A Detailed View on the Constituents of EPs® 7630

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- *Pelargonium sidoides*
- Geraniaceae
- oligomeric prodelphinidins
- benzopyranones
- sulfates
- secondary metabolites

Abstract

Extracts of *Pelargonium sidoides*, commonly known as EPs® 7630, are produced by extraction of milled roots with 11% (w/w) ethanol in water. This solvent leads to a spectrum of constituents which differs significantly from extracts obtained by extraction with non-polar solvents. EPs® 7630 is composed of six main groups of constituents, namely unsubstituted and substituted oligomeric prodelphinidins, monomeric and oligomeric carbohydrates, minerals, peptides, purine derivatives and highly substituted benzopyranones. The oligoprodelphinidins, frequently supposed to be compounds with unspecific tanning interactions, show, in contrast to other polyphenols, an amazing variety of substructures and connectivities which results in an uncommon diversity even at a low degree of polymerization. Three distinct purine derivatives, second messengers and probably intermediates of DNA synthesis, were identified and characterized by phytochemical means. The main benzopyranones of EPs® 7630 are highly oxygenated at the phenyl moiety (three to four oxygens) and in addition sulfated at distinct positions. A disulfate of 6,7,8-trihydoxybenzopyranone has been identified for the first time in plants. Taken together, these constituents amount to about 70 to 80% of the total weight of EPs® 7630, the active ingredient of the phytopharmaceutical Umckaloabo® (Iso Arzneimittel Ettlingen).

Introduction

Roots of *Pelargonium sidoides* DC. and *P. reniforme* Curt. (family Geraniaceae) are traditionally used in South African folk medicine for the treatment of a variety of diseases [1], [2]. At the end of the 19th century, this ethnomedicine came to Europe more or less by serendipity and has become a part of the pool of herbal medicinal products for a long period of time especially in Germany. *Pelargonium* ssp.-containing products have been available for almost 100 years. But only the modern phytopharmaceutical preparation EPs® 7630, a liquid herbal drug preparation from the roots of *Pelargonium sidoides* (1 : 8 – 10), extraction solvent: ethanol 11% (w/w), became a successful pharmaceutical product. It has been used in the treatment of colds and infections of the respiratory tract during the last 5 – 10 years enabled by new phytochemical [3], [4], pharmacological [5], [6], [7], [8], and clinical studies [9], [10], [11], as well as thorough documentation of pharmaceutical quality [12]. Over the last years, several papers have been published which describe constituents of *Pelargonium* species, such as phenols, phenolic acids, benzopyranones, flavonoids and flavan-3-ols as possible active ingredients. Unfortunately, these observations are not always applicable to the therapeutically used extract EPs® 7630. Reasons for this confusion include a sometimes unclear assignment of the plant material, failure to declare if a single plant genus or a mixture of *Pelargonium* species has been investigated, utilization of aerial parts [13] instead of roots or inappropriate use of non-polar extraction solvents [14] in place of aqueous ethanol. Therefore, it is the aim of this paper to present a comprehensive profile of the ingredients of EPs® 7630 to indicate which compounds are indeed contained in the extract and may consequently be responsible for its activity.

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Material and Methods

Chemicals and biochemicals

All solvents and chemicals were purchased from VWR or Sigma-Aldrich with required purity.

Plant material

Roots of *Pelargonium sidoides* were collected in South Africa (e.g., Eastern Cape). The dried material was tested in a battery of phytochemical and biochemical methods to confirm the quality and identity of the herbal material. Pharmacognosy was done by P. Riedl [DHU Arzneimittel GmbH & Co. KG, Karlsruhe (Germany)]. A voucher specimen of every lot is deposited in the Department of Pharmacognosy to be kept for ten years.

Extraction and isolation

EPs® 7630 was produced according to Erdelmeier et al. [15].

LC-DAD-MS analysis

LC-DAD-MS data were obtained with an Agilent 1100 series HPLC system consisting of an auto sampler, high pressure mixing pump, column oven and DAD detector connected to a Bruker Esquire HCT ion trap mass spectrometer. HPLC conditions: column: Phenomenex LUNA and Synergy, respectively; solvent system: A – 3 % MeOH in water + 1 % acetic acid, B – 70 % MeOH + 1 % acetic acid; gradient: 0 – 5 % B for 5 min, 5 – 100 % B for 40 min, 100 % B for 15 min; flow rate 1 mL/min; injection volume: up to 50 μL, sample concentration: about 1 mg/mL in eluent A. ESI conditions: alternating polarity with MS^2 fragmentation of the two most intensive signals.

NMR spectroscopy

^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker DPX200 spectrometer (200 MHz) in DMSO-d_6 unless otherwise mentioned. The chemical shifts refer to TMS (^{1}H NMR) or to DMSO-d_6 (^{13}C NMR, δ = 39.5 ppm), respectively.

Quantification of minerals

Sodium, magnesium, potassium, calcium and several other metals were analyzed by ICP-MS-AES at Mikroanalytisches Labor Pascher, An der Pulvermühle 1, D-53 424 Remagen–Bandorf, Germany.

Quantification of carbohydrates by HPLC-RI

Hydrolysis: A suspension of 2 mg extract in 400 μL 2 M trifluoroacetic acid (TFA) in water was heated in sealed glass vials to 120 °C for up to 90 min. The reaction mixture was three times evaporated by a nitrogen stream under sequential addition of water to remove all TFA. Finally, the residue was taken up in 800 μL of water, filtered, and the carbohydrates quantified by HPLC. Column: Rezex RPM Monosaccharide, 300 × 7.8 mm, 8 μm, ion form: Pb^{2+}, order Nr. Phenomenex OOH-0135-KO and precolumn AJO-4492, 4 × 3 mm; eluent: distilled water; flow rate: 0.6 mL/min; column temperature: 75 °C; detection: refractive index detector.

