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Bibenzyls and dihydroisocoumarins from white salsify (*Tragopogon porrifolius* subsp. *porrifolius*)

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Abstract

A phytochemical investigation of three accessions of *Tragopogon porrifolius* L. subsp. *porrifolius* (Asteraceae, Lactuceae) yielded three new bibenzyl derivatives, 5,4′-dihydroxy-3-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyloxybibenzyl, 2-carboxyl-3,4′-dihydroxy-5-β-D-xylopyranosyloxybibenzyl, tragopogonic acid (2′carboxyl-3′,5′,4″-trihydroxyphenylethanone) and three dihydroisocoumarin derivatives, including the new natural product 6-*O*-methylscorzocreticoside I. One of the isolated bibenzyl derivatives is considered to be a precursor to the biosynthesis of dihydroisocoumarins. Structures of new compounds were established by HR mass spectrometry, extensive 1D and 2D NMR spectroscopy, and CD spectroscopy. Moreover, radical scavenging activities of the polyphenolic compounds were measured using the 2,2-diphenyl-1-picrylhydrazyl assay; two of the bibenzyls showed moderate and two of the dihydroisocoumarins showed weak radical scavenging activities. The chemosystematic impact of bibenzyls and dihydroisocoumarins is discussed briefly.

Keywords: Tragopogon; Asteraceae; Tribe lactuceae; Subtribe scorzonerinae; Biosynthesis

1. Introduction

Tragopogon porrifolius L. subsp. *porrifolius* is an annual or biennial herb of 30–125 cm height with lilac to reddish-purple ligules, which is native to the Eastern and Central Mediterranean region and Asia Minor. The taxon was formerly widely cultivated as a vegetable in most parts of Europe and is therefore occurring as an introduced plant in the entire Mediterranean region and as a casual further North (von Weihe, 1972; Richardson, 1976). Usage as a vegetable decreased in Southern and Central Europe in recent decades. However, white salsify is still quite popular in the United Kingdom (Franke, 1997). The species *T. porrifolius* is subdivided into three subspecies: *T. p.* subsp. *australis* (Jord.) Nyman, *T. p.* subsp. *cupani* (Guss. ex DC.) I.B.K. Richardson, and *T. p.* subsp. *porrifolius*, which is the only subspecies used as a vegetable (Richardson, 1976). *T. porrifolius* proved to be a rich source of new acylated pentacyclic triterpene saponins (Warashina et al., 1991). Additionally, the ubiquitous quinic acid derivatives chlorogenic acid and 3,5-dicaffeoylquinic acid were identified in crude extracts of *T. porrifolius* (Zidorn et al., 2003).

In recent phytochemical investigations of the related genus *Scorzonera* (also Asteraceae, tribe Lactuceae,

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subtribe Scorzonerinae) a number of biogenetically related dihydroisocoumarins, benzophenone, and bibenzyl derivatives were detected in *S. cretica* Willd. (Paraschos et al., 2001) and *S. humilis* L. (Zidorn et al., 2000, 2002, 2003), respectively.

The present communication reports about new bibenzyl and dihydroisocoumarin derivatives from *T. porrifolius* s.str. and their radical scavenging activity.

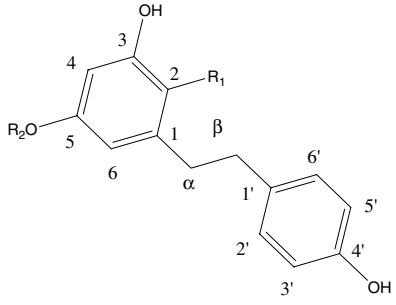
2. Results and discussion

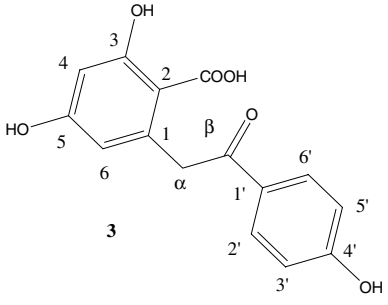
Bibenzyls **1–3** and dihydroisocoumarins **4–6** were isolated from *T. porrifolius* subsp. *porrifolius* collected in the wild in Andalusia/Spain (**1–2**, **6**), in Basilicata/Italy (**5**) and from plants originated from China and cultivated in Saxony-Anhalt/Germany (**3–4**), respectively. Compound **1a** was obtained by enzymatic splitting off of the sugar moiety and subsequent (unintended) decarboxylation of compound **1**. The structures were assigned using NMR, MS, UV, optical rotation data, and CD data.

