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Tyrolobibenzyls E and F from *Scorzonera humilis* and distribution of caffeic acid derivatives, lignans and tyrolobibenzyls in European taxa of the subtribe Scorzonerinae (Lactuceae, Asteraceae)

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Abstract

A chemosystematic study of the subtribe Scorzonerinae, a subtribe of the Lactuceae tribe of the Asteraceae family was performed, using the recently discovered tyrolobibenzyls as well as lignans and caffeic acid derivatives as diagnostic characters. In addition to the known compounds two new tyrolobibenzyls (E and F) were isolated and their structures were established by mass spectrometry and 1D and 2D NMR spectroscopy. Twenty four samples from rootstocks of seventeen different Scorzonerinae taxa, comprising members of three genera (*Podospermum*, *Scorzonera*, and *Tragopogon*), were analyzed. Tyrolobibenzyls A (**1**), B (**2**), C (**5**), D (**3**), E (**6**), and F (**4**) were identified in crude extracts by means of HPLC retention times, on-line UV spectra and on-line MS/MS spectra. Quantification of these compounds was performed by HPLC, using 2,2-bis-(4-hydroxyphenyl)-propane as an internal standard. Tyrolobibenzyls A–F were only detected in samples from *Scorzonera humilis*, while chlorogenic acid and 3,5-dicaffeoylquinic acid were detected in all samples investigated. In contrast, caffeoyl tartaric acid and cichoric acid were not detectable in any member of the subtribe Scorzonerinae.
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1. Introduction

The subtribe Scorzonerinae comprises the genera *Epilasia*, *Geropogon*, *Koelpinia*, *Pterachaenia*, *Scorzonera*, *Tourneuxia*, and *Tragopogon*, and encompasses a total of approximately 300 species (Bremer, 1994). For the European flora 28 species of *Scorzonera* — including *Podospermum* — (Chater, 1976) and 20 species of *Tragopogon* — including *Geropogon* — have been reported (Richardson, 1976). The Central European flora is comparatively poor in members of the Scorzonerinae: only nine species of *Scorzonera* (including two of *Podospermum*) and four species of *Tragopogon* are reported for Austria (Adler et al., 1994); one species of *Podospermum*, five of *Scorzonera* and five of *Tragopogon* for Germany (Jäger and Werner, 2002); and one species of *Podospermum*, two of *Scorzonera* and five of *Tragopogon* for Switzerland (Heitz, 1990).

Subaerial parts of *Scorzonera hispanica* L. and *Tragopogon porrifolius* L. are used as asparagus-like vegetables (Franke, 1997) and species from the genus *Scorzonera* were additionally employed as folk-medicinal plants (Siegmund, 1874). Previous phytochemical investigations of members of the Scorzonerinae resulted in the identification of dihydroisocoumarins from *Scorzonera cretica* (Paraschos et al., 2001); flavonoids from *S. columnae* (Menichini et al., 1994) and different *Tragopogon* species (Kroschewsky et al., 1969; Smolarz and Krzaczek, 1988); lignans from *S. hispanica* (Bryanskii et al., 1992a; Tolstikhina et al., 1999) and *S. humilis* (Zidorn et al., 2000b); a neolignane from *S. hispanica* (Tolstikhina and Semenov, 1998); phenolic acids from *S. hispanica* (Tolstikhina et al., 1988) and *Tragopogon orientalis* (Smolarz and Krzaczek, 1988); a sesquiterpene from *S. hispanica* (Zidorn et al., 2000a); sesquiterpene lactones from *S. hispanica* (Bryanskii et al., 1992b; Zidorn et al., 2000a); triterpene derivatives from *Koelpinia linearis*

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(Razdan et al., 1996; Shah et al., 1996; Koul et al., 2000), from *S. hispanica* (Tolstikhina et al., 1988), *S. tomentosa* (Öksuz et al., 1990), *T. montanus* (Lugovskaya and Plekhanova, 1980), *T. orientalis* (Krzaczek and Smolarz, 1988), *T. porrifolius* (Warashina et al., 1991), and *T. pratensis* (Miyase et al., 1992). Additionally, a new class of bibenzyl derivatives was recently isolated from *S. humilis* (Zidorn et al., 2000b, 2002b).

So far no reports deal with secondary metabolites from the genera *Epilasia*, *Geropogon*, and *Pterachaenia*.

