, Hungary).

3. Cytotoxicity assay

The cytotoxic effect of CDs was evaluated by a colorimetric cytotoxicity method i.e. the MTT test (Mosmann 1983). The test was performed as follows: HeLa cells in complete medium were seeded to 24-well plate at a final density of 8×10^4 cells/well. After 2–3 days the medium was removed, the cells were washed with PBS and the CD test solution was added. The cells were then incubated for 30 min at 37 °C in a 5% CO_2-air incubator. After incubation, the samples were removed, and the cells were washed twice with 1 ml PBS. At the end, 0.9 ml medium and 100 µl MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] solution (5 mg/ml) were added to each well. The plates were further incubated for 4 h, the MTT solution was removed and 2 ml of DMSO were added to dissolve the formed formazan crystals. The absorbance of each sample was recorded at 570 nm by a Shimadzu UV-1601 spectrophotometer. Data were expressed as the percentage of viable control cells calculated from the absorbance at 570 nm, corrected for background absorbance.

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A new arbutin derivative from the herb of *Myrothamnus flabellifolia* Welw

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The ethyl acetate soluble fraction of an acetone/water extract of the air-dried aerial parts from *Myrothamnus flabellifolia* Welw. (Myrothamnaceae) was fractionated by a combination of CC on Sephadex LH-20, MPLC on RP-18 material and LPLC on MCI-gel. This procedure has led to the isolation of 2,3-di-*O*-galloylarbutin, a new representative of the rare 2,3-diacylated glucopyranosides. The structure was elucidated with the help of 2D-NMR and ESI-MS experiments. Conformation of D-glucose was established by CZE (capillary zone electrophoresis).

Myrothamnus flabellifolia Welw. (Myrothamnaceae), a species growing in arid areas of Southern and Eastern Africa has a strong ability to survive regular periods of extreme dryness and high temperatures by dehydrating the vegetative tissue to an air-dry state. When getting in contact with water, the hydrated plant material rearranges colour and shape and is starting to flower within a short time period. Therefore, the plant is recorded to belong to the so-called desiccation-tolerant systems or resurrection plants (for review s. Moore et al. 2007). The plant is widely used in traditional South African medicine. Main diseases treated with *Myrothamnus* extracts are cough, colds, influenza, mastitis, haemorrhoids and a topical use for skin and mucosal irritations (Moore et al. 2007). From the phytochemical point of view the plant contains volatile oil with pinocarvone as main constituent (Viljoen et al. 2002), 3,4,5-tri-*O*-galloylquinic acid (Moore et al. 2005), a range of different flavan-3-ols and proanthocyanidins (Petereit et al. 2006) and arbutin (Suau et al. 1991).

The ethyl acetate soluble fraction of an acetone/water (7 + 3) extract of *Myrothamnus fabellifolia* was fractionated by a combination of column chromatography on Sephadex®LH-20, MPLC on RP-18 material and by a final purification by LPLC on MCI® gel. The purified compound **1** (yield 0.0025%, Fig. 1) was identified by ^1H and ^{13}C NMR as double galloylated arbutin. The key correlations were derived by 2D-NMR experiments (HBMC, Fig. 2) and proved the structure of 2,3-di-*O*-galloylarbutin. D-Glucose was established by CZE (capillary zone electrophoresis) after derivatization with *S*-(−)-1-phenylethylamine and reduction with sodiumcyanoborhydride (Noe and Freissmuth 1995). To the best of our knowledge, **1**

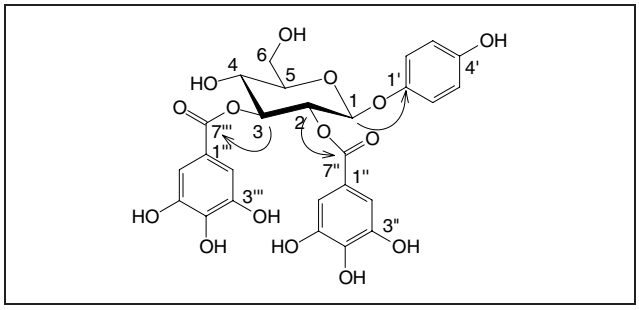

Fig. 1: Structure of 2,3-di-*O*-galloylarbutin (**1**); key correlations in the 2D-NMR experiment (HMBC, s. fig. 2) are marked with arrows

