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Identification and Quantification of Coumarins in *Peucedanum ostruthium* (L.) Koch by HPLC-DAD and HPLC-DAD-MS

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Supporting Information

ABSTRACT: The rhizomes of *Peucedanum ostruthium* (L.) Koch (masterwort) are traditionally used in the alpine region as ingredient of liqueurs and bitters, and as a herbal drug. A sensitive and specific high-performance liquid chromatography−diode-array detection−mass spectrometry (HPLC-DAD-MS) method has been developed for the simultaneous identification and quantification of its main coumarins, oxypeucedanin hydrate, oxypeucedanin, ostruthol, imperatorin, osthole, isoimperatorin, and ostruthin. Fast HPLC separation could be achieved on an Acclaim C18 column (150 mm × 2.1 mm i.d., 3 μm) using a mobile phase gradient of acetonitrile−water modified with 0.01% acetic acid. The quantification by HPLC-DAD was performed with imperatorin as external standard and validated to demonstrate selectivity, linearity, precision, and accuracy. The content of the main coumarins was quantitated in various batches of commercial and field-collected rhizomes of *Peucedanum ostruthium*, as well as in beverages prepared thereof.

KEYWORDS: *Peucedanum ostruthium*, HPLC-DAD-MS, validation, quantification, coumarins, oxypeucedanin hydrate, oxypeucedanin, osthole, imperatorin, isoimperatorin, ostruthol, ostruthin

INTRODUCTION

Comprising over 120 species, the genus *Peucedanum* is one of the most species-rich genera of the Apiaceae. While many members of the family Apiaceae are commonly used in cuisine, particularly as herbs and spices, this is rarely the case for the *Peucedanum* species. A notable exception is the masterwort (*Peucedanum ostruthium*). Its main distribution is in the alpine region, where the rhizomes have been traditionally used for the preparation of teas, liqueurs, and bitters.[1] Teas are typically prepared from dried and cut roots, which are commercially available in drugstores under the name "Radix Imperatoriae" or "Rhizoma Imperatoriae". Various tea recipes can be found, where masterwort is either the sole ingredient or is mixed with other herbal drugs. The masterwort roots are also popular for the preparation of liqueurs and bitters by means of cold soaking. Typically, the dried or fresh masterwort roots are cut, placed in a container, and covered with ethanol (38%). After an appropriate soaking time, usually several weeks, the preparation is filtered and diluted to reduce bitterness.

The usage of masterwort for the preparation of teas, liqueurs, and bitters is strongly linked to its important relevance in Austrian traditional medicine. These preparations are particularly recommended for gastro-intestinal diseases, but also for disorders of the cardiovascular system, the respiratory tract, and to treat tiredness.[1]

Coumarins represent a very interesting main compound class in *Peucedanum* species.[2] At least 1300 natural coumarins have been identified so far, mainly in the families of Rutaceae and Apiaceae.[3] Within the Apiaceae family, coumarin patterns can be used to distinguish between closely related species.[4] Members of this compound class are increasingly recognized as valuable health-promoting constituents of edible plants and herbal plant preparations due to their wide spectrum of biological activities. Recent studies on various coumarins have revealed, among others, antioxidant,[5] antimycobacterial,[6] anticoagulant, antitumoral, antiviral, antifungal, and antiinflammatory activities.[3,7−9] Coumarins from *Peucedanum ostruthium*, most notably ostruthol, have also been shown to act as inhibitors of acetylcholinesterase, which implicates a high potential for the treatment of Alzheimer's disease.[10]

However, coumarins and furanocoumarins have also been linked to phototoxic, mutagenic, carcinogenic, and hepatotoxic effects.[11−13] In 2004, the European Food Safety Authority (EFSA) established a tolerable daily intake of coumarin (1,2-benzopyrone) of 0.1 mg/kg body weight based on its hepatotoxicity.[14] Very high concentrations of coumarin have been found in cassia cinnamon, which lead to public discussions in Germany and Austria regarding the safety of cinnamon-containing foods.[11,15] Although relevant data on other coumarin derivatives are scarce, it is not unlikely that they are also toxicologically relevant or may interact with drugs such as warfarin.[16] Consequently, there is a need for the quantification of the content of coumarins in food and herbal remedies, like those containing *Peucedanum ostruthium*.

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Up to now, there are no qualitative or quantitative standards for the quality control of the commercial drug "Radix/Rhizoma Imperatoriae". Drugstores and distributors of this herbal drug specify the content of essential oil as a quality feature, but the content of the coumarins is typically not determined. This potentially important information is thus not available to the consumers.