Quantification of monosaccharides by anthrone colour reaction

The method is described in Ref. [16].

Quantification of peptides

Analysis of amino acids (AAs) was performed by HPLC and fluorimetric detection using a DIONEX P680 pump, DIONEX ASI-100 auto sampler, and a Merck-Hitachi F-1050 fluorescence detector. Excitation wavelength: 335 nm; emission: 445 nm; column: Luna (1812) injection volume 100 μL; eluent A: 0.05 M NaH_2PO_4 pH 5.5/MeOH (80/20; v/v), eluent B: 0.05 M NaH_2PO_4 pH 5.5/MeOH (20/80; v/v), gradient: 0 to 63 min, 0 → 100 % B; flow rate 0.5 mL/min. Samples dissolved in ethanol/water solutions were hydrolyzed with 1.5 N HCl at 100 °C for 1.5 h and the reaction mixture neutralized with 10 M NaOH. With a 0.4 M boric acid buffer, adjusted with NaOH solution to pH 10.4, samples were further diluted. AAs were detected after precolumn derivatization with *o*-phthaldialdehyde reagent (OPA, 600 μL sample + 240 μL reagent + 60 μL 0.4 M boric acid buffer pH 10.4). Quantification was established by linear regression with a calibration curve of arginin.

Quantification of oligoprodelphinidins

Oligoprodelphinidins were quantified by reaction with molybdato reagent according to the described DAB 2000 method for total phenolics.

Purine derivatives

500 g dried roots from *Pelargonium sidoides* were extracted with 5 kg 11.2 % aqueous ethanol. The mixture was filtered, the filtrate evaporated under vacuum to remove the organic solvent. The remaining water phase was freeze-dried. An aliquot (50 g) of the lyophilisate was dissolved in water, filtered over HP20, and the filtrate chromatographed on PVP. Fractions containing the purines were concentrated and the anions adsorbed on a strongly basic ion exchanger. The resin was eluted with 1 N hydrochloric acid, the eluate neutralized (pH 5) and freeze-dried. The residue was taken up in MeOH, the suspension filtered, and the filtrate evaporated to remove the organic solvent. The remaining aqueous phase was separated by HPLC (column: Hibar Lichrospher 100, RP-18; eluent: water/acetonitrile/acetic acid (99 : 1 : 1; v/v/v); detection: UV 254 nm).

cAMP: 185 mg (0.037 % isolated from dried roots, 0.64 % of EPs® 7630 by HPLC analysis); was identified by matching ^{1}H- and ^{13}C-NMR data of Aldrich-Library 3,229 A and commercially available material (Sigma A-9501 lot 22K1762).

cGMP: 240 mg (0.048 % of dried roots, 0.68 % of EPs® 7630), was identified by comparison of ^{1}H- and ^{13}C-NMR data with those of commercially available material (Fluka 51055 lot 402 854/1 34 502 041).

1-Methyl-cGMP: 85 mg (0.017 % of dried roots, 0.35 % of EPs® 7630); was identified by analysis of ^{1}H- and ^{13}C-NMR data (data are not available in the literature). The extra methyl group was located by an HMBC experiment. ^{1}H NMR ($NaOD/D_2O$, 200 MHz): δ = 3.44 (3H, s, 1-Me, HMBC to C-2, C-6), 4.22 – 4.68 (6H, m, H-2′ to H-5′), 5.82 (1H, s, H-1′, HMBC to C-4, C-8), 7.87 (1H, s, H-8, HMBC to C-4, C-5, C-6); ^{13}C NMR ($NaOD/D_2O$, 50 MHz): δ = 31.4 (CH_3, 1-Me), 70.5, 70.6 (CH_2, C-5′), 74.9, 75.0 (CH, ribosyl-H), 77.5, 77.6 (CH, ribosyl-H), 82.0, 82.1 (CH, ribosyl-H), 97.4 (CH, C-1′), 118.8 (C, C-5), 140.8 (CH, C-8), 151.8 (C, C-4), 157.6 (C, C-2), 161.5 (C, C-6).

Fractionation of EPs® 7630

Ultrafiltration: 400 g EPs® 7630 were taken up in 710 g water and 996 g 96 % EtOH, the suspension filtered (Seitz Supra 1500), and the solution submitted to successive steps of ultrafiltration across membranes with molecular cut-offs of 30 kDA, 10 kDa

and 3 kDa. The final filtrate was evaporated on a vacuum evaporator to remove the organic solvent and finally freeze-dried (130 g, 32.5 %).

Benzopyranones

Milled dried roots from *Pelargonium sidoides* (15 kg) were extracted with water. The aqueous extract was saturated with ammonium sulfate and extracted three times with 2-butanone/ethanol (3 : 2; v/v). The combined organic phases were evaporated, the residue taken up in water and the solution chromatographed over HP20 by reducing the polarity of the eluent to 10 % ethanol. For further separation of the compounds **1**, **2**, **3**, **4**, **5a**, **12a** and **12b,** the fractions were treated as described below.

7-Hydroxy-5,6-dimethoxy-2*H*-1-benzopyran-2-one (umckalin, 1): The fractions containing **2** were concentrated and decomposed to **1** at elevated temperature (50 °C, 3 h), washed with water and recrystallized from water/methanol: yield: 18.1 g, m. p. 147 – 148.5 °C (146 – 147 °C, Ref. [11]); ^{1}H- and ^{13}C-NMR in acetone-d_6 corresponded to the data of Ref. [17]. For convenience NMR values are presented in DMSO-d_6 in ▶ **Table 2**.