In detail, HRMS of compound **1** in the positive mode showed a $[M + Na]^+$ signal at $m/z = 429.11787$ (calculated for $C_{20}H_{22}O_9Na$, $m/z = 429.11615$), which indicated a molecular formula of $C_{20}H_{22}O_9$. ^1H NMR data (Table 1) included six signals assignable to a β-D-xylopyranosyl moiety (Lavaud et al., 1996; Hiradate et al., 1999), two signals with double intensity of an A_2B_2 spin system assignable to a *para*-substituted aromatic moiety, two further aromatic signals in meta position two each other ($J = 2.5$ Hz) and signals assignable to an ethane-bridge linking the two aromatic systems. ^{13}C NMR spectra (Table 1) also evidenced the presence of a bibenzyl (Kunz and Becker, 1992, 1994) and a xylose moiety. Additionally, a signal at $\delta_C = 177.3$ ppm was observed, which, in congruence with the MS data, was assigned to a carboxylic acid moiety. Conclusively, **1** consisted of a β-D-xylopyranosyl moiety and a bibenzyl moiety substituted with a carboxyl function and three additional oxygen atoms. HSQC and HMBC experiments – important correlations are depicted in Fig. 1 – in combination with ^1H NMR coupling patterns established the substitution pattern of the bibenzyl moiety, the β-configuration of the xylopyranose moiety, and that the sugar moiety was attached to O-5 of the bibenzyl moiety. Conclusively, compound **1** was established as 2-carboxyl-3,4′-dihydroxy-5-β-D-xylopyranosyloxybibenzyl, a new natural compound.

Compound **1a**, which was obtained from **1** by enzymatic degradation employing cellulase, was identified by ESI mass spectrometry, which showed a quasi molecular peak at $m/z = 229$ $[M - H]^-$, and ^1H NMR spectroscopy and by comparing the ^1H NMR results with literature data (El-Feraly, 1984) as 3,5,4′-trihydroxybibenzyl (dihydroresveratrol). Thus, the enzymatic degra-

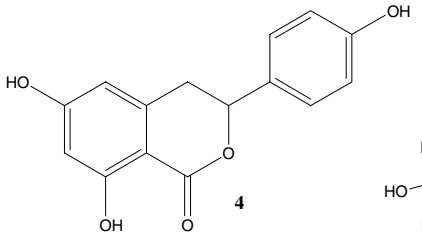
Structures of bibenzyl derivatives (**1-3**) and dihydroisocoumarins (**4-6**) from *T. porrifolius* subsp. *porrifolius*.[a]





3

1: R_1 = COOH, R_2 = β-D-xylopyranosyl
1a: R_1 =H, R_2 = H
2: R_1 = H, R_2 = α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl



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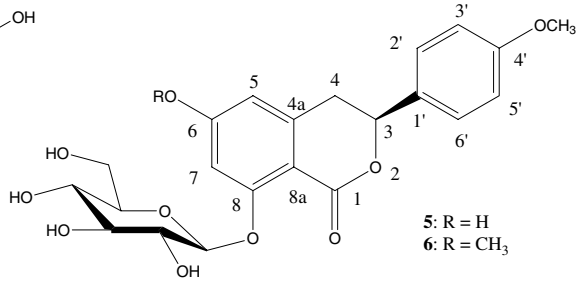

5: R = H
6: R = CH_3

[a] Numbering of compound **3** is arbitrary and follows bibenzyl numbering.