Several methods have been published for the quantification of coumarins in foods and herbal drugs. For instance, HPLC methods have been developed for the determination of coumarins in citrus products[17] and in *Angelicae dahuricae* Radix.[18,19] GC-MS has been applied for the quantification of coumarins in bergamot oil,[20] citrus fruits, and vegetables of the Apiaceae family.[21] However, to the best of our knowledge, there is no method available for the quantification of the main coumarins in masterwort roots. Therefore, the aim of this study was to establish a specific HPLC method for this purpose and to quantify the main coumarins in commercial and field-collected samples of *Peucedanum ostruthium* and preparations thereof. Furthermore, the method was validated according to the ICH guidelines[22] on the basis of specificity, linearity, precision, and accuracy.

MATERIALS AND METHODS

Reagents and Chemicals. Methanol (MeOH), dichloromethane, and acetonitrile (MeCN) were HPLC-grade (VWR, Vienna, Austria). Glacial acetic acid was purchased from Carl Roth (Karlsruhe, Germany). Water was distilled by an IKA-Dest M3000 automatic water distillation apparatus (IKA, Staufen, Germany). Imperatorin, isoimperatorin, oxypeucedanin, and oxypeucedanin hydrate were all of 99% purity and purchased from Herboreal Ltd. (Edinburgh, UK). We obtained osthole with a purity of 98.9% from Chromadex (Irvine, USA).

Ostruthol and ostruthin were isolated from the dichloromethane extract of *Peucedanum ostruthium* rhizomes using preparative HPLC. The purity was confirmed to be >98% by HPLC with DAD and ELSD detection as well as by NMR.

Samples and Preparations of *Peucedanum ostruthium* Rhizomes. Six samples of "Radix/Rhizoma Imperatoriae" were purchased from various companies in Austria and Germany (two batches from Kottas Pharma GmbH, Vienna, Austria; one each from KWIZDA, Vienna, Austria; Alfred Richter GmbH & CO KG, Kufstein, Austria; Alfred Galke GmbH, Gittelde, Germany, and Herba Chemosan, Vienna, Austria). Another two batches were field-collected and identified by Dr. Saukel (dried root material collected in 2008 at the Weisspriachtal, Salzburg, Austria, 1200 m above sea level, and dried root material collected in 2008 at Gontal, Carinthia, Austria, 1800 m above sea level). Voucher specimens are deposited at the Department of Pharmacognosy, University of Vienna, Austria.

For exhaustive extraction of the coumarins, 0.5 g dried and powdered material was processed with 20 mL of dichloromethane using reflux extraction for 15 min at 40 °C. Subsequently, the extracted solution was filtered, evaporated to dryness, and redissolved in 20 mL of MeOH.

In order to quantify the coumarin content in traditional formulations, water extract and liqueurs were prepared and analyzed. The water decoction was obtained by extraction of 0.5 g dried and powdered drug with 20 mL of water for 15 min in a water bath at 100 °C. This procedure was chosen for a direct comparison of the extraction efficiency of water with dichloromethane. In the case of a traditional masterwort tea, cut pieces would be used instead of the powder. The extracted solution was filtered, centrifuged, and used directly for HPLC measurements. Furthermore, we investigated four traditionally prepared liqueurs produced in different years from roots of various sample locations. The roots of the masterwort have been cut, put into a bottle, filled up with ethanol (38%), and kept for at least 4 weeks. For the analysis, the solution was filtered, centrifuged, and used directly for HPLC measurements. The liqueur obtained in the above-described way is typically diluted 1:10−1:15 with ethanol (38%) before consumption.

Identification and Quantification of the Coumarins by HPLC-MS and HPLC-DAD. An HPLC instrument from Shimadzu (Kyoto, Japan) consisting of a CBM-20A system controller, a DGU-20A5 membrane degasser, an LC-20AD solvent delivery unit, an SIL-20AC HT autosampler, a CTO-20AC column oven, and an SPD-M20A photodiode array detector was used for quantification. Data analysis was performed using the chromatography software LCsolution Ver.1.2 (Shimadzu). Chromatographic separation was achieved on a 150 mm × 2.1 mm i.d., 3 μm, Acclaim 120 C18 reversed-phase column, with a 10 mm × 2.1 mm i.d., 5 μm, Acclaim 120 C18 guard column from Dionex (Germering, Germany), at 38 °C and a flow rate of 0.5 mL/min. Water and MeCN, both modified with 0.01% acetic acid, were used as mobile phases A and B, respectively. Gradient elution was performed as follows: 25−37% of B in 6 min, 37−45% of B in 8 min, 45−65% of B in 10 min, 65−95% of B in 1 min, and isocratic at 95% of B for 5 min. The injection volume was 5 μL for all samples. The DAD collected data from 190 to 400 nm.