5,6-Dimethoxy-7-(sulfooxy)-2*H*-1-benzopyran-2-one (umckalin sulfate, 2): The fractions containing **2** and **4** were adjusted to pH 7.5 with diluted potassium hydroxide solution and concentrated. Purification was performed by column chromatography on Sepabeads Sp-825 (water → 20 % ethanol), adjustment to pH 7.5 again and a further chromatography on LH-20 (methanol) gave the monopotassium salt of **2**, which was recrystallized from ethanol/water: yield: 8.5 g slightly yellow powder. ^{1}H- and ^{13}C-NMR (DMSO-D_6) see ▶ **Table 2** (Ref. [18] gives NMR data in CD_3OD).

7,8-Dihydroxy-5,6-dimethoxy-2*H*-1-benzopyran-2-one (3): The fractions containing **4** were concentrated and decomposed to **3** at elevated temperature (50 °C, 3 h). Purification was performed by column chromatography on HP-20 (20 % ethanol → 35 % ethanol) and on Sepabeads Sp-850 (10 % ethanol → 30 % ethanol). Crude **3** was washed with ethanol and recrystallized from water: yield: 2.4 g, m. p. 202 – 203 °C. ^{1}H- and ^{13}C-NMR (DMSO-d_6) see ▶ **Table 2**.

7-Hydroxy-5,6-dimethoxy-8-(sulfooxy)-2*H*-1-benzopyran-2-one (4): Chromatography as described for compound **2** afforded the monopotassium salt of **4**: yield: 4.4 g yellow crystals. ^{1}H- and ^{13}C-NMR see ▶ **Table 2**

8-Hydroxy-5,6,7-trimethoxy-2*H*-1-benzopyran-2-one: To define the position of the sulfate group in **4,** the compound was methylated with MeI/K_2CO_3 in DMF at 60 °C for 1 h. The intermediate sulfate was saponified by addition of concentrated HCl, the resulting phenol extracted with ethyl acetate, and the organic solvent evaporated under vacuum. The product was finally purified by chromatography over silica (eluent: ethyl acetate) and crystallization from methanol. ^{1}H- and ^{13}C-NMR data were identical to those of Ref. [19].

6,8-Bis(sulfooxy)-7-hydroxy-2*H*-1-benzopyran-2-one (**5a** and **b**,**c**)

Tripotassium salt 5a: The fractions containing **5** were adjusted to pH 10.7 with dilute potassium hydroxide solution, and diluted with ethanol. The precipitate was collected, washed with ethanol/water 1/1 and dried. The tripotassium salt of **5a** (yield: 33.2 g) was obtained. ^{1}H- and ^{13}C-NMR see ▶ **Table 3**.

Dipotassium salt 5b: 5a was dissolved in water, adjusted to pH 7 and diluted with ethanol. The solid was filtered off, washed with ethanol/water 1/1 and dried to afford the dipotassium salt of **5b**. ^{1}H- and ^{13}C see ▶ **Table 3**.

Mixed ammonia/potassium salt (5c): 28.25 g of the above ultrafiltrate were chromatographed on 480 g LH_{20} (5 cm × 92 cm) using 1 % MeOH in water as eluent. Fractions of approx. 50 mL were collected, analyzed by TLC (ethyl acetate/acetic acid/formic acid/water = 50 : 11 : 11 : 27). Fractions of similar composition were combined. The fraction containing the compound at R_f = 0.6 (vials 39 to 43) was freeze dried and the residue extracted 2 times with 20 mL 50 % MeOH to remove polar impurities. The formed solid was collected on a glass frit, washed two times with 20 mL MeOH and finally dried at 40 °C *in vacuo* to give 250 mg (0.9 %) of disulfate **5b**. ^{1}H- and ^{13}C-NMR see ▶ **Table 3**; ESI-MS (pos. ion mode): m/z = 355.0 → 275.0 → 194.9; ESI-MS (neg. ion mode): m/z = 272.9 → 192.9; elemental analysis, calcd. as mixed ammonia potassium salt: C 25.53, H 1.27, K 15.39, N 1.10, S 15.1; found: C 25.28, H 1.56, K 15.7, N 0.84, S 13.3.

6,7-Dihydroxy-8-(sulfooxy)-2*H*-1-benzopyran-2-one (12a) and 7,8-dihydroxy-6-(sulfooxy)-2*H*-1-benzopyran-2-one (12b): The fractions containing **12a** and **12b** were adjusted to pH 7.5, concentrated and purified by column chromatography on LH-20 (methanol). The mono potassium salts of **12a** and **12b** were separately crystallized from methanol to afford 435 mg of **12a** and 285 mg of **12b.** ^{1}H- and ^{13}C see ▶ **Table 3**.

6,7,8-Trihydroxy-2*H*-1-benzopyran-2-one (13): Crude compound **5a** (20 g) was dissolved in hot water, filtered, acidified with HCl and stirred for 20 h at 40 to 50 °C. Compound **13** precipitated on cooling and was recrystallized from water; yield: 5.9 g; m. p. 260 °C (dec.; ref. 20: 258 °C); ^{1}H- and ^{13}C-NMR in acetone-d_6 correspond to those in Ref. [11].