In this communication we report on isolation and structure elucidation of two new bibenzyl derivatives from *Scorzonera humilis* L. and on HPLC-MS and HPLC-DAD systems to identify and quantify bibenzyls in crude methanolic extracts and on the occurrence of these compounds in European members of the subtribe Scorzonerinae.

2. Results and discussion

2.1. New tyrolobibenzyls

The HPLC data from extracts from *S. humilis* samples revealed that besides known tyrolobibenzyls A–D (Fig. 1) two additional, polar compounds with very similar on-line MS fragmentation patterns were detectable. These compounds (**4** and **6**, Fig. 2) were isolated by silica gel column chromatography (CC), Sephadex LH-20 CC and semi-preparative HPLC. Compound **6** showed on-line MS signals at *m*/*z* 595 $[M–1]^-$ and 433 $[M–glucose–H]^-$, congruent with a molecular formula of $C_{28}H_{36}O_{14}$. The fragment at *m*/*z* 433 had the same mass as the quasi molecular ion peak observed for tyrolobibenzyl C (**5**), leading to the assumption that **6** was a derivative of **5** with an additional glucose moiety. This assumption was corroborated by NMR data (Table 1) showing signals for two glucose moieties and signals for a bibenzyl moiety nearly super-imposable to those observed for compound **5** (Zidorn et al., 2000b). Minor shift differences observed for the tetra-substituted benzene ring of the bibenzyl moiety in comparison with compound **5** and HMBC crosspeaks from the two anomeric glucose protons to carbons C-3 and C-6, respectively, established the structure of **6** as the 6-*O*-β-D-glucosyl derivative of tyrolobibenzyl C. The new natural compound **6** was named tyrolobibenzyl E in analogy to the already known bibenzyl derivatives from *S. humilis*.

On-line MS spectra of compound **4** displayed signals at *m*/*z* 621 $[M–H]^-$ and 459 $[M–glucose–H]^-$, indicating that **4** might be a derivative of tyrolobibenzyl B (**2**) with an additional glucose moiety. NMR data (Table 2) were congruent with that assumption, showing signals assignable to a bibenzyl moiety, similar to those observed for compound **2** (Zidorn et al., 2000b) and signals assignable to two glucose moieties. ^1H NMR results indicated that the anomeric carbons of the glucose moieties were linked with a carbonyl ($\delta_{H\text{-}1''}$ 5.39 ppm) and a phenolic moiety ($\delta_{H\text{-}1'''}$ 4.93 ppm), respectively. A HMBC experiment proved that the esterified glucose moiety was attached in the same position as in compound **2**. The connectivity of the second glucose

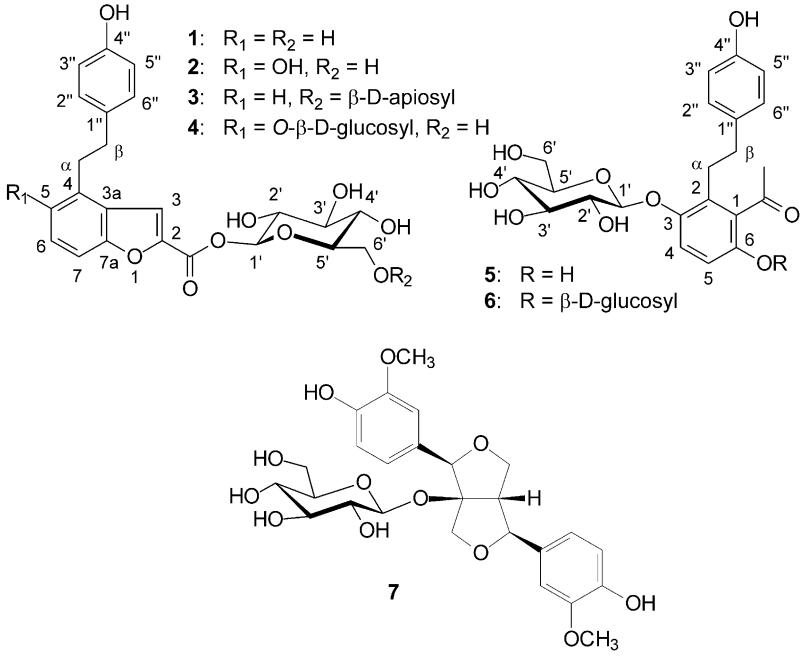

Fig. 1. Structures of tyrolobibenzyls A (**1**), B (**2**), C (**5**), D (**3**), E (**6**), F (**4**) and of the lignane pinoresinol-1-yl-D-glucopyranoside (**7**) isolated from *S. humilis*.