Tentative identification of the main coumarins was facilitated by HPLC-DAD-MS. These analyses were performed on an UltiMate 3000 RSLC-series system (Dionex) coupled to an HCT 3D quadrupole ion trap mass spectrometer equipped with an orthogonal ESI source (Bruker Daltonics, Bremen, Germany). HPLC separation was carried out as described above. The eluent flow was split roughly 1:8 before the ESI ion source, which was operated as follows: capillary voltage, −4.0 kV; nebulizer, 20 psi (N_2); dry gas flow, 6 L/min (N_2); and dry temperature, 350 °C. The mass spectrometer was operated in an automated data-dependent acquisition (DDA) mode where each MS scan (m/z 100−700) was followed by MS^2 scans (m/z 40−700, fragmentation amplitude of 0.7 V) of the two most intense precursor ions, and MS^3 scans (m/z 40−700, fragmentation amplitude of 1.0 V) of the most intense fragment ion in each MS^2 scan. Helium was used as collision gas, the isolation window was set to 4 m/z, and three spectra were averaged per scan.

The identity of the main compounds was confirmed either by comparison with reference compounds (**1**, **2**, and **4**−**6**, see Figure 1) or by isolation and structural characterization by 1D and 2D NMR and MS (**3** and **7**).

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) using $CDCl_3$ [$\delta(^1H)$ = 7.26 ppm and $\delta(^{13}C)$ = 77.00 ppm] or CD_3OD [$\delta(^1H)$ = 3.31 ppm and $\delta(^{13}C)$ = 49.00 ppm] as solvent. ^1H and ^{13}C NMR data of **3** and **7** were in accordance with previously published data.[23−25]

Imperatorin (**4**) was chosen as an external standard for the quantification of the identified major coumarins by HPLC-DAD. Stock solutions of **4** in MeOH were prepared in a concentration of 2 mg/mL and further diluted in MeOH to obtain the appropriate working solutions for external calibration and validation. The detector signal at 310 nm was used for the quantification of the coumarins. As the structures and therefore the UV spectra and absorption coefficients at 310 nm differ between **4** and the six other coumarins, a UV-response factor was calculated for each substance. For that, **1**−**7** were separately weighed and dissolved in MeOH to make stock solutions of 0.25 mg/mL. Those stock solutions were mixed and further diluted with MeOH by a factor of 10. The purity of the coumarins, including the two isolated compounds **3** and **7**, was confirmed by HPLC analysis (UV detection at 205, 254, and 310 nm).

Validation of the Analytical Method. The specificity of the method was shown by testing the peak purity of the analytes in the samples on the basis of the online UV and MS spectra. The precision (i.e., repeatability of measurements) was determined by at least triplicate