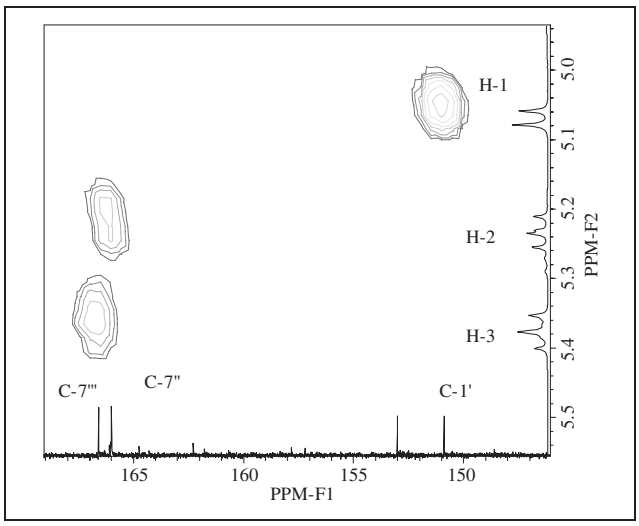

Fig. 2: HMBC of **1**

has not been described from a natural source before, whereas the synthetic product was already known (Haslam and Uddin 1968). Compound **1** extends the range of natural occurring 2,3-acylated glycopyranosides and opens the field for speculation of its role in the biosynthetic pathway of hydrolyzable tannins especially ellagitannins which are constituents of *M. flabellifolia* (unpublished results) and potential substituted ellagitannins. Moreover, this arbutin derivative may act effectively against oxidative stress during the long-time-dehydration period to reduce rate of peroxidation of membrane lipids.

3. Experimental

3.1. Plant material

Dried plant material originating from Northern Province, Republic of South Africa and harvested in August 2004, was obtained from Myro AG, Switzerland. Identification was done by Dr. R. Plüss (Myro AG) and A. Hensel. Identification parameter were resurrection activity and general morphology (Glen et al. 1999). A voucher specimen is retained in the documentation file of the Institute of Pharmaceutical Biology and Phytochemistry (Myro 1). The total hydrochinone content (3.2%) was determined photometically as "Emerson-reaction product" according to the monograph "Bärentraubenblätter (bearberry leaf)" ("Uvae ursi folium" – European Pharmacopoeia 2001).

3.2. General

TLC: All fractions were tested by TLC on silica gel plates [Merck, Darmstadt]; system: EtOAc/HAc/H_2O (90 + 5 + 5); spots were visualized by spraying with "Naturstoff reagent", vanillin/HCl or anisaldehyde/H_2SO_4-reagent. NMR and MS: NMR-spectra were recorded on a Varian AS 400 in CD_3OD; ESI-MS were measured on a Finnigan LCQ.

3.3. Extraction and isolation

The dry pulverised drug material (1 kg) was exhaustively extracted with Me_2CO/H_2O (7 + 3, 8 l) and the combined extracts evaporated *in vacuo* to 2 l, filtered to remove the precipitated chlorophyll, concentrated and defatted with petrol. Successive extractions with EtOAc (5 l) gave, on evaporation of solvent, a solid of 40.4 g. A portion (22.4 g) of the EtOAc fraction was subjected to chromatography on Sephadex® LH-20 (60 × 660 mm; eluents: EtOH 28 l, MeOH 8.7 l, Acetone-water 1.5 l) which afforded 20 subfractions (first 1080 ml of eluent discarded). Subfractions 1–3 and 5–20 containing flavonoid glycosides and proanthocyanidins to be published. Subfraction 4 (653 mg, 4750–5930 ml) was applied to MPLC (RP-18, 18–32–100 µm, 36 × 500 mm, Besta) using MeOH 25% (20 ml/frs.). Subfraction I (1270 ml) yielded 198 mg. Subfraction I was subjected to MCI®-gel chromatography (CHP 20 P, Mitsubishi Corp., Tokyo, 30 × 480 mm) with a 15–50% MeOH linear gradient (10 ml/min.) to afford **1** (frs. 66–88, 25 mg).

3.4. Structural data

^{1}H NMR [MeOD, 400 MHz, δ 3.30 (ppm)]: 3.62 (1 H, dt, J = 9.4, 6.0, 1.8 Hz, H-5), 3.80 (1 H, dd, J = 12.2, 6.0 Hz, H-6b), 3.82 (1 H, t, J = 9.4 Hz, H-4), 3.97 (1 H, dd, J = 12.2, 1.8 Hz, H-6a), 5.14 (1 H, d, J = 8.2 Hz, H-1), 5.31 (1 H, dd, J = 8.2, 9.4 Hz, H-2), 5.45 (1 H, t, J = 9.4 Hz, H-3), 6.65 (2 H, d, J = 8.8 Hz, H-3′/5′), 6.85 (2 H, d, J = 8.8 Hz, H-2′/6′), 6.97 (2 H, *s*, H-2″/6″), 7.02 (2 H, s, H-2‴/6‴).
^{13}C NMR [MeOD, 100 MHz, δ 49.0 (ppm)]: 62.14 (C-6), 69.60 (C-4), 73.37 (C-2), 76.86 (C-3), 78.21 (C-5), 102.23 (C-1), 110.29 (C-2″/6″), 110.37 (C-2‴/6‴), 116.69 (C-3′/5′), 119.74 (C-2′/6′), 120.82 (C-1″), 121.10 (C-1‴), 139.87 (C-4‴), 140.03 (C-4″), 146.31 (C-3‴/5‴), 146.37 (C-3″/5″), 152.14 (C-1′), 154.25 (C-4′), 167.24 (C-7″), 167.84 (C-7‴). ESI-MS (neg. Mode): m/z 575.18 $[M - H]^-$ is in agreement with a molecular formula of $C_{26}O_{15}H_{24}$.

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