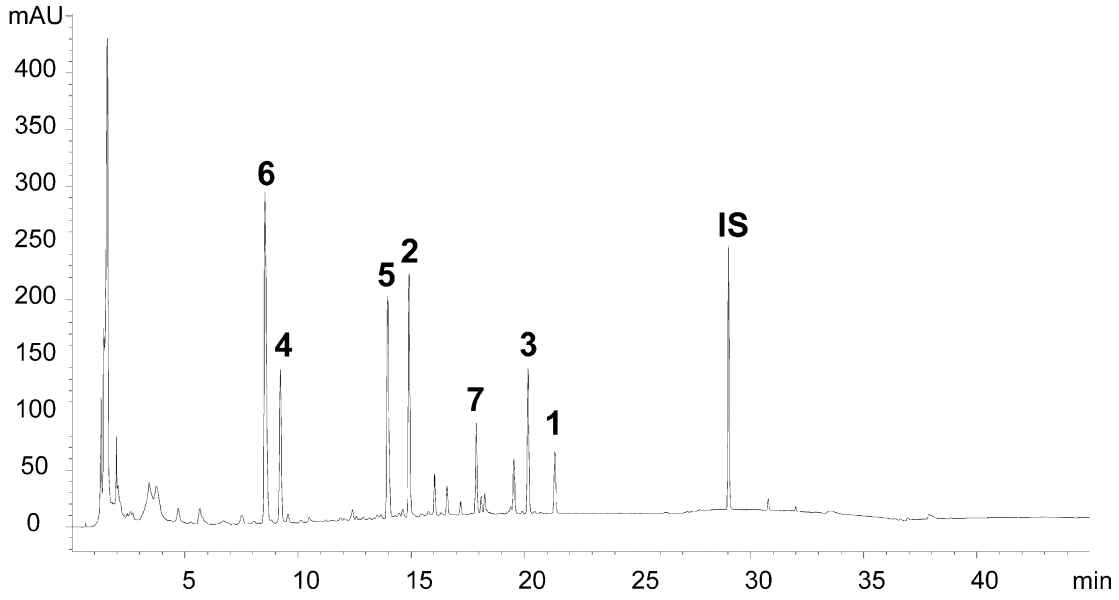

Fig. 2. HPL-chromatogram of a methanolic extract of subaerial parts from *S. humilis* L.HPLC parameters: Mobile phase A: H_2O, mobile phase B: MeCN; linear gradient: 0 min: 8% B, 5 min: 8% B, 25 min: 32% B, 30 min: 60% B, 35 min. 95% B; stop time: 45 min; post time: 15 min; flow rate: 1.00 ml/min; oven temperature: 55 °C; column: Zorbax SB-C18 4.6 × 150 mm (particle size 3.5 μm); guard column: Merck LiChroCart 4 × 4 mm packed with LiChrospher RP18 material (5 μm particle size); injection volume: 2 μl; detection wavelength: 280 nm. For compounds **1**–**7** see Fig. 1. IS = internal standard: 2,2-bis-(4-hydroxyphenyl)-propane.

moiety to the oxygen in position 5 of the bibenzyl moiety was established indirectly by the observed ^{1}H NMR shift differences for protons H-6 (δ_H 7.58) and H-7 (δ_H 7.23) in comparison with the corresponding signals of compound **2** ($\delta_{H\text{-}6}$ 7.14, $\delta_{H\text{-}7}$ 7.10) and directly by a weak HMBC cross-peak from the anomeric proton H-1‴ to C-5. Thus, **4** was the 5-*O*-glucosyl derivative of tyrolobibenzyl B (**2**), a new natural compound named tyrolobibenzyl F.