Table 1
^{1}H NMR data of bibenzyl derivatives **1**–**3**[a]

Position	^{1}H NMR			^{13}C NMR		
	1	**2**	**3**	**1**	**2**	**3**
Bibenzyl moiety						
COOH				177.3		171.2[d]
1				148.9	145.0	141.8
2		6.29 1H, *m*[e]		112.0	108.6	110.9
3				165.2	158.9	166.0
4	6.37 1H, *d* (2.5)	6.30 1H, *m*[e]	6.18 1H, *d* (2.5)	102.8	102.0	102.4
5				161.2	158.9	161.2
6	6.24 1H, *d* (2.5)	6.29 1H, *m*[e]	6.10 1H, *d* (2.5)	111.5	110.4	111.9
α	3.35 1H, *m*[e]		4.64 2H, br *s*[c]	39.2	39.3	45.6
	3.23 1H, *m*[e]					
β	2,77 2H, *m*[e]	2,75 2H, *m*[e]		38.1	37.7	200.1
1′				134.9	133.2	130.8
2′,6′	7.02 2H, *d* (8.5)	6.97 2H, *d* (8.5)	7.95 2H, *d* (8.5)	130.5	130.2	132.0
3′,5′	6.67 2H, *d* (8.5)	6.68 2H, *d* (8.5)	6.83 2H, *d* (8.5)	115.9	115.8	116.0
4′				156.1	155.8	163.2
Xylose moiety						
1″	4.77 1H, *d* (7.0)	4.83 1H,[b]		102.1	100.7	
2″	3.41 1H, *m*[e]	3.62 1H, *m*		74.6	71.9	
3″	3.42 1H, *m*[e]	3.54 1H, *m*[e]		77.6	78.8	
4″	3.55 1H, *m*[e]	3.53 1H, *m*[e]		71.0	71.0	
5″	3.90 1H, *dd* (11.0, 5.0)	3.89 1H, *m*		66.9	66.5	
	3.33 1H, *m*[e]	3.28 1H, *m*				
Rhamnose moiety						
1‴		5.24 1H, *d* (1.5)			102.3	
2‴		3.94 1H, *m*[e]			71.9	
3‴		3.94 1H, *m*[e]			71.9	
4‴		3.39 1H, *m*			73.7	
5‴		3.97 1H, *m*[e]			69.6	
6‴		1.28 3H, *d* (6.0)			17.8	

[a] Measured in CD_3OD (^{1}H at 300 MHz, ^{13}C at 75 MHz) and referenced to solvent residual and solvent signals at 3.31 ppm (^{1}H) and 49.0 ppm (^{13}C), respectively.
[b] Coupling pattern concealed by H_2O signal.
[c] Weak signal due to exchange of ^{1}H to ^{2}H.
[d] Signal only weak; J_4 correlation from H-4.
[e] Overlapping signals.

dation not only resulted in a loss of the sugar moiety but also in subsequent decarboxylation of the aglycon of compound **1**. Decarboxylation of the aglycon was probably promoted by the instability of the 2,5-dihydroxybenzoic acid moiety due to its enhanced capacity to form stable mesomeric forms (Li and Brill, 2003).

HRMS of compound **2** in the positive mode showed a $[M + H]^+$ signal at $m/z = 509.20444$ (calculated for $C_{25}H_{33}O_{11}$, $m/z = 509.20174$), which indicated a molecular formula of $C_{25}H_{32}O_{11}$. ^{1}H NMR and ^{13}C NMR data of **2** (Table 1) were similar to compound **1** and also indicated the presence of a bibenzyl and a β-D-xylopyranosyl moiety. In contrast to compound **1**, the ^{13}C NMR signal for the carboxyl group was missing and an ^{1}H NMR signal for an additional aromatic proton was present. Furthermore, ^{1}H and ^{13}C NMR signals for an α-L-rhamnopyranosyl moiety were detectable (Lavaud et al., 1996; Hiradate et al., 1999). ^{1}H NMR coupling patterns, HSQC, and HMBC experiments established that the aglycon of compound **2** was 3,5,4′-trihydroxybibenzyl (dihydroresveratrol, **1a**). HMBC correlations proved that the anomeric carbon of the rhamnopyranosyl moiety was linked via O-35″ to the xylopyranosyl moiety and that the anomeric carbon of the xylopyranosyl moiety was linked via O-3 to the bibenzyl moiety. Thus, the structure of compound **2** was established as 5,4′-dihy droxy-3-α-L-rhamnosyl-(1″→3′)-β-D-xylopyranosyloxybibenzyl, which represents another new natural bibenzyl derivative.