6,8-Bis(sulfooxy)-7-methoxy-2*H*-1-benzopyran-2-one (6): 38 mg **5b** (0.088 mmol) were dissolved in 4 mL 10 % MeOH in water and successively treated with a 200-fold excess of 1 M diazomethane in ether. The reaction mixture was washed twice with 1 mL diethyl ether and freeze-dried, the residue agitated with 2 times 10 mL isopropanol, and the solid residue dried under vacuum at 40 °C to afford 6; yield: 34 mg (86.7 %); elemental analysis, calcd. as dipotassium salt monohydrate: C 25.97, H 1.74; found: C 26.09, H 2.01; ^{1}H- and ^{13}C-NMR see ▶ **Table 3**.

6,8-Dihydroxy-7-methoxy-2*H*-1-benzopyran-2-one (7): 33 mg (0.074 mmol) **6** were dissolved in 1 % aqueous $KHSO_4$ and heated for 7 h at 100 °C. The reaction mixture was extracted 3 times with 20 mL ethyl acetate, the combined organic phases washed with 10 mL saturated NaCl solution, dried over Na_2SO_4 and evaporated to afford **7**; yield: 14 mg (90.6 %); ^{1}H- and ^{13}C-NMR see ▶ **Table 3.**

6,8-Dibenzyloxy-7-methoxy-2*H*-1-benzopyran-2-one (8): ^{1}H-NMR ($CDCl_3$): δ = 7.55 (d, J = 9.5 Hz, H-4), 7.58 – 7.34 (m, 2 × C_6H_5), 6.72 (s, H-5), 6.32 (d, J = 9.5 Hz, H-3), 5.24 (s, 8-OCH_2), 5.13 (s, 6-OCH_2), 3.95 (s, 7-OCH_3); ^{13}C-NMR ($CDCl_3$): δ = 160.3 (C-2), 149.1 (C-6), 147.0 (C-7), 143.5 (C-8a), 143.3 (C-4), 140.1 (C-8), 136.8, 136.3 (C-1′, C-1″), 128.7, 128.5, 128.4, 127.3 (C-2′/6′, C-3′/5′, C-2″/6″, C-3″/5″), 128.24, 128.20 (C-4′, C-4″), 115.2 (C-3), 114.3 (C-4a), 106.4 (C-5), 76.0 (8-OCH_2), 71.5 (6-OCH_2), 61.6 (7-OCH_3).

Methyl 3′,5′-bis(sulfooxy)-2′,4′-dimethoxycinnamate (10): 530 mg of disulfate **5b** (1.18 mmol) were dissolved in 50 mL 10 % MeOH in water and successively treated with a 200-fold excess of 1 M diazomethane in ether. The water phase was washed 3 times with 25 mL diethyl ether and then freeze-ried (620 mg). 250 mg of crude product were chromatographed over 80 g LH_{20} (1.6 × 41 cm, about 7 mL per fraction) using water as eluent. Fractions 27 and 28 were combined and freeze-dried to afford compound **10**; yield: 77 mg (13.3 %); elemental analysis, calcd. as dipotassium salt dihydrate: C 27.37, H 3.06; found: C 27.31, H 2.77; ^{1}H-

and ^{13}C-NMR see **Table 4**; ESI-MS (pos. ion mode): *m/z* = 415.1 → 383.0 → 303.1 → 223.0; ESI-MS (neg. ion mode): *m/z* = 413.0 → 333.1 → 252.9 → 238.3.

***E*- and *Z*-Methyl 3′,5′-dihydroxy-2′,4′-dimethoxycinnamate (11):** A solution of 30 mg (0.060 mmol) **10** dissolved in 15 mL 3% MeOH was acidified by addition of 0.75 mL acetic acid and kept 7 h at 100 °C. Afterwards the reaction mixture was extracted with 50 mL TBME, the organic phase washed 2 times with 30 mL water, dried over Na_2SO_4 and evaporated to afford **11**; yield: 5.5 mg (36.0%); ^{1}H- and ^{13}C-NMR of **11a** see **Table 4**; ^{1}H-NMR **11b** (DMSO-d_6, 600 MHz): δ = 3.68 (3H, s, 2′-OCH_3), 3.72 (3H, s, 1-OCH_3), 3.74 (3H, s, 4′-O CH_3), 6.34 (1H, d, *J* = 16.2 Hz, H-2), 6.62 (1H, s, H-6′), 7.72 (1H, d, *J* = 16.2 Hz, H-3), 9.04, 9,18 (2H, broad, Ar-OH); ESI-MS (pos. ion mode): *m/z* = 277.1 → 223.0 → 207.9; ESI-MS (neg. ion mode): *m/z* = 252.9 → 237.9 → 222.9.

Results and Discussion

EPs® 7630 is produced by extraction of dried roots of *Pelargonium sidoides* with 11% aqueous ethanol. This leads to a specific mixture of polar compounds derived from the primary and secondary metabolism of the plant (**Fig. 1**).

About 40% of the dried extract can be attributed to oligomers of the proanthocyanidin family, mainly based on gallocatechin and epigallocatechin entities connected by B- and A-type bonds (**Fig. 2**). Additionally, some oligomers show mono-derivatization at as yet undefined positions [4] of the phenolic functions. The detected isomers of oligoprodelphinidin dimers implement a diversity of extraordinary broadness. It seems that at least all currently known stereoisomers (**Table 1**) are present in the extract. Additional isomers may be present but still await structure elucidation [4]. Oligomers of higher masses (up to 16 mer can be detected with electrospray MS, see **Fig. 3**) and similar connectivities can be expected to be contained in EPs® 7630 indicating that *Pelargonium sidoides* prodelphinidins represent a rich diversity of structures.