2.2. Comparative HPLC investigations

Methanolic extracts from rootstocks (Chater, 1976) of all available 24 samples representing 17 taxa from the subtribe Scorzonerinae—including all Austrian taxa—were analyzed by two different HPLC systems (a) for the occurrence of bibenzyl derivatives and (b) for the occurrence of caffeic acid derivatives. HPLC results are summarized in Table 3 and collection data and full scientific names of all investigated taxa are given in Table 4. Tyrolobibenzyls A–F (Fig. 1) were only found in extracts obtained from *S. humilis*. The absolute amounts of these compounds found in two samples from different parts of the Northern Tyrol varied pronouncedly (Table 3). Especially the two new compounds **4** and **6** were found in considerable concentrations only in the plant samples from the Außerfern region (Vils), with compound **6** being the prevalent bibenzyl in that extract. In contrast compounds **4** and **6** were only observed in trace amounts in the *S. humilis* sample from the Leutasch region. Whether that variation is due to regional or seasonal differences will be the subject of further studies.

The caffeic acid derivatives chlorogenic acid (**8**) and 3,5-dicaffeoyl quinic acid (**9**) were present in all investigated extracts. Caffeoyl tartaric acid derivatives caffeoyl tartaric acid (**10**) and cichoric acid (**11**) were not detectable in any of the investigated members of the Scorzonerinae. This again highlights the potential of these tartaric acid derivatives as chemosystematic markers to differentiate between different genera of the Lactuceae tribe (Zidorn et al., 2002a).

3. Experimental

3.1. Plant material

The collection data of the plant material are given in Table 4. Nomenclature, plant identification, and taxon delimitation are based on Jäger and Werner (2002) and Heitz (1990) for Central Europe, Pignatti (1997) for Italy and Diaz de la Guardia and Blanc Lopez (1987) for Spain. Plant material was collected and identified by the corresponding author.

3.2. Voucher specimens

Voucher specimens of all investigated populations (Table 2) are preserved in the Herbarium of the Institut für Pharmazie, Innsbruck. Voucher codes are included in Table 4.

Table 1
^{1}H and ^{13}C NMR spectra spectral data of tyrolobibenzyl E (**6**)[a]

Position	^{13}C NMR	^{1}H NMR
Bibenzyl moiety		
1	135.0	
2	130.2	
3	152.7	
4	118.4	7.24 1H, *d* (9.0)
5	115.8	7.09 1H, *d* (9.0)
6	150.0	
$CH_3\underline{C}O$	208.0	
$\underline{C}H_3CO$	32.7	2.35 3H, *s*
α	31.3	2.83 1H, *m*
		2.74 1H, *m*
β	36.6	2.74 1H, *m*
		2.74 1H, *m*
1′	134.6	
2′	130.6	6.99,[e] 6.96[e] 1H
3′	116.1	6.67,[e] 6.64[e] 1H
4′	156.5	6.67,[e] 6.64[e] 1H
5′	116.1	6.99,[e] 6.96[e] 1H
6′	130.6	
Glucose moiety I		
1″	103.2	4.91 1H, *d* (7.5)
2″	75.2[b]	3.53 1H, *m*[f]
3″	78.3[c]	3.35-3.471H, *m*[f]
4″	71.4[d]	3.35 1H, *m*[f]
5″	78.2[c]	3.35-3.47 1H, *m*[f]
6″	62.6	3.91 1H, *dd* (12.0, 2.0)[g]
		3.71 1H, *dd* (12.0, 5.0)[h]
Glucose moiety II		
1‴	103.2	4.85 1H, *d* (7.5)
2‴	74.9[b]	3.38 1H, *m*[f]
3‴	78.5[c]	3.35-3.47 1H, *m*[f]
4‴	71.5[d]	3.42 1H, *m*[f]
5‴	78.2[c]	3.35-3.47 1H, *m*[f]
6‴	62.6	3.87 1H, *dd* (12.0, 2.0)[g]
		3.67 1H, *dd* (12.0, 5.5)[h]

[a] All assignments are based on HSQC and HMBC experiments.
[b] Signals might be interchangeable in pairs.
[c] Signals might be interchangeable in pairs
[d] Signals might be interchangeable in pairs
[e] Most intense signals of an AA′XX′ spin system
[f] Signals not resolved.
[g] Signals might be interchangeable in pairs
[h] Signals might be interchangeable in pairs

Table 2
^{1}H and ^{13}C NMR spectra data of tyrolobibenzyl F (**4**)[a]