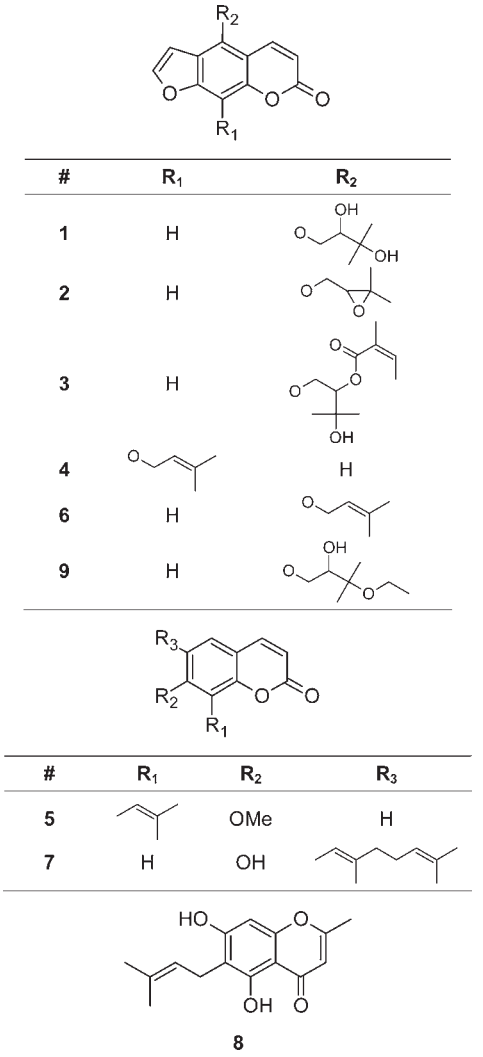

Figure 1. Structure of the main coumarins in *Peucedanum ostruthium*. Linear furanocoumarins: oxypeucedanin hydrate (**1**), oxypeucedanin (**2**), ostruthol (**3**), imperatorin (**4**), isoimperatorin (**6**), and oxypeucedanin ethanolate (**9**). Simple courmarins: osthole (**5**) and ostruthin (**7**). Chromones: peucenin (**8**).

analysis of sample R1 (Kottas Pharma GmbH, Vienna, Austria, 2008) on three different days, whereby the sample was independently prepared on each day. Intra- and interday variability was evaluated for all seven main coumarins and expressed by the relative standard deviations (RSD). The accuracy of the method was estimated by means of recovery experiments with all seven coumarins. Therefore, an aliquot of the dry sample drug (R1) was spiked with 2.08 mg of each compound (dissolved in MeOH) per g drug. After evaporation of the methanol, the sample was analyzed in pentuplicate. The sensitivity and linear range of the method were shown for the external standard imperatorin as well as for the mixture of the seven reference coumarins. The concentration range was evaluated for imperatorin starting at very high concentrations (2 and 1.5 mg/mL) and further stepwise dilution by a factor of 2 or 10 down to the limit of detection. The Pearson product−moment correlation coefficient (r) of the calibration curve was evaluated. The lower limit of detection (LLOD) and quantification (LLOQ) values were demonstrated as the concentration where the signal-to-noise ratio (S/N) had a defined value (for LLOD, $S/N \sim 3$, and for LLOQ $S/N \sim 10$, where N is the height of the noise measured at the retention time of the reference peak when the blank is analyzed, and S is the height of the signal of the reference).

RESULTS AND DISCUSSION

Optimization of the HPLC Method. In addition to the essential oil components, coumarins represent an interesting compound class within the Apiaceae family. Recent studies showed their broad spectrum of activities and their potency as new drug leads.[3,5−8,26−30]

Currently, there are no investigations on the coumarin content of *Peucedanum ostruthium*, but several HPLC methods for the analysis of coumarins were published. Different methods from literature using gradient as well as isocratic elution were tested for the analysis of the coumarins in *Peucedanum ostruthium*.[4,31] Unfortunately, even with simple adaptations, none of the tested methods provided sufficient peak purity for the accurate quantification of the main coumarins as determined by HPLC-MS. Therefore, a novel method was developed using a C18 column with smaller particle size and a higher temperature. Baseline separation (resolution >1.5) of all relevant components (Figure 1) in the apolar extract of the dried root material of *Peucedanum ostruthium* could be achieved on the Acclaim C18 column with a gradient of H_2O and MeCN containing 0.01% acetic acid in just 30 min (Figure 2).

Identification of the Main Coumarins in *Peucedanum ostruthium* Rhizomes. On the basis of the HPLC fingerprint, 7 major coumarin constituents (**1**−**7**) were selected for the quantitative analysis (Figures 1 and 2). Identification of these components was based on their characteristic MS^n spectra (Table 1). The $[M + H]^+$ ions of the linear hydroxyfuranocoumarin derivatives all showed neutral loss of their oxygen-bound side chain to yield the fragment ion at m/z 203 (protonated hydroxyfuranocoumarin). Further fragmentation of this ion in MS^3 allows the differentiation of 5- and 8-hydroxyfuranocoumarins via the ratio of the intensities of the fragment ions at m/z 175 and m/z 159 as described previously.[32] In the case of **3**, an intense fragment ion corresponding to the protonated (dehydrogenated) side chain is obtained at m/z 185. The fragmentation of **5** has also been described, and **7** shows the analogous neutral loss of the side chain followed by the loss of CO.[33] The identity of **1**, **2**, and **4**−**6** was further confirmed by comparison of their retention times and UV spectra with authentic references. In addition, **3** and **7** were isolated by semipreparative HPLC in order to facilitate comprehensive structure elucidation by NMR spectroscopy. Several less prominent compounds, such as the chromone **8**, were identified by LC-MS only (data not shown). Khaled et al.[24] also reported **1**−**8** as the main compounds of the dried roots but without any quantitative analyses. The coumarin glycosides reported in the study by Khaled et al.[24] were not detected in any of the investigated samples, including a methanolic extract of dried roots that was enriched in more polar compounds.