The second large group of EPs® 7630 constituents are typical members of the primary plant metabolism, which are extracted because of the very polar conditions employed. To enable the quantification of carbohydrates, amino acids and peptides, and minerals, extracts were heated in acidic media to yield quantifiable low molecular weight compounds. In case of carbohydrates, monosaccharides like glucose, galactose, mannose, and fructose as well as inositol and mannitol were individually quantified by HPLC-RI. In addition, carbohydrates were quantified by photometry after a colour reaction with anthrone. Both assays gave a total amount of 10 – 12% carbohydrates in the dry extract.

Minerals and inorganic salts contribute to the composition of EPs® 7630 in a comparable amount. Cations were detected as

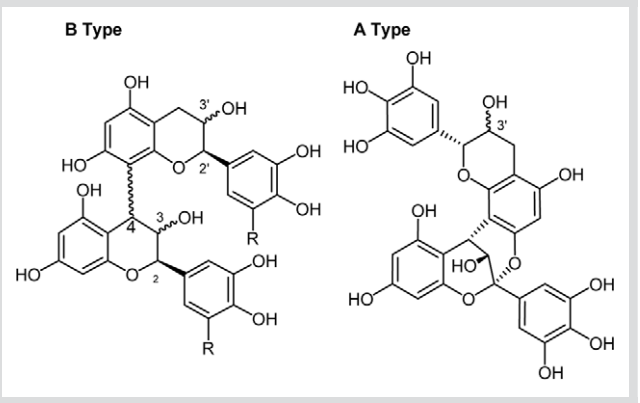

Fig. 2 General structures of B- and A-type oligomeric prodelphinidins.

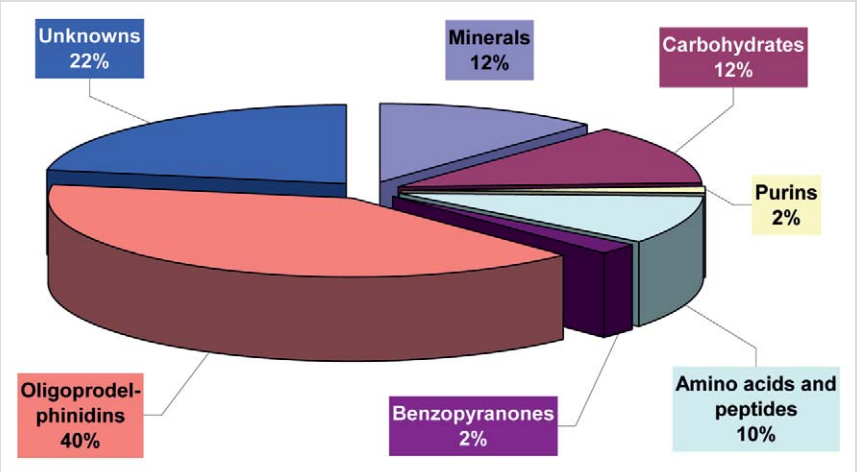

Fig. 1 Compositional profile of EPs® 7630.

B-type bonding		A-type bonding	
Name	**Configuration**	**Name**	**Configuration**
Prodelphinidin B1	2*R*,2′*R*,3*R*,3`*S*,4*R*	Prodelphinidin A1	3′*R*
Prodelphinidin B2	2*R*,2′*R*,3*R*,3′*R*,4*R*	Prodelphinidin A2	3′*S*
Prodelphinidin B3	2*R*,2′*R*,3*S*,3′*S*,4*S*		
Prodelphinidin B4	2*R*,2′*R*,3*S*,3′*R*,4*S*		
Prodelphinidin B9	2*R*,2′*R*,3*R*,3′*R*,4*S*		

Table 1 Constitutions of known prodelphinidin dimers (for numbering see Fig. **2**)

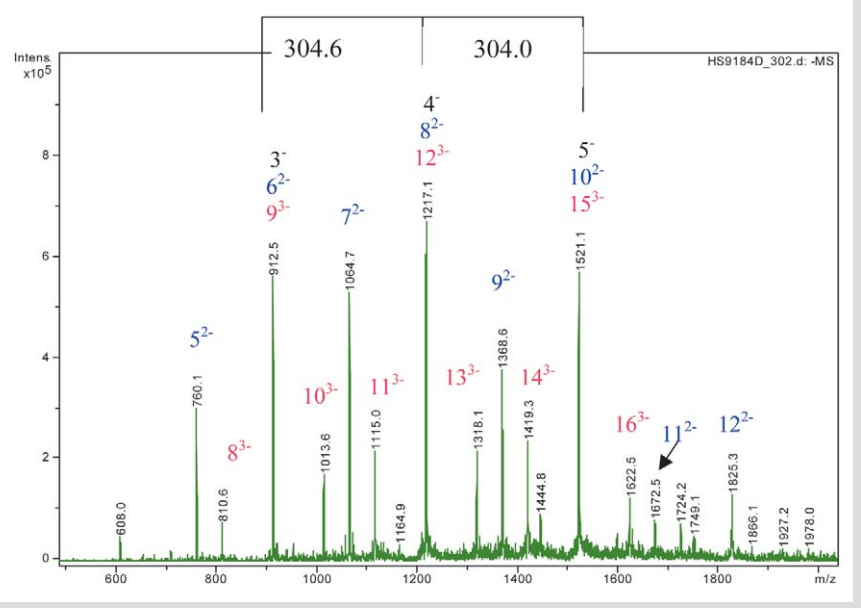

Fig. 3 Infusion electrospray mass spectrum of a prodelphinidin polymer. The electrospray signals are composed of singly charged (black colour, n-), doubly charged (blue colour; n^{2-}), and triply charged ions (red colour; n^{3-}). Therefore, three different classes of overlapping signals are visible. Signals at *m/z* = 912.5, 1217.1, and 1521.1 are assembled from singly, doubly and triply charged ions. Signals at *m/z* = 760.1, 1064.7, 1368.6, and 1672.5 show doubly charged species. Signals at *m/z* = 810.6, 1013.6, 1150.0, 1318.1, 1419.3, and 1622.5 are assignable to triply charged oligomers. From all this data results a repeating unit of 304 Da (dehydroprodelphinidin), the mass difference between singly charged ions.