Position	^{13}C NMR	^{1}H NMR
Benzofuran moiety		
$\underline{C}OO$	168.5	
2	166.5	
3	93.9	6.05 1H, *s*
3a	115.0	
4	127.6	
5	153.8	
6	122.0	7.58 1H, *d* (9.0)
7	116.4	7.23 1H, *d* (9.0)
7a	151.3	
p-Hydroxyphenylethyl moiety		
α	29.6	3.60 2H, *m*[f]
β	36.4	2.80 1H, *m*[f]
		2.71 1H, *m*[f]
1′	134.6	
2′	130.4	7.22,[e] 7.20[e] 1H
3′	115.7	6.72,[e] 6.70[e] 1H
4′	156.1	
5′	115.7	6.72,[e] 6.70[e] 1H
6′	130.4	7.22,[e] 7.20[e] 1H
Glucose moiety I		
1″	100.9	5.39 1H, d (8.0)
2″	74.1[b]	3.59 1H, *m*[f]
3″	78.7[c]	3.57 1H, *m*[f]
4″	71.4[d]	3.64 1H, *m*[f]
5″	78.0[c]	3.42 1H, *m*[f]
6″	62.3	3.95 1H, *dd* (12.0, 2.0)[g]
		3.75 1H, *dd* (12.0, 5.5)[h]
Glucose moiety II		
1‴	103.0	4.93 1H, *d* (8.0)
2‴	75.0[b]	3.57 1H, *m*[f]
3‴	78.3[c]	3.49 1H, *m*[f]
4‴	71.3[d]	3.41 1H, *m*[f]
5‴	78.0[c]	3.42 1H, *m*[f]
6‴	62.3	3.88 1H, *dd* (12.0, 2.0)[g]
		3.70 1H, *dd* (12.0, 5.5)[h]

[a] All assignments are based on HSQC and HMBC experiments. ^{13}C NMR data are derived from indirect (HMBC, HSQC) experiments.
[b] Signals might be interchangeable in pairs.
[c] Signals might be interchangeable in pairs.
[d] Signals might be interchangeable in pairs.
[e] Most intense signals of an AA′XX′ spin system.
[f] Signals not resolved.
[g] Signals might be interchangeable in pairs.
[h] Signals might be interchangeable in pairs.

3.3. Abbreviations of political districts and states

AB = Abruzzo, And = Andalucia, BL = Burgenland, CA = Calabria, LB = Lombardia, NW = North Rhine-Westphalia, NÖ = Niederösterreich, OT = Otago, T = Tirol, TC = Toscana, TR = Trentino-Südtirol, VE = Venetia; A = Austria, B = Belgium, D = Germany, E = Spain, I = Italy, NZ = New Zealand.

3.4. Isolation of tyrolobibenzyls E (**6**) and F (**4**)

Freeze-dried subaerial parts of *S. humilis* (72.9 g) were exhaustively extracted with MeOH to yield 26.9 g crude extract after evaporating the solvent in vacuo. The crude extract was fractionated by silica gel CC using a gradient of CH_2Cl_2 and MeOH. The most polar fractions containing **6** and **4** were further separated by repeated Sephadex LH-20 CC using mixtures of MeOH/$(CH_3)_2CO$ and H_2O (3/1/1, v/v/v) as an eluant. Finally enriched fractions of **6** (44.7 mg) were purified by reversed phase (RP18) semi-preparative HPLC using a gradient of H_2O and MeCN. Column: Waters XTerra Prep MS C18, 7.8 × 100 mm, particle size: 5 μm (No. 186001156) ; column temperature: 25 °C; guard column: Merck Lichrospher 100 RP-18, particle size 5 μm (Nr.: 50931); HPLC system consisted of: Dionex P580 pump, Dionex ASI-100 autosampler, Dionex UVD170U

Table 3
Quantification results (mg/g dry plant material) of compounds **1**–**11** in subaerial parts of taxa from the subtribe Scorzonerinae[a]