Quantification of the Main Coumarins in the *Peucedanum ostruthium* Rhizome. The content of the main coumarins was quantitated in various batches of commercial and field-collected rhizomes of *Peucedanum ostruthium* as well as in liqueurs and in a water decoction. For this, the HPLC-DAD method has been validated using the external standard imperatorin (**4**). With an injection volume of 5 μL, the linear range for **4** was determined to span concentrations from 61 ng/mL to 1.0 mg/mL with an linear regression equation of $y = 23850014x$ and an r of 0.99996. The calibration curves for the other coumarins were also linear over the investigated concentration range of approximately 0.2 μg/mL to 0.2 mg/mL (Table 2). Relative response factors to **4** were

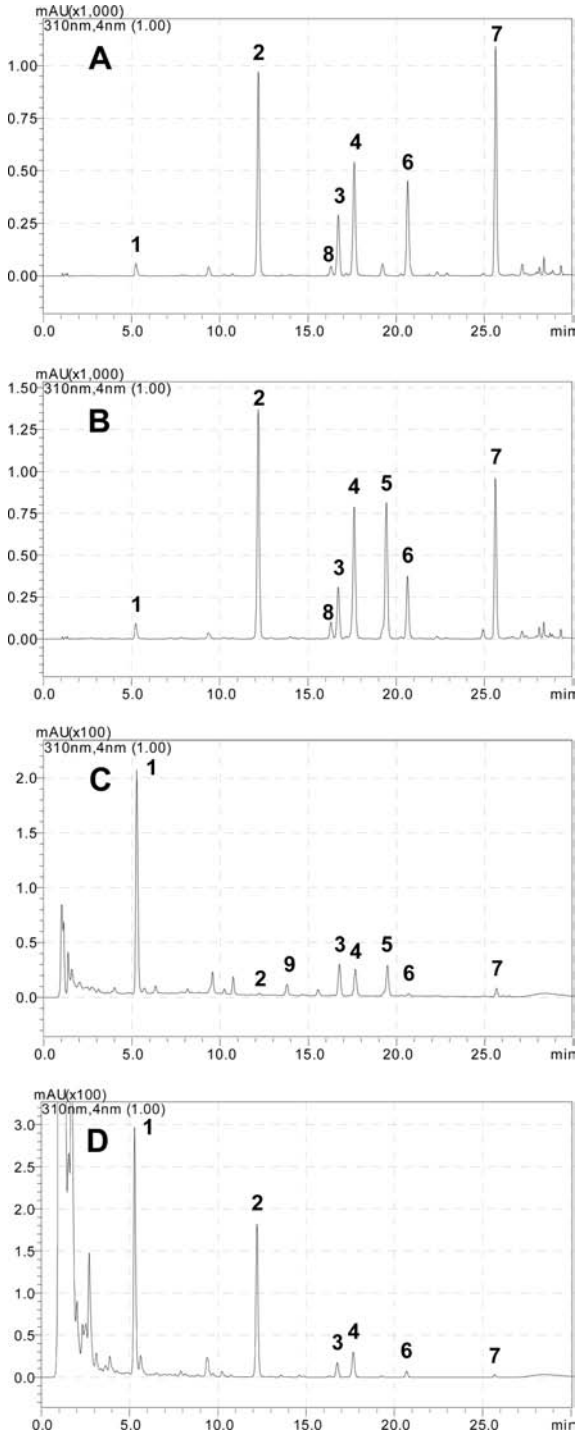

Figure 2. HPLC chromatograms (310 nm) of (A) the dichloromethane extract of commercial *Peucedanum ostruthium* rhizome (Kottas 2010), (B) the dichloromethane extract of the *Peucedanum ostruthium* rhizome from a wild collection at Weisspriachtal, Salzburg, (C) a liqueur of *Peucedanum ostruthium* rhizome, and (D) a water decoction of the *Peucedanum ostruthium* rhizome (prepared from sample Kottas 2010). Peak numbers refer to the compounds shown in Figure 1.

determined for **1**−**3** and **5**−**7** (Table 2). The LLOD of the external standard was 15 ng/mL ($S/N = 3.6$), whereas the LLOQ was found to be 61 ng/mL ($S/N = 8.6$). Data on the precision expressed as intra- and interday RSD were determined from three independently prepared samples of one batch and are given in Table 3. The interval between the first two and the third analysis was around seven months, which also indicates the high stability of the coumarins upon storage of the dried root material. The higher RSD values for **5** can be explained by the presence of an only partly resolved impurity that requires peak splitting during data analysis. The accuracy and extraction efficiency were estimated by an analysis of a sample spiked with (relatively small) amounts of all seven coumarins (Table 4).