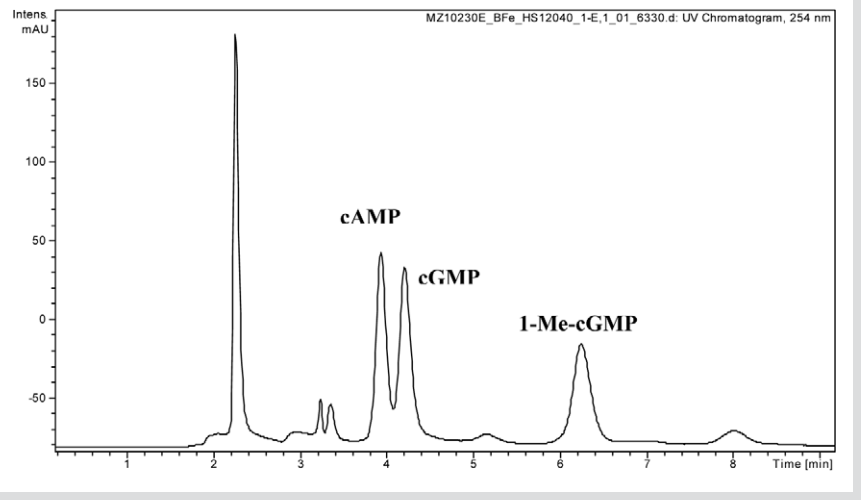

Fig. 4 HPLC-UV chromatogram of purine derivatives. Column: Phenomenex Synergy 4 µm Hydro-RP 150 × 2 mm; Eluent: MeCN/H_2O/HCOOH = 5 : 95 : 0,3; isocratic: 10 min; flow 0.2 mL/min; detection: UV 254 nm.

such with ICP-MS: potassium (~4%), sodium (~1.2%), and magnesium (~0.4%). Approx. 40 transition metals were analyzed but were below 0.1%. Anions were quantified by ion chromatography giving sulfate (~4.5%), phosphate (~2%), and chloride (~1%). All ions together add up to 10 to 14%. This was in good agreement with combustion experiments, where a total mineral content of 10 to 12% was found.

Peptides, amino acids and their derivatives constitute another substantial part of EPs® 7630. Main components detected after hydrolysis include arginine (Arg: ~4%), glutamine/glutamic acid (Glx: ~2%) and asparagine/aspartic acid (Asx: ~2%), whereas the other amino acids together sum up to about 2% of the dried material.

Less abundant constituents of EPs® 7630 which, however, can easily be detected in the UV-chromatogram at 254 nm (**Fig. 4**), are purine derivatives. Along with cAMP [21] and cGMP [22], a typical second messenger more often known from bacterial and mammalian cells, 1-methyl-cGMP, was identified as a quite uncommon derivative. This compound has not been reported from plants before [23]. Overall, these compounds amount up to almost 2% of dried EPs® 7630.

The most characteristic secondary metabolites of *Pelargonium sidoides* are benzopyranones, especially umckalin **1** [14]. Other members of this group include 5,6-dimethoxy-7,8-dihydroxycoumarin **3** and 6,7,8-trihydroxycoumarin **13**. A remarkable feature is that predominant amounts of these compounds occur as their sulfated derivatives (**Fig. 5**).

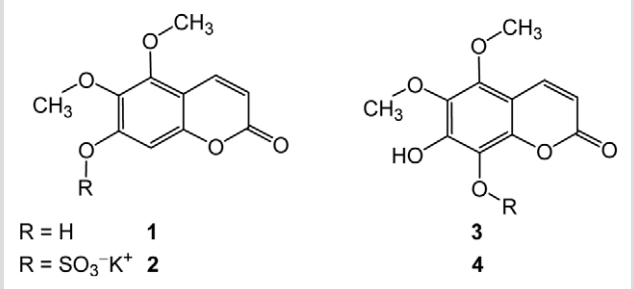

Fig. 5 Structures of major 5,6,7-trihydroxybenzopyran derivatives and their sulfates.