ESIMS of compound **3** in the negative mode showed signals at $m/z = 287$ $[M - H]^-$ and 245 $[M - H - CO_2]^-$, which together with ^{13}C NMR data (Table 2) indicated a molecular formula of $C_{15}H_{12}O_6$. ^{1}H NMR data of compound **3** indicated the presence of an aromatic A_2B_2 spin system, a tetrasubstituted aromatic system with the two protons in meta position to each other

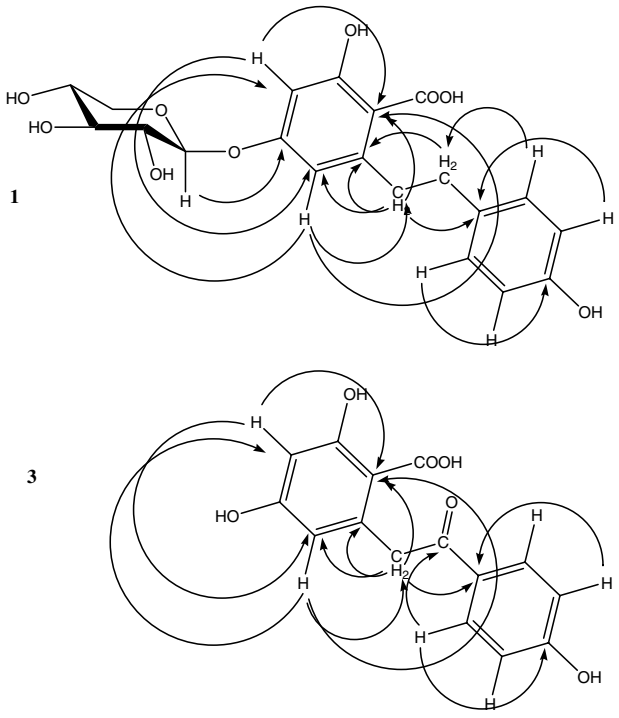

Fig. 1. Important HMBC correlations observed for compounds **1** and **3**.

Table 2
NMR data of compounds **6**[a]

Position	^{1}H NMR	^{13}C NMR
1		164.4
3	5.48 1H, *dd* (10.5, 3.5)	79.9
4	3.31 1H, *m*	36.5
	3.16 1H, *dd* 16.5 (3.5)	
4a		145.1
5	6.64 1H, *d* (2.5)	108.4
6		162.3
7	6.88 1H, *d* (2.5)	103.0
8		166.7
8a		108.5
1′		132.0
2′,6′	7.40 2H, *d* (8.5)	128.7
3′,5′	6.96 2H, *d* (8.5)	115.0
4′		161.6
6-OCH_3	3.88 3H, *s*	56.1
4′-OCH_3	3.81 3H, *s*	55.8
Glucose		
1″	4.93 1H, *d* (7.5)	103.2
2″	3.60 1H, *m*	74.6
3″	3.60 1H, *m*	78.7
4″	3.41 1H, *m*	71.1
5″	3.50 1H, *m*	77.9
6″	3.92 1H, *dd* (12.0, 2.5)	62.2
	3.69 1H, *dd* (12.0, 6.0)	

[a] Measured in CD_3OD (^{1}H at 300 MHz, ^{13}C at 75 MHz) and referenced to solvent residual and solvent signals at 3.31 ppm (^{1}H) and 49.0 ppm (^{13}C), respectively.

and an methylene moiety. Furthermore, ^{13}C NMR data indicated the presence of a carboxylic acid and a ketone moiety. HSQC and HMBC (Fig. 1) established that compound **3** was the β-keto derivative of the aglycon of compound **1**. This compound, which we name tragopogonic acid (systematic name: 2,4-dihydroxy-6-[2-(4-hydroxy-phenyl)-2-oxo-ethyl]-benzoic acid), also represents a new natural compound. It is proposed that tragopogonic acid **3** is a biosynthetic precursor or by-product in the biosynthesis of dihydroisocoumarins, which are also present in *T. porrifolius*. Interestingly, methyl 4,6-dimethoxy-2-(3,4-dimethoxybenzoyl)-methyl-benzoate, a compound which is related to tragopogonic acid **3** has been reported as a by-product in an investigation aiming at the chemical syntheses of unsymmetrical 3,4-disubstitued isocoumarin derivatives (Rossi et al., 2003).

