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Analysis of phenolic glycosides and saponins in *Primula elatior* and *Primula veris* (primula root) by liquid chromatography, evaporative light scattering detection and mass spectrometry

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Abstract

This paper describes the first liquid chromatographic method suitable for the simultaneous determination of bioactive compounds, saponins and phenolic glycosides, present in *Primula elatior* and *Primula veris*, including the NMR data of primulaverin and primeverin. Optimum separations were obtained with a Synergi 4 μm Fusion RP 80 Å column, using 0.025% TFA in water and 5% acetonitrile in methanol as mobile phase. Saponins were detected by evaporative light scattering detection (ELSD), whereas the phenolic glycosides were monitored by UV at 210 nm. The method was validated for repeatability ($\sigma_{rel} \leq 4.5\%$), precision (intra- and inter-day variation ≤5.0%), accuracy (recovery ≥97.1%) and sensitivity (LOD ≤22 ng (UV) and ≤38 ng (ELSD) on-column, respectively). LC–MS experiments in negative APCI mode allowed a final peak assignment. Both *Primula* species could easily be differentiated by their saponin pattern. The total saponin content was highest in *P. veris* roots (max. 14.9%), the aerial parts or *P. elatior* contained significantly less amounts; primeverin (0.64–1.42%) showed to be the most dominant phenolic glycoside.

Keywords: *Primula veris*; *Primula elatior*; ELSD; Mass Spectrometry; Saponins; Phenolic glycosides

1. Introduction

In the current, fourth edition of the European Pharmacopoeia two species are listed as source for primula (formerly primrose) root, *Primula elatior* L. Hill. (vernacular name: oxlip) and *Primula veris* (L.) (vernacular name: cowslip) [1]. Both plants, belonging to the Primulaceae family, are hardy perennial herbs native to Europe and temperate Asia; they can be distinguished by height (*P. elatior* is slightly taller) and coloration of flowers (oxlip has sulphurous-yellow, cowslip has pale-yellow flower heads). Hybridisation occurs quite often, which is no problem as the roots and rhizomes of both species are officinally used as Primulae radix [2].

The main indication for primula root is the treatment of respiratory tract problems, such as cough, asthma, bronchitis and catarrh. Responsible for these actions are secretolytic and secretomotoric triterpenoid saponins (Fig. 1), like priverosaponin B-22-acetate (**3**), primulasaponin I (**5**) and II (**4**), which are present in the plant material in rather high amounts up to 12% [3]. Safety and efficacy of primrose extracts rich in saponins have been demonstrated in a number of pharmacological studies, which showed potent anti-asthmatic, anti-inflammatory and anti-viral properties [4–7]. Phenolic glycosides (Fig. 1), mainly primulaverin (**1**) and primeverin (**2**), are characteristic compounds for the genus *Primula*. They degrade during storage in presence of the enzyme primverase, resulting in the typical fragrance of the drug [2]. Thus, they not only serve as marker compounds but also as indicators of the age of the plant material.

Analytical methods for the analysis of primula root are quite rare, most likely because the saponins possess no chromophore and therefore are difficult to detect by UV. Besides determinations by TLC [8] and spectrophotometry [9,10], only one HPLC method for the analysis of primula saponins has been reported [11]. Being published nearly 20 years ago, the latter is still the most recent analytical report on Primulae radix, but it does not enable the determination of all relevant saponins or the simultaneous analysis of saponins and phenolic glycosides. In addition the method was not validated. Thus, the study presented herein aimed to improve the established HPLC methodology in respect to sensitivity, number of analytes, validity and ease of operation. By means of ELSD (which previously showed to be ideal for the analysis of low absorbing compounds; [12]) and MS, the

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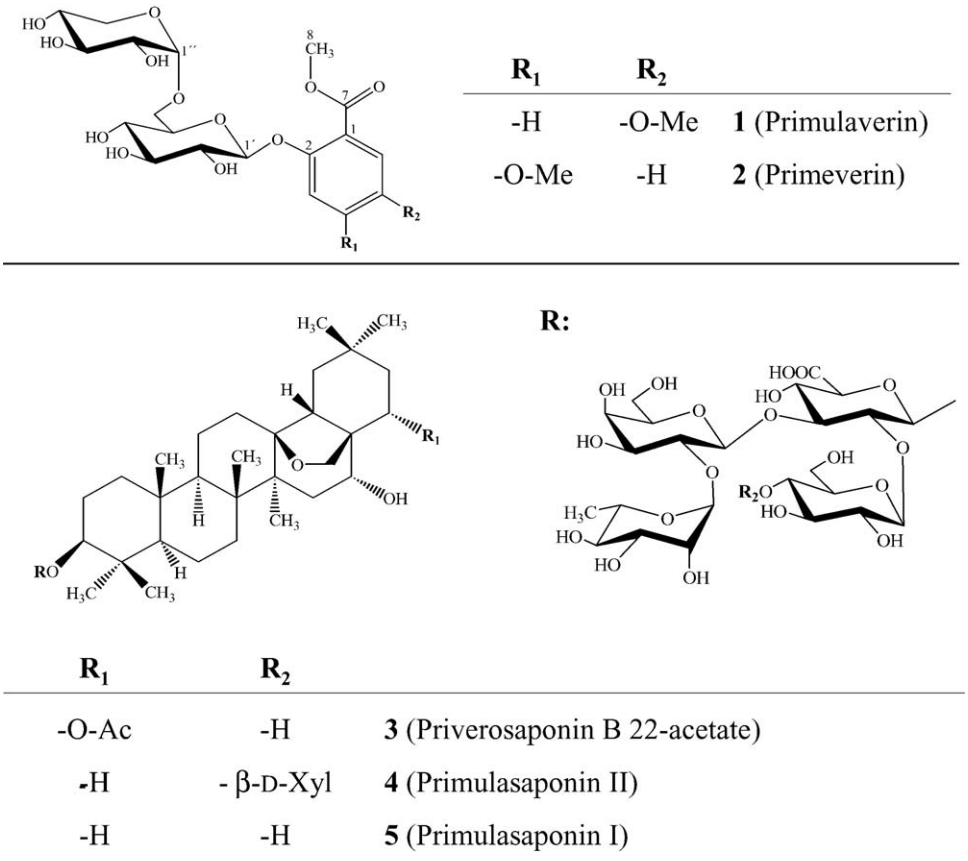