With one exception (collected sample from Weisspriachtal, Salzburg, 2008; R7), variations of the coumarin content between the drug samples were rather low for a biological material with relative standard deviations lower than ±30% (Table 5). In the dichloromethane extracts of these root samples (R1−R6 and R8), **7** was quantitated as the main coumarin (38−41% of the total coumarin content), followed by **2** (17−20%), **4** (12−15%), **3** (12−14%), **6** (9−10%), **1** (2−7%), and **5** composing just about 1%. The sample collected from Weisspriachtal in 2008 (R7) differed mainly by a significantly higher absolute and relative

Table 1. MS^n Fragmentation Patterns of the Main Coumarins in *Peucedanum ostruthium* Rhizome Extracts and Preparations

#[a]	$[M + H]^+$	main fragment ions (>10% rel. int.)
1	305.0	MS^2 [305.0]: 202.8
		MS^3 [202.8]: 174.9, 173.8, 158.9, 146.9, 131.0
2	287.0	MS^2 [287.0]: 202.8
		MS^3 [202.8]: 174.8, 173.8, 172.8, 158.8, 146.9, 130.9, 114.1
3	387.1	MS^2 [387.1]: 369.1, 305.0, 184.9, 166.9
		MS^3 [369.1]: 287.0, 268.9, 202.8, 166.9
		MS^3 [184.9]: 166.9, 83.3
4	270.9	MS^2 [270.9]: 202.8
		MS^3 [202.8]: 174.8, 158.8, 146.9, 130.9
5	245.0	MS^2 [245.0]: 188.8
		MS^3 [188.8]: 160.9, 158.8, 130.9
6	270.9	MS^2 [270.9]: 202.8
		MS^3 [202.8]: 158.8, 146.9, 130.9
7	299.1	MS^2 [299.1]: 174.8
		MS^3 [174.8]: 146.9
8	261.0	MS^2 [261.0]: 204.8
9	333.1	MS^2 [333.1]: 202.8
		MS^3 [202.8]: 174.9, 158.9, 157.8, 146.9, 130.9

[a] Numbers refer to the compounds shown in Figure 1.

Table 2. Relative UV-Response Factors to Imperatorin (f_i), Linear Regression Equations, and Correlation Coefficients (r or Pearson Product-Moment) for the Seven Coumarins

#[a]	f_i [b]	linear regression equation[b]	r[b]
1	1.7	$y = 12295600x$	0.99112
2	0.8	$y = 26169348x$	0.99571
3	1.7	$y = 12558206x$	0.99760
4		$y = 23850014x$	0.99996
5	0.6	$y = 33689612x$	0.99997
6	0.8	$y = 28921459x$	0.99779
7	1.5	$y = 14131201x$	0.99304

[a] Numbers refer to the compounds shown in Figure 1. [b] The values were determined from 5 replicates performed in one day.

content of **5** (8.8 mg/g and 11.1%, respectively) and a somewhat higher content of **2** and **4**. The total coumarin content (i.e., sum of the contents of the seven quantitated coumarins) in the dichloromethane extracts represented 68−78%, whereas the amount on gram dry weight of the drug made up 4−8%.

Analysis of Liqueurs and a Water Decoction Prepared from the *Peucedanum ostruthium* Rhizomes. In Austrian traditional medicine, particularly in the alpine region, the rhizomes of *Peucedanum ostruthium* have been and are still used for the preparation of teas, liqueurs, and bitters. To investigate whether significant amounts of the different coumarins can be extracted with the rather polar solvents used for the preparation of liqueurs (ethanol/water) and teas (hot water), we have analyzed four traditionally prepared liqueurs and a tea-like water decoction made of one of the commercial "Radix/Rhizoma Imperatoriae" samples. The analysis was performed with the same method optimized for the dichloromethane extracts, and no sample preparation except filtration and centrifugation was conducted. Interestingly, all seven coumarins could be detected and quantified in the four different liqueurs, although the absolute content differed very strongly (Table 6). In comparison to the other extracts, the preparation of the liqueurs was not standardized. Neither the amount of drug per bottle nor the "extraction time" was identical between the batches. Furthermore, the liqueurs were made with cut root material, which most likely has an influence on the extraction rates of the compounds. Still, the relative content of the different coumarins was surprisingly similar, with the most polar coumarin **1** found in the highest concentration (64−85%), followed by **3** (5−17%), **4** (5−9%), **5** (1−5%), **7** (1−4%), **6** (0.5−2%), and **2** (0.4−2%). In the water decoction, the most dominant coumarin was again **1**, comprising 64%, followed by **2** (21%), **3** (6%), **4** (6%), **7** (2%), and **6** (2%).