Taxon	Sample	**1** (s_x)	**2** (s_x)	**3** (s_x)	**4** (s_x)	**5** (s_x)	**6** (s_x)	**7** (s_x)	**8** (s_x)	**9** (s_x)	**10** (s_x)	**11** (s_x)
Podospermum												
P. canum	CZ-20000517A-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
P. laciniatum	CZ-20000517A-2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
Scorzonera												
aristata	CZ-20000622A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	RS-20010801A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	CZ-20010809A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
austriaca	CZ-99-00381	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
baetica	CZ-19970426A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
hispanica	CZ-20020525C-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	CZ- HW98-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
aff. hispanica	CZ-200204D-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
humilis	CZ-98-00386	1.35 (0.05)	4.92 (0.01)	2.83 (0.02)	3.46 (0.10)	5.64 (0.01)	9.02 (0.04)	1.96 (0.03)	+	+	n.d.	n.d.
	CZ-99-00034	1.09 (0.00)	3.80 (0.04)	1.41 (0.01)	0.30 (0.01)	2.56 (0.03)	0.58 (0.01)	0.49 (0.03)	+	+	n.d.	n.d.
parviflora	CZ-20020524D-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
purpurea	CZ-20020524E-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
rosea	CZ-20000623A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
trachysperma	CZ-20020331C-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	CZ-20020402B-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
Tragopogon												
dubius	CZ-20000512A-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	CZ-20001117A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
orientalis	CZ-20020608A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
porrifolius	CZ-20000513A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
	CZ-20000517A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
pratensis	CZ-20010628A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
samaritani	CZ-20010721B-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	+	n.d	n.d.

[a] Amounts of compounds **1**–**7** were estimated by ratio of peak areas to areas of 2,2-bis-(4-hydroxyphenyl)-propane as internal standard, standard deviation (s_x) are given in brackets; n.d. not detectable; compounds **8**–**11** were investigated qualitatively: n.d. not detectable, + detectable.

UV-detector, and a Gilson 206 fraction collector; detection wavelength: 280 nm; injection volume: 100 μl flow rate: 3.0 ml/min; linear gradient: 0 min 8% MeCN, 10 min 8% MeCN, 15 min 10% MeCN. Compound **6** was collected between 6.5 and 10.1 min. In eight successive runs 5.7 mg of pure compound **6** were obtained.

Sephadex CC fractions containing **4** (862 mg) were again chromatographed on Sephadex to yield an enriched fraction of 654 mg. This was further fractionated by semi-preparative HPLC using the instrumentation described above and the following HPLC system: detection wavelength 280 nm; flow rate: 3.0 ml/min; linear gradient: 0 min 5% MeCN, 10 min 8% MeCN, 15 min 10% MeCN. Compound **4** was collected between 14.0 and 16.0 min. In eight successive runs 7.6 mg of a mixture of **4** and **6** (3/2) were obtained. This mixture was finally separated by semi-preparative HPLC using the system described above to yield 0.5 mg of compound **4**.

3.5. NMR spectroscopy

NMR spectra were recorded on a Varian-Unityplus-500 spectrometer at 500 and 125 MHz, respectively. Spectra were recorded in MeOH-d_4 and referenced to solvent residual signals and solvent signals at $\delta_H = 3.31$ ppm and $\delta_C = 49.0$ ppm, respectively.

3.6. Tyrolobibenzyl E (6)

Compound **6** was obtained as an amorphous solid, decomposing under gas emission above 148 °C. FTIR ν_{max}^{ZnSe}cm^{-1}: 3388, 2928, 2880, 1685, 1611, 1598, 1514, 1472, 1256, 1076, 897; ESIMS see 3.10; NMR data are given in Table 1.

3.7. Tyrolobibenzyl F (4)

Compound **4** was obtained as an amorphous solid, decomposing without gas emission above 154 °C. FTIR ν_{max}^{ZnSe}cm^{-1}: 3350, 2919, 2848, 1596, 1515, 1460, 1359, 1253, 1072, 1045; ESIMS see 3.10; NMR data are given in Table 2.

3.8. Extraction of the plant material

Dry plant material (500 mg) was mixed with 5.0 ml of a stock solution of 2,2-bis-(4-hydroxyphenyl)-propan containing 40.0 mg of the internal standard per 100 ml MeOH and sonicated three times for 15 min with 30 ml MeOH. The extract was brought to dryness in vacuo, re-dissolved in 2.00 ml of MeOH, filtered and used for HPLC analysis. Employing this procedure between 96 and 98% of the lignane and tyrolobibenzyl content was extracted and it was therefore considered to be an exhaustive extraction.