Fig. 1. Structures of compounds **1**–**5**.

distribution of phenolic glycosides and saponins in *P. veris* and *P. elatior* and different plant parts was investigated.

2. Experimental

2.1. Materials

Compounds **1**–**5** were isolated by chromatographic techniques from commercial plant material. Identity and purity (>95%) of the isolated compounds were confirmed by TLC, HPLC, MS, 1D- and 2D-NMR experiments. A final structural assignment of **3**–**5** was achieved by a comparison of the obtained NMR shifts with literature values [13–15]; for compounds **1** and **2** no NMR data has been published (see Section 2.2). Besides **1**–**5** another major compound (**6**) was present in all root samples analyzed. It shows similar properties like the aforementioned saponins (e.g. mass range and polarity, no UV absorption), with a molecular weight of *m*/*z* 1178; thus, it was assumed to be a saponin. A saponin with this molecular mass has not been reported from primula so far and its isolation and structural elucidation is currently in progress.

Plant material (*P. veris*: PV-1 to PV-3; *P. elatior*: PE-1 to PE-3) was collected between May and June 2004 in several locations in Austria (Wies: PE-1, PV-1; Innsbruck: PE-2, PE-3, Landeck: PV-3) and Germany (Merseburg: PV-2) and authenticated by Prof. Dr. C. Zidorn (Institute of Pharmacy, University of Innsbruck, Austria). Voucher specimens of all samples are deposited at the same institution.

Solvents (acetonitrile, methanol) were of HPLC grade and purchased from Merck (Darmstadt, Germany); water was purified by nanopure filtration prior to HPLC use.

2.2. NMR data of primulaverin and primeverin

NMR spectra of primulaverin (**1**) and primeverin (**2**) were recorded on a Bruker-AM-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C). Both substances were dissolved in DMSO-d_6 and spectral data were referenced to solvent residual signals at $\delta_H = 2.50$ ppm and $\delta_C = 39.5$ ppm.

1: ^{1}H NMR: *methoxy salicylic acid ester moiety*; δ 7.32 (1H, d, $J = 9.0$ Hz, H-3), 7.13 (1H, d, $J = 3.0$ Hz, H-6), 7.10 (1H, dd, $J = 9.0$, 3.0 Hz, H-4), 3.80 (3H, s, H-8), 3.75 (3H, s, H-9), *primverosyl moiety*: δ 4.70 (1H, d, $J = 7.5$ Hz, H-1′), 4.19 (1H, d, $J = 7.5$ Hz, H-1″), 3.97 (1H, brd, $J = 11.0$ Hz, H-6$_a$′), 3.68 (1H, dd, $J = 11.0$, 5.5 Hz, H-5$_a$″), 3.56 (1H, dd, $J = 11.0$, 6.5 Hz, H-6$_b$′), 3.49 (1H, t, $J = 6.5$ Hz, H-5′), 3.26 (3H, m*, H-2′,3′,4″), 3.17 (1H, d, $J = 5.0$ Hz, H-4′), 3.06 (1H, dd, $J = 8.0$, 4.5 Hz, H-3″), 3.00 (1H, dd, $J = 7.5$, 4.5 Hz, H-2″), 2.95 (1H, d, $J = 11.0$ Hz, H-5$_b$″); ^{13}C NMR: *methoxy salicylic acid ester moiety*; δ 166.3 (C-7), 153.6 (C-5), 150.2 (C-2), 122.3 (C-1), 119.0 (C-4), 118.7 (C-3), 113.9 (C-6), 55.3 (C-9), 51.7 (C-8), *primverosyl moiety*: δ 103.6 (C-1″), 101.7 (C-1′), 76.1 (C-3″), 76.0 (C-3′), 75.6 (C-