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Proanthocyanidins and a phloroglucinol derivative from *Rumex acetosa* L.

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ABSTRACT

From the ethyl acetate soluble fraction of an acetone–water extract of the aerial parts of *Rumex acetosa* L. (Polygonaceae), a variety of monomeric flavan-3-ols (catechin, epicatechin, epicatechin-3-O-gallate), A- and B-type procyanidins and propelargonidins (15 dimers, 7 trimers, 2 tetramers) were isolated with 5 so far unknown natural products. *Dimers*: procyanidin B1, B2, B3, B4, B5, B7, A2, epiafzelechin-(4β→8)-epicatechin, epiafzelechin-(4β→8)-epicatechin-3-*O*-gallate (new natural product), epiafzelechin-(4β→6)-epicatechin-3-*O*-gallate (new natural product), epiafzelechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate, B2-3′-*O*-gallate, B2-3,3′-di-*O*-gallate, B5-3′-*O*-gallate, and B5-3,3′-di-*O*-gallate. *Trimers*: procyanidin C1, epiafzelechin-(4β→8)-epicatechin-(4β→8)-epicatechin (new natural product), epicatechin-(4β→8)-epicatechin-(4β→8)-catechin, cinnamtannin B1, cinnamtannin B1-3-*O*-gallate (new natural product), tentatively epicatechin-(2β→7, 4β→8)-epiafzelechin-(4α→8)-epicatechin (new natural product), and epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate.
Tetramers: procyanidin D1 and parameritannin A1. All compounds were elucidated by ESI-MS, CD spectra, 1D- and 2D-NMR experiments as free phenols or peracetylated derivatives and, in part, after partial acid-catalysed degradation with phloroglucinol.
A more abundant proanthocyanidin polymer was also isolated, purified and its chemical composition studied by ^{13}C NMR.
In addition a so far unknown phloroglucinolglycoside (1-*O*-β-D-(2,4-dihydroxy-6-methoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside) was isolated.

1. Introduction

Rumex acetosa L. (Polygonaceae) is a perennial plant worldwide distributed in areas with temperate climate. The aerial parts of this so called “sorrel” are used within food technology and for phytotherapeutic use. Medicinal applications are related to the tannin content of the material, leading to adstringent effects which are useful for treatment of diarrhoe and skin irritations. Modern phytotherapeutic preparations with nationally registrated drug status in Europe contain extracts of *R. acetosa* for treatment of acute and chronic infections of the upper respiratory system [1].

The aerial parts have been reported to contain flavonoids (rutin, hyperoside, quercitrin, quercetin-3-*O*-glucuronide, avicularin, vitexin, orientin, isoorientin and their acetyl derivatives) [[2] and literature cited therein], 1,8-dihydroxyanthraquinones (chrysophanol and its 8-*O*-glucoside, physcion, physcionanthrone, emodin and its 8-*O*-glucoside, emodinanthrone, aloeemodin, acetoxyaloeemodin) [3,4] oxalic acid, flavan-3-ols with catechin and epicatechin [5], phenolic acids (gallic acid, protocatechuic acid, ferrulic acid, *p*-coumaric acid) and higher amounts of polysaccharides from the rhamnogalacturonan and arabinogalactan type with immunstimulating and antiphlogistic properties [6].

Despite the fact that the aerial parts of *R. acetosa* contain substantial amounts of tannins it seems interesting that no phytochemical details are published on the respective structural features.

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2. Experimental

2.1. Plant material

Dried plant material of *R. acetosa* L. (Herba Rumicis acetosae conc., Ch-B.: 43146115) was obtained from Caesar & Loretz GmbH, Hilden, Germany. Identification was performed by microscopic investigations. A voucher specimen is retained in the documentation file of the Institute of Pharmaceutical Biology and Phytochemistry under the code *Rumex* 1.

2.2. General experimental procedures

NMR spectra of the peracetylated derivatives were recorded in $CDCl_3$ (δ 7.26 and 77.00 ppm) on a Varian Unity plus 600, a Varian INOVA 500 or a Varian m400 spectrometer. Spectra of free-phenolic compounds were recorded in MeOD (δ 3.31 and 49.05 ppm) on a Varian m400 spectrometer. Assignment of rotameric signals are marked with H_R and C_R. MS data were obtained on a Quattro LC mass spectrometer. CD spectra were measured with a Jasco J-815 CD spectrometer in MeOH. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter in MeOH. Analytic TLC was carried out on silica gel aluminium plates (0.2 mm, Merck) using ethyl acetate/water/formic acid (90:5:5) as solvent. Compounds were visualized as red coloured spots by spaying with vanillin–HCl reagent. Preparative TLC of peracetylated compounds was performed on silica gel glass plates (0.5 mm, Merck) using toluene/acetone (7:3) as solvent. Acetylation of compounds was performed in pyridine/acetic acid anhydride (1:1) at room temperature for 24 h in the dark. Acid degradation with phloroglucinol was performed according to the method described by Fletcher et al. [7].

Zone capillary electrophoresis of the carbohydrate part from **28** was performed on a PACE 50101 Beckmann Coulter CE (Palo Alto, U.S.A) with 50 mM sodium borate buffer and 4.4 M acetonitrile at pH 10.3 on a capillary with 50 μm i.d. over 77 cm. Injection 1–5 s, detection 200 nm. Enantioselective separation of (+) and (−)-catechin with 1 mg test sample in MeOH/H_2O (8:2) in a buffer with 20 mM NaH_2PO_4, 20 mM Na_2HPO_4 (pH 7.0), 20 mM γ-hydroxypropylcyclodextrin, 100 mM sodiumdodecylsulfat according to Noe and Freissmuth [8].