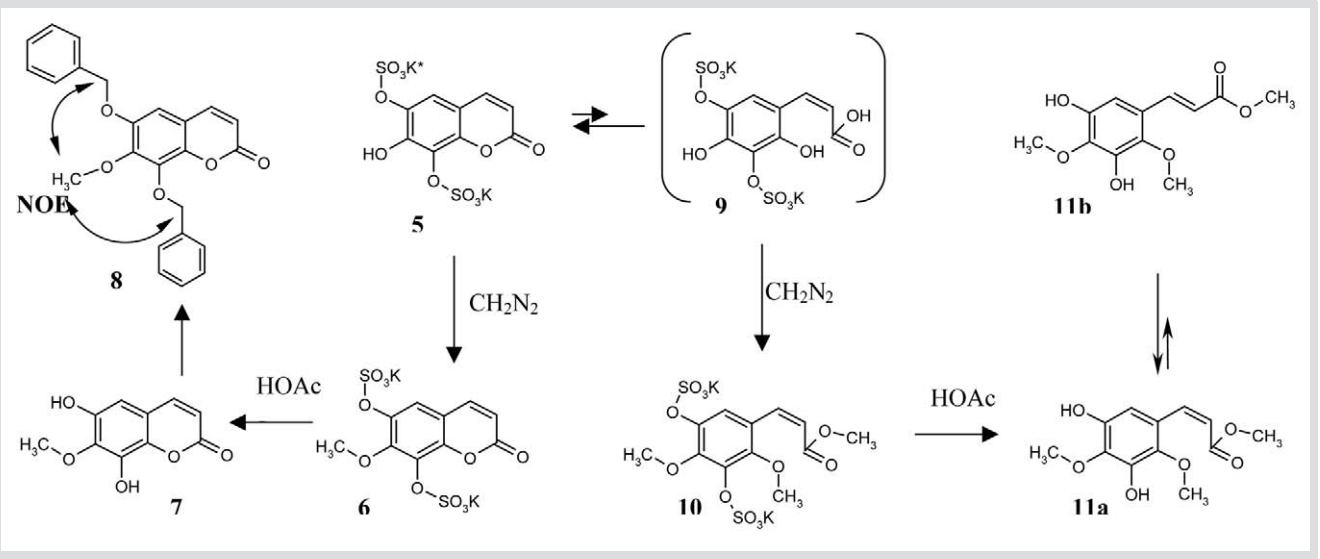

Fig. 6 Derivatization reactions for the structure elucidation of **5**.

Compound	1	2[a]	3	4[a]
H-3	6.19 (d, 9.7)	6.32 (d, 9.7)	6.20 (d, 9.7)	6.07 (d, 9.5)
H-4	7.96 (dd, 9.7, 0.5)	8.02 (d, 9.7)	7.97 (d, 9.7)	7.91 (d, 9.5)
5-OC$\underline{H}_3$	3.94 (s)	3.94 (s)	3.84 (s)	3.88 (s)
6-OC$\underline{H}_3$	3.77 (s)	3.81 (s)	3.78 (s)	3.79 (s)
7-OH	10.64 (s)	–	9.6 (br. s)	9.95 (br. s)
H-8	6.60 (d, 0.5)	7.30 (s)	–	–
8-OH	–	–	9.6 (br. s)	–
C-2	160.3	160.1	160.2	160.4
C-3	111.1	113.2	111.2	109.2
C-4	139.3	138.9	139.6	139.5
C-4a	105.6	108.3	105.0	103.5
C-5	149.3	149.5	141.5	145.7
5-O$\underline{C}H_3$	61.8	61.9	62.1	61.9
C-6	137.2	139.6	137.8	138.7
6-O$\underline{C}H_3$	60.5	61.0	60.6	60.3
C-7	155.5	151.0	144.4	152.7
C-8	98.7	103.2	129.3	125.6
C-8a	150.8	148.9	139.6	144.6

[a] Sulfates **2** and **4** were measured as potassium salts.
All spectra were recorded in DMSO-d_6.

Table 2 ^{1}H- and ^{13}C-NMR shifts (^{1}H with multiplicity and coupling constants) of the benzopyranones **1** – **4**

A new derivative that appeared in standard chromatograms at very low retention times was characterized by mass spectroscopy and elemental analysis as a disulfate. Its structure was elucidated by chemical and spectroscopic means (**Fig. 6**). Reaction of the compound with diazomethane introduced a methyl group and located the free phenol function. Hydrolysis of both sulfate groups afforded **7**. The two phenolic groups were successively alkylated with benzyl bromide. NOE correlation spectroscopy showed spatial proximity of the benzylic methylene protons with the methoxy group at C-6 [15].

Methylation of **5** under slightly basic conditions afforded a cinnamate **10**, which in turn lead to dihydroxy-dimethoxy derivative **11** after removal of the sulfate groups. HMBC correlation spectroscopy confirmed the proposed substitution pattern (see **Table 4**). The compound forms a stable mixture of *Z*- and *E*-isomers (**11a** and **11b**) in a ratio of 3 : 1. It is important to notice that the ^{1}H- and ^{13}C-NMR data of compound **5** are extremely sensitive to changes in the ion sphere. Already the incorporation of some ammonia (**5c**) leads to chemical shift differences (see **Table 3**) that were not expected from less polar compounds. An extreme change in chemicals shifts is observed in the tripotassium salt **5a** that was isolated at pH ~11. Thus, spectral interpretation for compounds must be taken with care as long the number and character of counterions is not known.

Additional benzopyranones **12** and **13** were products of stepwise removal of sulfate moieties (**Fig. 6**). Although the sulfated benzopyranones show very prominent and characteristic signals in HPLC-UV chromatograms, only about 2% of dry weight is attributed to this class of compounds.