Compound **4** was identified on the basis of its ESIMS and NMR spectra as the simple dihydroisocoumarin derivative thunberginol C, which has been reported from *Hydrangea macrophylla* (Thunb.) Ser. var. *thunbergii* Makino (Yoshikawa et al., 1992). Like the compound isolated from *Hydrangea*, the substance isolated from *T. porrifolius* also showed no optical activity and no circular dichroism (CD). Therefore, compound **4** was identified as a racemic mixture with regard to the C-3 stereocenter.

Compound **5** was identified as scorzocreticoside I (6-*O*-β-D-glucopyranosyl-4′-*O*-methyl-thunberginol C), employing mass spectrometry, 1D and 2D NMR spectroscopy, CD spectroscopy, and by comparing the obtained data with literature data of the compound, which was recently reported from *Scorzonera cretica* (Paraschos et al., 2001).

HRMS of compound **6** in the positive mode showed a $[M + H]^+$ signal at $m/z = 463.16020$ (calculated for $C_{23}H_{27}O_{10}$, $m/z = 463.16042$), which indicated a molecular formula of $C_{23}H_{26}O_{10}$. ^{1}H NMR and ^{13}C NMR data (Table 2) and comparison with literature data of scorzocreticoside I (**5**) (Paraschos et al., 2001), established that **6** was the formerly unknown 6-*O*-methyl derivative of scorzocreticoside I. The absolute configuration was proven to be identical with that of scorzocreticoside I by CD spectrometry. The CD spectrum of compound **6** also showed a negative cotton effect at 283 nm. In accordance with published CD data for 6-hydroxymellein (Krohn et al., 1997) this effect is assignable to the $n \rightarrow \pi^*$ transition of the carbonyl group of the dihydroisocoumarin chromophore. The negative cotton effect indicates (*S*)-configuration at position C-3, which is in congruence with the configuration assigned to scorzocreticoside I (**5**) (Paraschos et al., 2001).

The occurrence of chiral natural products, which have different stereochemistry in a given asymmetric centre, in the same taxon is pretty unusual. However, the fact that not only both stereoisomers of compound

4 were found in *T. porrifolius*, but that also dihydroisocoumarins from *H. macrophylla* (Thunb.) Ser. subsp. *serrata* (Thunb.) Makino (Hashimoto et al., 1987) belong to both possible groups of stereoisomers with regards to the configuration at C-3, implies that the biosynthesis of dihydroisocoumarins starting from 2-carboxy-bibenzyl derivatives is not sterically controlled. The fact that aglyca like thunberginol C are usually isolated as racemic mixture, while glycosides are usually obtained as one or the other isomer is easily explained by the fact that the two different C-3 epimers of the dihydroisocoumarin aglyca are enantiomers, while glycosides which differ in their stereochemistry only in position C-3 of the aglycon moiety are diastereomers. Such diastereomers are easily separated employing phytochemical standard techniques like silica gel, Sephadex, and reversed phase column chromatography, while enantiomers are not separable using these techniques unless chiral column materials are used.

Radical scavenging activities of compounds **1–6** were assessed employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in comparison to the reference compounds ascorbic acid, caffeic acid, and chlorogenic acid. DPPH assay results are summarized in Table 3. Compounds **2** and **3** showed moderate radical scavenging activity and compounds **4** and **5** showed weak radical scavenging activity. However, none of the new natural products showed a higher level of radical scavenging activity than any of the reference compounds. The limited radical scavenging activity of the tested compounds might be explained by their lack of *ortho*-dihydroxylated aromatic moieties (Rice-Evans et al., 1996).

The detection of bibenzyls and dihydroisocoumarin derivatives in *T. porrifolius* and the fact that biogenetically related or even identical substances (compound **5**) were also recently reported for taxa from the related genus *Scorzonera* (Zidorn et al., 2000, 2002, 2003; Paraschos et al., 2001), which also belongs to the subtribe Scorzonerinae of the Lactuceae tribe of the Asteraceae, highlights the close phylogenetic relationship of the genera *Scorzonera* and *Tragopogon*. Furthermore, it indicates that more members of these classes of compounds are to be expected from other so far uninvestigated taxa of the subtribe Scorzonerinae.