Table 4
Origin of plant material

Taxon	Location	Coordinates	Altitude (a.m.s.l.)	Sample
Podospermum				
P canum C.A. Meyer	I/AB/between Collelungo and Avezzano	N 41°56′; E 13°32′	735	CZ-20000517A-3
P. laciniatum (L.) DC.	I/AB/between Collelungo and Avezzano	N 41°56′; E 13°32′	735	CZ-20000517A-2
Scorzonera				
aristata Ramond ex DC.	I/LB/between Breno and Passo Croce Domini	N 45°55′; E 10°21′	1750	CZ-20000622A-1
	I/TR/Seceda NE St. Ulrich	N 46°36′; E 11°45′	2300	RS-20010801A-1
	I/VE/betw. Rifugio Fuciade and Pas de la Civéde	N 46°23′; E 11°49′	2060	CZ-20010809A-1
austriaca L.	A/NÖ/Kobenzl near Mödling	N 48°05′; E 16°16′	330	CZ-99-00381
baetica (Boiss.) Boiss.	E/And/between Alozaina and Coin	N 36°41′, W 4°50′	250	CZ-19970426A-1
hispanica L.	A/NÖ/near Gumpoldskirchen	N 48°02′; E 16°15′	340	CZ-20020525C-1
	B/market product (De Maeyer, Reg.-Nr.: 32134)	not available (n.a.)	n.a.	CZ- HW98-1
aff. hispanica L.	I/CA/between San Sosti and Lungro	N 39°40′; E 16°02′	430	CZ-20020404D-1
humilis L.	A/T/E Vils	N 47°33′; E 10°39′	810	CZ-98-00386
	A/T/SE Leutasch-Unterkirchen	N 47°24′; E 11°24′	1040	CZ-99-00034
parviflora Jacq.	A/BL/SW Sulzhof, ENE Siegendorf	N 47°46′; E 16°34′	160	CZ-20020524D-1
purpurea L.	A/BL/ESE Siegendorf, S Königsschüssel	N 47°46′; E 16°33′	200	CZ-20020524E-1
rosea Waldst. et Kit.	I/LO/Passo del Maniva near Colombano	N 45°49′; E 10°24′	1575	CZ-20000623A-1
trachysperma Guss.	I/CA/S La Mandria (close to Francavilla Marittima)	N 39°48′; E 16°20′	230	CZ-20020331C-1
	I/CA/near Cropolati	N 39°30′; E 16°46′	360	CZ-20020402B-1
Tragopogon				
dubius Scop.	I/TC/E Civitella in Val di Chiana	N 43°24′; E 11°44′	300	CZ-20000512A-6
	NZ/OT/Bendigo Road SW Bendigo	S 44°56′; E 169°18′	210	CZ-20001117A-1
orientalis L.	I/T/W Zirl at the transformer station	N 47°16′; E 11°12′	600	CZ-20020608A-1
porrifolius L.	I/TC/Sinalunga	N 43°12′; E 11°45′	270	CZ-20000513A-1
	I/AB/between Collelungo and Avezzano	N 41°56′; E 13°32′	735	CZ-20000517A-1
pratensis L.	D/NW/Schneeberg NW Aachen	N 50°46′; E 06°01′	220	CZ-20010628A-1
samaritani Heldr. et Sart.	I/TC/Pratomagno NE Loro Ciuffenna	N 43°38′; E 11°39′	1250	CZ-20010721B-1

3.9. Origin of reference compounds

Tyrolobibenzyls A–D and pinoresinol-1-yl-D-glucopyranoside were isolated from *Scorzonera humilis* as reported before (Zidorn et al., 2000b, 2002b). Chlorogenic acid was purchased from C. Roth (Karlsruhe, Germany), the internal standard 2,2-bis-(4-hydroxyphenyl)-propane from Fluka AG (Buchs, Switzerland), caffeoyl tartaric acid and cichoric acid were a generous gift from Prof. Dr. R. Bauer (Graz, Austria), and 3,5-dicaffeoyl quinic acid was isolated from *Leontodon hispidus* L. (Zidorn and Stuppner, 2001).

3.10. HPLC analyses

Instrumentation: Hewlett-Packard 1090 Liquid Chromatograph equipped with autosampler and DAD detector; HPLC parameters for the analyses of bibenzyls: Mobile phase A: H_2O, mobile phase B: MeCN; linear gradient: 0 min: 8% B, 5 min: 8% B, 25 min: 32% B, 30 min: 60% B, 35 min. 95% B; stop time: 45 min; post time: 15 min; flow rate: 1.00 ml/min; oven temperature: 55 °C; column: Zorbax SB-C18 4.6 × 150 mm (particle size 3.5 μm); guard column: Merck LiChroCart 4 × 4 mm packed with LiChrospher RP18 material (5 μm particle size); injection volume: 2 μl; detection wavelength: 280 nm. Retention times (min): 21.3 (**1**), 14.9 (**2**), 20.2 (**3**), 9.2 (**4**), 14.0 (**5**), 8.5 (**6**), 17.9 (**7**), 29.0 internal standard [2,2-bis-(4-hydroxyphenyl)-propan]. Amounts of compounds **1**–**7** were estimated by ratio of peak areas to areas of 2,2-bis-(4-hydroxyphenyl)-propane. All analyses were performed in triplicate.