EPs® 7630 is a modern herbal medicinal product with demonstrated clinical effectiveness [24], [25], [26], [27], [28], [29], [30], [31]. According to present investigations, the oligomeric prodelphinidins, benzopyranones, carbohydrates, minerals, amino acids and peptides, and purines add up to 70 to 80% of the dry matter of the extract, leaving 30 to 20% of yet unknown constituents. Although secondary metabolites are generally believed to

Table 3 ^{1}H- and ^{13}C-NMR shifts (^{1}H with multiplicity and coupling constants) of the benzopyranones **5** – **13**

Compound	5a[a]	5b[a]	5c[a]	6	7	12a[b]	12b[b]	13
H-3	5.59 (d, 9.1)	6.11 (d, 9.4)	5.90 (d, 9.3)	6.31 (d, 9.5)	6.31 (d, 9.5)	6.23 (d 9.5)	6.22 (d, 9.5)	6.17 (d, 9.5)
H-4	7.62 (d, 9.1)	7.91 (d, 9.4)	7.82 (d, 9.3)	7.99 (d, 9.5)	7.88 (d, 9.5)	7.89 (d, 9.5)	7.92 (d, 9.5)	7.84 (d, 9.5)
H-5	7.03 (s)	7.43 (s)	7.26 (s)	7.55 (s)	6.59 (s)	6.86 (s)	7,09 (s)	6.56 (s)
6-OH		–	–	–	9.54 (s)	9.55 (br s)		9.41 (s)
7-OH						9.55 (br s)	9.43 (br s)	9.41 (s)
7-O**C**H_3		–	–	3.92 (s)	3.78 (s)			
8-OH		–	–	–	9.70 (s)		9.43 (br s)	9.41 (s)
C-2	161.9	160.5	161.1	160.1	160.2	160.3	160.4	160.6
C-3	102.4	110.5	107.0	113.9	114.3	112.4	112.1	111.6
C-4	145.1	144.9	145.0	144.4	144.6	144.5	145.0	145.0
C-4a	101.5	108.7	105.7	113.1	114.5	111.0	110.5	110.4
C-5	115.5	115.1	115.3	114.8	103.4	108.7	111.0	103.2
C-6	142.2	140.0	140.9	143.2	147.3	143.8	138.4	142.9
C-7	162.9	149.6	n. d.	148.9	139.6	143.4	142.1	138.9
7-O**C**H_3	–	–	–	60.8	60.2	–	–	
C-8	147.7	128.7	129.5	133.6	138.2	128.4	133.5	132.9
C-8a	130.4	144.8	146.0	144.7	137.1	141.7	140.5	138.1

[a] The disulfates **5a** – **c** were measured as potassium salts containing different phenolic counterions; **5a** was a potassium phenolate, **5b** the free phenol, and **5c** a mixed phenol/ammonium phenolate form.

[b] Sulfates **12a** and **12b** were measured as potassium salts.

All spectra were recorded in DMSO-d_6.

Table 4 ^{1}H- and ^{13}C-NMR shifts (^{1}H with multiplicity and coupling constants), and HMBC correlations of cinnamic acid derivatives **10** + **11b**

#	10	11a
H-2	5.95 d, 10.5	5.95; d; 12.6
H-3	6.97 d, 10.5	6.97; d; 12.6
H-6′	7.36, s	6.55; s
1-O**C**H_3	3.64 s	3.63 s
4′-O**C**H_3	3.77 s	3.72 s
2′-O**C**H_3	3.83 s	3.60 s
5′-OH	–	8.81 br
3′-OH	–	9.00 br
C-1	166.5 (H-3; 2-OCH_3)	166.4 (2-OCH_3)
C-2	118.9 (H-3)	119.0 (H-3)
C-3	136.7 (H-5)	136.7 (H-5)
C-1′	121.5 (H-3)	112.8 (H-3)
C-2′	148.8 (H-4, H-5, 8a-OCH_3)	143.3 (8a-OCH_3)
C-3′	139.2	137.5 (8-OH, 6-OH)
C-4′	147.5(H-5, 7-OCH_3)	145.9 (7-OCH_3)
C-5′	141.4 (H-5)	139.9 (8-OH)
C-6′	118.6 (H-5, H-4)	106.3 (H-5, H-4; 6-OH)
1-O**C**H_3	51.3	51.1
2′-O**C**H_3	60.5	60.7
4′-O**C**H_3	60.8	59.8

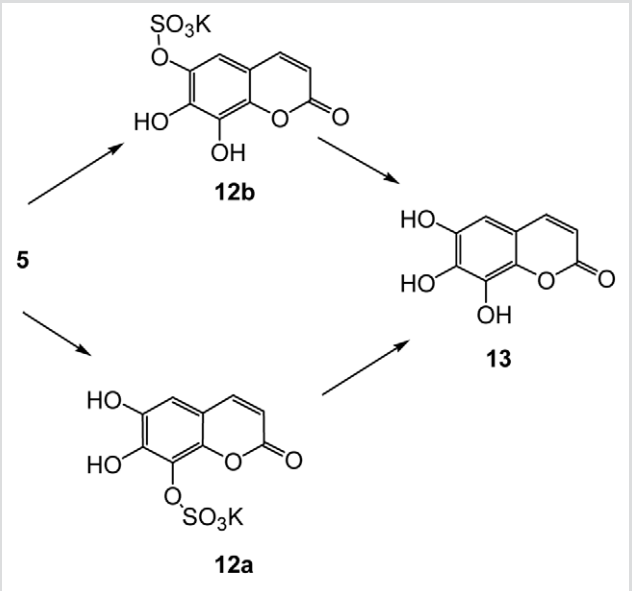

Fig. 7 Hydrolysis of disulfate **5**.

be responsible for the pharmacological effects of herbal extracts, the bulk of primary metabolites are at least modifiers of solubility and, consequently, of bioavailability. In addition, the stability for sulfated benzopyranones appears to be enhanced in the extract, whereas these compounds decompose rather quickly when they are isolated. Therefore, EPs® 7630 seems to contain a unique combination of bioactive molecules and a matrix of primary metabolites which together form an effective herbal medicinal product.

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