Table 3
Radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH = assay (IC_{50} values))[a]

Compound	IC_{50} (μg/ml)	IC_{50} (μM/ml)
Ascorbic acid	2.49 (0.32)	14.13 (1.84)
Caffeic acid	1.78 (0.03)	9.88 (0.19)
Chlorogenic acid	1.28 (0.38)	3.61 (1.07)
1	>200	–
2	8.93 (0.94)	17.66 (1.86)
3	14.22 (3.79)	49.3 (13.2)
4	115.6 (12.6)	424.6 (46.3)
5	59.15 (7.38)	131.9 (16.5)
6	>200	–

[a] Standard deviations are indicated in brackets.

3. Experimental

3.1. Plant material

One accession of *T. porrifolius* subsp. *porrifolius* was collected N Villanueva de la Concepcion/Malaga/Andalusia/Spain in April 2003 (coordinates: N36°56′, W 04°32′; alt.: 800 m) from plants growing in the wild in an abandoned olive grove. This collection was initially erroneously assigned to *Tragopogon hybridus* L. (Salvenmoser, 2004). A voucher specimen is preserved in the Herbarium of the Institut für Pharmazie, Innsbruck (voucher code: CZ-20030418C-1). A second collection of *T. porrifolius* s.str. was made in April 2004 in Maratea-Massa/Potenza in the South Italian Basilicata region (coordinates: N 39°58′, E 15°45′; alt.: 450 m; voucher code: CZ-20040423D-1). The third accession of *T. porrifolius* subsp. *porrifolius* consisted of plants grown from seeds obtained from the Botanical Garden Beijing/P. R. China in the experimental fields of the IPK-Gatersleben (Saxony-Anhalt/Germany; voucher code: TRA 4, Herbarium GAT).

3.2. Extraction and isolation

3.2.1. Isolation of compounds *1–2* and *6* from Spanish plant material

2-Carboxyl-3,4′-dihydroxy-5-β-D-xylopyranosyloxybibenzyl **1** (61.5 mg), 5,4′-dihydroxy-3-α-L-rhamnosyl-(1″→3′)-β-D-xylopyranosyloxybibenzyl **2** (4.5 mg), 6-*O*-methyl-scorzocreticoside I **6** (1.0 mg) were isolated from the EtOAc phase (3.32 g) of the methanolic extract (36.6 g) from air-dried subaerial parts (128 g) of *T. porrifolius* by silica gel 60 (230–400 mesh) column chromatography (CC) using gradients of CH_2Cl_2 and MeOH, Sephadex LH-20 CC using MeOH as an eluant, and successive semi-preparative RP-18 HPLC using gradients of H_2O and MeCN.

3.2.2. Enzymatic preparation of compound *1a* from *1*

Compound **1** (20 mg) was dissolved in 0.2 ml of EtOH, mixed with 1.8 ml of H_2O adjusted with CH_3COOH to pH 5 and 20 mg of cellulase (Sigma, St. Louis, USA). The mixture was kept for two days at 37 °C. Compound **1a** was purified by partitioning with EtOAc and Sephadex LH-20 CC of the EtOAc layer.

3.2.3. Isolation of compounds *3–4* from German plant material

Tragopogonic acid **3** (7.5 mg) and thunberginol C **4** (5.6 mg) were isolated from the EtOAc phase (5.62 g)

of the methanolic extract (165 g) of freeze-dried subaerial parts (791 g) of *T. porrifolius* by silica gel 60 (230–400 mesh) column chromatography (CC) using a gradient of CH_2Cl_2 and MeOH and Sephadex LH-20 CC using MeOH as an eluant.

*3.2.4. Isolation of compound **5** from Italian plant material*

Scorzocreticoside I **5** (21.3 mg) was isolated from the EtOAc phase (3.31 g) of the methanolic extract (43.9 g) of air-dried subaerial parts (129 g) of *T. porrifolius* by silica gel 60 (230–400 mesh) column chromatography (CC) using a gradient of CH_2Cl_2 and MeOH and Sephadex LH-20 CC using MeOH as an eluant.