Obviously, the extraction efficiency for the different coumarins with hot water and 38% ethanol is strongly dependent on the polarity of the compounds. However, this alone does not explain the extremely high content of **1** and the very low content of **2** in the liqueurs. In a recent study, it was shown that **1** is formed by hydrolytic ring-opening of **2** during sample preparation.[34] In the case of the liqueurs, where the plant material is kept in the solvent for a long time (up to two years in our case), the majority of **1** is presumably a transformation product of **2**. This hypothesis is supported by the tentative identification of **9** in the liqueurs, which most likely results from ethanolytic epoxide ring-opening as a second transformation product of **2** (Figure 2C). Analogously, oxypeucedanin methanolate was observed in MeOH extracts of *Peucedanum ostruthium* rhizomes (data not shown). Transformation of **2** to the hydrate has most likely also occurred in water decoction, but the reaction is less complete due to the rather short extraction and processing time before analysis. Theoretically, **1** could also result from hydrolysis of the ester function of **3**, but no experimental evidence for this pathway was observed.

Table 3. Intra- and Interday Repeatability of the Method

#[a]	mean level ± SD [mg/g][b]	intraday repeatability RSD [%][c]	interday repeatability RSD [%][d]
1	3.48 ± 0.11	1.02	7.61
2	9.90 ± 0.06	0.94	0.96
3	7.59 ± 0.08	0.79	0.89
4	6.82 ± 0.09	0.89	1.15
5	0.25 ± 0.02	1.36	9.86
6	4.84 ± 0.22	1.00	4.78
7	22.15 ± 0.91	0.46	4.47

[a] Numbers refer to the compounds shown in Figure 1. [b] Coumarin content in a Radix Imperatoriae sample (R1, Kottas Pharma GmbH, Vienna, Austria, 2008) as quantitated from the dichloromethane extract (n = 11). [c] Average of the RSD values (n = 3−5, each) obtained on three different days. [d] RSD of the three average values (n = 3−5, each) obtained on three different days.

Table 4. Recovery of the Seven Coumarins

#[a]	R1 unspiked mean level ± SD [mg/g][b]	R1 spiked mean level ± SD [mg/g][c]	recovery [%]
1	3.23 ± 0.02	5.29 ± 0.08	99
2	9.82 ± 0.06	11.81 ± 0.10	95
3	7.63 ± 0.07	9.46 ± 0.07	87
4	6.88 ± 0.05	8.67 ± 0.09	86
5	0.23 ± 0.00	1.88 ± 0.02	79
6	4.60 ± 0.04	6.34 ± 0.11	84
7	21.21 ± 0.11	24.05 ± 0.28	136

[a] Numbers refer to the compounds shown in Figure 1. [b] Coumarin content in an unspiked Radix Imperatoriae sample (R1, Kottas Pharma GmbH, Vienna, Austria, 2008) as quantitated from the dichloromethane extract (n = 5). [c] Coumarin content in a spiked aliquot of sample R1 (2.08 mg each of **1**-**7** per g drug) as quantitated from the dichloromethane extract (n = 5).

Table 5. Coumarin Content in Different Samples of Radix Imperatoriae as Quantitated from the Dichloromethane Extracts