HPLC system for the analyses of caffeic acid derivatives: Mobile phase A: H_2O/MeOH/CH_3COOH 84.5/15.0/0.5 (v/v/v), mobile phase B: MeCN/MeOH/CH_3COOH 84.5/15.0/0.5 (v/v/v); linear gradient: 0 min: 0% B, 5 min: 0% B, 20 min: 15% B, 45 min: 25% B, 50 min. 25% B, 51 min. 95% B; stop time: 65 min; post time: 15 min; flow rate: 1.00 ml/min; oven temperature: 35 °C; column: Zorbax SB-C18 4.6 × 150 mm (particle size 3.5 μm); guard column: Merck LiChroCart 4 × 4 mm packed with LiChrospher RP18 material (5 μm particle size); injection volume: 2 μl; detection wavelength: 350 nm. Retention times (min): 10.2 (**8**), 23.7 (**9**), 4.6 (**10**), 10.3 (**11**), 52.2 internal standard [2,2-bis-(4-hydroxyphenyl)-propan] (detectable only at 280 nm).

3.11. HPLC–MS analyses of bibenzyls

LC–MS analyses employing the HPLC system described above yielded only poor ionization of the bibenzyls. Therefore 0.15% acetic acid (v/v) was added to both solvents, otherwise the parameters given above were used. Instrumentation: Hewlett Packard HP-1100 Liquid Chromatograph employed with a DAD-detector coupled with a Bruker Esquire 3000plus ion trap LC/MS^n.

Retention times (min): 22.1 (**1**), 15.8 (**2**), 20.8 (**3**), 10.0 (**4**), 14.8 (**5**), 9.1 (**6**), 18.5 (**7**), 29.0 [2,2-bis-(4-hydroxyphenyl)-propane] (internal standard).

MS parameters: ESI, negative ionization mode, spray voltage: 4.5 kV, nebulizer: 15 psi, dry gas (N_2) 10 l/min, dry temperature: 350 °C, scanning range: *m*/*z* 100–1500.

Online MS fragmentation patterns observed for compounds **1**–**7** (relative signal-intensities are indicated in brackets): **1**: *m*/*z* 503 (80) $[M+CH_3COOH–H]^-$, 443 (100) $[M–H]^-$, 281 (56) [M–glucose–H]$^-$; **2**: *m*/*z* 519 (36) $[M+CH_3COOH–H]^-$, 459 (100) $[M–H]^-$, 297 (28) [M–glucose–H]$^-$; **3**: *m*/*z* 575 (100) $[M–H]^-$, 281 (12) [M–apiosylglucose–H]$^-$; **4**: *m*/*z* 681 (100) $[M+CH_3COOH–H]^-$, 621 (8) $[M–H]^-$, 459 (36) [M–glucose–H]$^-$; **5**: *m*/*z* 433 (100) $[M–H]^-$, 271 (8) [M–glucose–H]$^-$; **6**: *m*/*z* 655 (56) $[M+CH_3COOH–H]^-$, 595 (100) $[M–H]^-$, 433 (16) [M–glucose–H]$^-$; **7**: *m*/*z* 535 (100) $[M–H]^-$; 2,2-bis-(4-hydroxyphenyl)-propane (internal standard) 455 (8) $[2M–H]^-$, 227 (100) $[M–H]^-$. Fragmentation of the $[M–H]^-$ ions (MS/MS) yielded the following signals: **1**: *m*/*z* 281 [M–glucose–H]$^-$ (100); **2**: *m*/*z* 297; **3**: *m*/*z* 281 [M–apiosylglucose–H]$^-$ (100); **4**: *m*/*z* 459 [M–glucose–H]$^-$ (100); **5**: *m*/*z* 271 [M–glucose–H]$^-$ (100); **6**: *m*/*z* 433 [M–glucose–H]$^-$ (100); **7**: *m*/*z* 355 [M–glucose–H_2O–H]$^-$ (100), 179 [$C_6H_{12}O_6$–H] (46).

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