3.3. Physical data of new compounds

*3.3.1. 2-Carboxyl-3,4′-dihydroxy-5-β-D-xylopyranosyloxybibenzyl (**1**)*

Amorphous yellowish compound, glass transition above 205 °C; $[\alpha]_D^{20}$ −22° (MeOH; *c* 1.32); FTIR ν_{max}^{ZnSe} cm^{-1}: 2361, 2341, 1919, 1869, 1844, 1829, 1792, 1772, 1750, 1734, 1717, 1699, 1684, 1670, 1653, 1636, 1617, 1576, 1569, 1559, 1541, 1521, 1507, 1497, 1489, 1473, 1457, 1436, 1419, 1396, 1387, 1374, 1363, 1340, 1105; HRMS: *m/z* = 429.11787 $[M + Na]^+$; calculated for $C_{20}H_{22}O_9$ Na: 429.11615.

*3.3.2. 5,4′-Dihydroxy-3-α-L-rhamnosyl-(1″→3′)-β-D-xylopyranosyloxybibenzyl (**2**)*

Amorphous yellowish compound, glass transition above 179 °C; $[\alpha]_D^{20}$ −39° (MeOH; *c* 0.235); FTIR ν_{max}^{ZnSe} cm^{-1}: 2921, 2536, 2361, 2341, 2149, 1943, 1919, 1890, 1868, 1844, 1829, 1792, 1772, 1749, 1734, 1717, 1699, 1684, 1670, 1653, 1636, 1576, 1559, 1541, 1521, 1507, 1498, 1489, 1473, 1457, 1437, 1419, 1375, 1292, 1251, 1203, 1177, 1078, 1038. HRMS: *m/z* = 509.20444 $[M + H]^+$; calculated for $C_{25}H_{33}O_{11}$: 509.20229.

*3.3.3. Tragopogonic acid (**3**)*

Colourless crystals, decomposing above 161 °C; FTIR ν_{max}^{ZnSe} cm^{-1}: 3200 (br), 2934, 2697, 2589, 2355, 2332, 2140, 1653, 1597, 1463, 1373, 1313, 1273, 1167, 1111, 1049, 1020, 926, 917.

*3.3.4. 6-O-Methylscorzocreticoside I (**6**)*

Colourless needles, decomposing above 206 °C; $[\alpha]_D^{20}$ −12° (MeOH; *c* 0.0685); FTIR ν_{max}^{ZnSe} cm^{-1}: 1713, 1608, 1587, 1519, 1443, 1357, 1325, 1313, 1277, 1247, 1221, 1197, 1185, 1164, 1150, 1102, 1079, 1038, 980, 955, 902. HRMS: *m/z* = 463.16020 $[M + H]^+$; calculated for $C_{23}H_{27}O_{10}$: 463.16043.

3.4. Radical scavenging activity

Methanolic solutions of test compounds were mixed with a methanolic solution of DPPH (Sigma-Aldrich, Steinheim, Germany). The final DPPH concentration was 40 mg/l. Compounds **1**–**6** were tested in final concentrations of 1, 2, 5, 10, 20, 50, 100, and 200 μg/ml, respectively. After incubation in 96 well-plates, the reaction mixture (250 μl) was kept in the dark at ambient temperature (25 °C) for 30 min. Then, the optical density of the test mixtures in comparison to DPPH and pure methanol was measured using a Hidex Chameleon plate reader at 515 nm. IC_{50} values for each replicate were calculated using the following formula: $IC_{50} = [(50 - LP)/(HP - LP) * (HC - LC)] + LC$. LP = low percentage, i.e., highest percent inhibition less than 50%; HP = high percentage, i.e., lowest percent inhibition greater than 50%; HC = high concentration, i.e., concentration of test substance at the high percentage, LC = low concentration, i.e., concentration of test substance at the low percentage. All compounds and concentrations were assayed in triplicate and mean values were calculated for each compound. Ascorbic acid (Merck, Darmstadt, Germany), caffeic acid (Fluka, Buchs, Switzerland), chlorogenic acid (Roth, Karlsruhe, Germany), and DPPH (Sigma-Aldrich, Steinheim, Germany) were obtained commercially.

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