2.3. Extraction and isolation

The dried, cut plant material (2.5 kg) was exhaustively extracted with cold acetone/water (7:3, 15 l, Ultraturrax®). The combined extracts were evaporated *in vacuo*, filtered to remove the precipitated chlorophyll, defatted with petroleum benzene and freeze-dried to yield the crude extract (252 g). This extract was partitioned between water and EtOAc. After removal of solvent, the residues were lyophilized to yield 215 g H_2O-soluble fraction (W) and 36 g EtOAc-soluble fraction (E). 35 g of E were fractionated by column chromatography over Sephadex® LH-20 (900×55 mm) using stepwise gradient elution with increasing polarity (ethanol (18 l)-methanol (14 l)-acetone/water 7:3 (5 l)) to give 13 fractions. Fractions were monitored by TLC. Further fractionation was performed using a combination of CC on MCI-Gel® CHP-20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan, 2.5×50 cm), MPLC on RP18 material (3.6×50 cm, 18–32 μm; Besta Technik, Wilhelmsfeld, Germany), MLCCC (Ito Multilayer Coil Separator Extractor, P.C. Inc. Potoay, Maryland, U.S.A., 325 ml column, 1.6 mm i.D. at 800 rpm and 1 ml/min flow rate) with EtOAc–water (1:1, upper phase) as mobile phase, FCPC (Fast Centrifugal Partition Chromatography) on a CRC Kromaton system (Kromaton Technologies, Angers, France) at 1.600 rpm, 25 ml/min flow rate with water–EtOH–hexane–EtOAc (7:2:1:8, upper phase) as mobile phase, preparative HPLC on Silica Uptishere Diol, 6 μm, 250×21.2 mm or prep. TLC.

A portion of the above Sephadex-fraction 2 (3.8 g) was purified by MLCCC and CC on MCI-Gel (20–80% MeOH linear gradient; system 1) followed by MPLC (20–80% MeOH linear gradient) to yield **28** (102 mg). A part of fraction 3 (1.7 g) was fractionated by MLCCC followed by purification on MCI-Gel (system 1) to yield **1** (36 mg) and **2**. Compound **2** was finally purificated by prep. HPLC (ACN/MeOH/water-gradient) to yield 26 mg. A portion of fraction 4 (510 mg) was fractionated using a step gradient on MCI-Gel (20–50% MeOH, 50% MeOH isocratic, 50–80% MeOH; system 2) followed by prep. TLC of a peracetylated subfraction of impure **8** to yield **8a** (30 mg).

A part of Sephadex-fraction 5 (1 g) was at first fractionated using MCI-Gel (system 1, MeOH 20%, 2 L, than MeOH 80% 2 L, cleaning with MeOH 100%500 mL) which yielded two proanthocyanidin containing subfractions (a and b). Subfraction a contained compounds **4**–**7** which were isolated as their peracetates **4a**–**7a** after preparative TLC (KG 60 F_{254}, 0.5 mm layer, mobile phase toluene:acetone (7:3 V/V) of the peracetylated subfraction a. Subfraction b was purified by MPLC (system 2, with MeOH 20%, 2 L, than MeOH 80%, 2 L, than cleaning with MeOH 100%, 500 mL) yielding pure compound **3** (204 mg) An additional slightly red spot was observed in an accompanying MPLC subfraction after spraying with vanillin/HCl reagent. Complete acetylation of this subfraction and purification with prep. TLC (KG 60 F_{254}, 0.5 mm layer, mobile phase toluene:acetone (7:3 V/V) yielded **19a** (15 mg).

Parts of Sephadex-fraction 6 (300 mg) were separated by FCPC (water–EtOH–hexane–EtOAc 7:2:1:8 (upper phase) followed by peracetylation of all subfractions yielded the peracetates of **9**, **13**, **14**, **15** and **20** (**9a**, 14 mg; **13a**, 17 mg; **14a**, 12 mg; **15a**, 14 mg; **20a**, 25 mg).

Sephadex-fraction 7 (300 mg) was fractionated by FCPC (H_2O/EtOH/hexane/EtOAc 7:2:1:8 (upper phase) to give pure **10** (32 mg), **24** (10 mg), **11** (22 mg), **23** (38 mg) and **21** (18 mg). A subfraction showed next to spots from **23** and **21** a third slightly red spot on the TLC plate. After peracetylation of that subfraction followed by preparative TLC (conditions see above) the peracetate **16a** (7 mg) was isolated. A portion of Sephadex-fraction 8 (1.8 g) was subfractionated by FCPC (H_2O/EtOH/hexane/EtOAc 7:2:1:8 (upper phase). From the resulting subfractions compounds **17** (80 mg), **12** (520 mg), **23** (560 mg) and compound **26** (15 mg) were isolated in a pure state. Sephadex-fraction 9 (400 mg) was again fractionated by the above described FCPC system to yield pure **25** (69 mg) and **27** (45 mg). 1.5 g of Sephadex-fraction 11 was fractionated by FCPC (H_2O/MeOH/EtOAc 5:2:5) to give **18** (194 mg) from subfraction 1. Compound **22** was enriched in