	roots (coumarin content in mg/g dry weight ± SD)[b]								
#[a]	R1	R2	R3	R4	R5	R6	R7	R8	average R1−R8
1	3.48 ± 0.11	1.58 ± 0.03	3.78 ± 0.04	2.19 ± 0.02	3.93 ± 0.07	3.44 ± 0.02	2.41 ± 0.01	3.08 ± 0.02	2.99 ± 0.83
2	9.90 ± 0.06	12.72 ± 0.63	9.96 ± 0.17	6.91 ± 0.05	9.80 ± 0.06	10.29 ± 0.01	17.21 ± 0.07	13.11 ± 0.06	11.24 ± 3.08
3	7.59 ± 0.08	8.73 ± 0.36	7.69 ± 0.16	5.28 ± 0.08	8.09 ± 0.08	7.39 ± 0.20	8.96 ± 0.04	9.22 ± 0.09	7.88 ± 1.24
4	6.82 ± 0.09	10.07 ± 0.41	7.45 ± 0.04	4.88 ± 0.06	7.04 ± 0.06	6.82 ± 0.12	14.27 ± 0.03	11.42 ± 0.25	8.60 ± 3.08
5	0.25 ± 0.02		0.51 ± 0.00	0.32 ± 0.00	0.41 ± 0.01	0.43 ± 0.02	8.81 ± 0.04	0.49 ± 0.01	1.60 ± 3.18
6	4.84 ± 0.22	6.50 ± 0.39	4.96 ± 0.09	3.96 ± 0.10	5.04 ± 0.04	5.02 ± 0.04	4.98 ± 0.05	6.99 ± 0.03	5.29 ± 0.97
7	22.15 ± 0.91	25.05 ± 0.11	22.83 ± 0.11	16.47 ± 0.13	23.74 ± 0.11	23.22 ± 0.09	21.08 ± 0.09	31.31 ± 0.21	23.23 ± 4.13

[a] Numbers refer to the compounds shown in Figure 1. [b] Samples: R1, Kottas Pharma GmbH, Vienna, Austria, 2008; R2, Kottas Pharma GmbH, Vienna, Austria, 2010; R3, Herba Chemosan, Vienna, Austria, 2009; R4, KWIZDA, Vienna, Austria, 2009; R5, Alfred Galke GmbH, Gittelde, Germany, 2009; R6, Alfred Richter GmbH & CO KG, Kufstein, Austria, 2009; R7, dried root material collected in 2008 at the Weisspriachtal in Salzburg, Austria; and R8, dried root material collected in 2008 at the Gontal in Carinthia, Austria; n = 3, except for R1 where n = 11.

Table 6. Coumarin Content in Different Beverages Prepared from the *Peucedanum ostruthium* Rhizome

	liqueurs[b] mean level ± SD in [μg/mL]				water extract[c] mean level ± SD in [μg/mL]
#[a]	S1	S2	S3	S4	T
1	125.21 ± 0.31	511.49 ± 3.34	2574.75 ± 5.14	2574.04 ± 3.82	180.96 ± 2.68
2	0.71 ± 0.09	12.88 ± 1.54	34.36 ± 0.62	82.16 ± 3.38	60.40 ± 1.22
3	21.43 ± 0.38	133.58 ± 0.98	146.84 ± 2.17	268.79 ± 9.61	18.43 ± 0.86
4	11.69 ± 0.19	56.65 ± 0.56	164.30 ± 2.27	323.01 ± 2.36	15.94 ± 0.57
5	8.17 ± 0.10	35.47 ± 0.88	26.89 ± 0.29	66.53 ± 4.37	
6	0.93 ± 0.13	4.89 ± 0.66	22.11 ± 0.48	60.75 ± 2.67	4.90 ± 0.21
7	4.53 ± 0.29	35.15 ± 0.25	33.98 ± 0.43	126.70 ± 7.00	4.92 ± 0.16

[a] Numbers refer to the compounds shown in Figure 1. [b] Samples: S1, liqueur prepared from roots from Gontal in Carinthia, 2007; S2, liqueur prepared of roots from at the Königsalm in Lower Austria, Austria, 2008; S3, liqueur prepared of roots from Weisspriachtal in Salzburg, 2009; and S4, liqueur prepared of roots from the Riedingtal in Salzburg, Austria, 2009; $n = 3$. [c] T, water decoction of R1; $n = 3$.

Overall, the coumarin content of the liqueurs and the water decoction is potentially high enough to exert biological effects upon consumption. Considering that the liqueurs analyzed in this study are typically diluted 1:10 to 1:15 before consumption to reduce bitterness, low milligram quantities of coumarins, mostly oxypeucedanin hydrate (**1**), are taken up with a typically applied volume of 20−40 mL. The concentration of coumarins in the water decoction is most likely higher than in traditional masterwort teas due to differences in the way of preparation. Estimating that traditional tea preparations are less concentrated by a factor of about 10 compared to liqueurs, similar amounts of coumarins are ingested since much larger volumes of tea (approximately 250 mL) are consumed.

In conclusion, the above-described method is well suited for fast profiling and quantification of the main coumarins for quality control of the drug "Radix Imperatoriae" and various preparations thereof.

■ ASSOCIATED CONTENT

Supporting Information. Calibration function of **4**, ^{1}H, ^{13}C, and 2D NMR spectra of compounds **3** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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