

LIGNAN GLUCOSIDES AND SEROTONIN PHENYLPROPANOIDS FROM THE SEEDS OF *Leuzea carthamoides*⁺

Juraj HARMATHA^{1,*}, Miloš BUDĚŠÍNSKÝ², Karel VOKÁČ³, Milan PAVLÍK,
Karel GRÜNER and Věra LAUDOVÁ

*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic,
Flemingovo nám. 2, 166 10 Prague 6, Czech Republic; e-mail: ¹ harmatha@uochb.cas.cz,
² budesinsky@uochb.cas.cz, ³ vokac@uochb.cas.cz*

Received December 30, 2006

Accepted March 5, 2007

Two lignan glucosides, the known tracheloside and the new carthamoside, together with their two known aglycones, trachelogenin and (7E,8'S)-7,8-didehydroarctigenin (cf. carthamogenin), were isolated from the seeds of *Leuzea carthamoides*. Additional four phenylpropanoid derivatives of tryptamine, the *E* and *Z* isomers of *N*-feruloylserotonin and *N*-isoferuloylserotonin, were also isolated and identified. Their structure determination and identification were performed by spectroscopic, mainly NMR analysis.

Keywords: *Leuzea carthamoides*; Asteraceae; Lignans; Phenylpropanoids; Tracheloside; Carthamoside; *N*-Feruloylserotonin; *N*-Isoferuloylserotonin; Glycosides; Natural compounds.

Leuzea carthamoides (Willd.) DC., syn. *Rhaponticum carthamoides* (Willd.) Iljin (Asteraceae) is an endemic species of southern Siberia, but currently also cultivated and widely produced as a medicinal plant in Eastern and Central Europe. This plant is known for the content of various ecdysteroids¹, insect moulting hormone analogues. It has been used as a rich source of ecdysteroids for our phytochemical^{2,3}, chemotransformation⁴ and phototransformation^{5,6} studies. The compounds were mainly utilised in specific bioassays reflecting their affinity to the insect ecdysteroid receptor^{7,8}. The pharmacological potency and utilisation of *L. carthamoides* has been so far attributed to a high content and rich structural variability of ecdysteroids^{9,10}. The ecdysteroid content promoted utilisation of *L. carthamoides* for phytotherapeutic¹¹, dietary¹² or cosmetic¹³ purposes. Biological effects of various preparations produced from this species^{1,11}

+ Part 60 in the series Plant Substances; Part 59 see ref.³

may be also influenced by other biologically active constituents, which have been so far less intensively studied compared with phytoecdysteroids. Few years ago, the presence of a series of guaianolides in the genus *Leuzea* was reported¹⁴. These bioactive sesquiterpene lactones are widely distributed, and thus characteristic in the whole subtribus Centaureinae¹⁵. Identification of various flavonoids and flavonoid glycosides was also occasionally published by several authors, as summarised in ref.¹⁶ and additionally in ref.¹⁷. The range of *Leuzea* phenylalkanooids¹⁸ was enlarged by reporting the presence of a stilbene derivative¹⁹ and four serotonin phenylpropanoids²⁰.

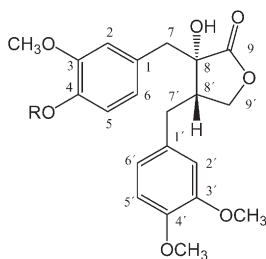
Our phytochemical interest in biologically active ecdysteroid agonists and antagonists^{21,22} and in insect growth regulating phenylpropanoids²³ led us to analyse and compare the content and composition of the above mentioned compounds in different organs of this plant. Seeds were selected for investigation because of their high content and structural variability of ecdysteroids²⁴ and promising content of phenylpropanoids^{16–20}. Seeds were in our case extracted and separated in a similar procedure as described earlier for roots^{2,3}. Besides ecdysteroids, we isolated and identified from polar ecdysteroid-containing fractions also lignan glycosides and rather rare serotonin phenylpropanoids. Identification of these compounds is here described.

RESULTS AND DISCUSSION

Lignan glycosides **1** and **2** were isolated from the methanol extract, initially prepared for recovery of a series of expected and also new ecdysteroids^{2,3}. Compound **1** was the major non-steroid constituent of this fraction. In its IR spectrum, the presence of a five-membered lactone ring (1765 cm^{-1}), aromatic ring (1515 , 1591 and 1606 cm^{-1}) and OH groups (3532 and 3431 cm^{-1}) were detected. HR-MS confirmed the composition $\text{C}_{27}\text{H}_{34}\text{O}_{12}$ with $[\text{M} + 1]^+$ 551 and main fragments at m/z 388 $[\text{M} - \text{Glc}]$ (for the lignan aglycone), m/z 151 (for the dimethoxybenzyl fragment) and m/z 136 (for the hydroxy-(methoxy)benzyl fragment). The ^1H NMR spectrum confirmed the presence of three aromatic methoxy groups (singlets at δ 3.802, 3.800 and 3.797) and the β -D-glucose moiety (anomeric H-1 as doublet at δ 4.86 with $J(1,2) = 7.6\text{ Hz}$). Complete analysis of ^1H and ^{13}C NMR spectra (Table I) and the optical rotation $[\alpha]_D^{20} -50.2$ proved the identity of compound **1** with the tracheloside²⁵.

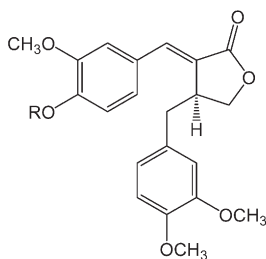
The second major non-steroid constituent, compound **2**, showed in the IR spectrum the presence of OH groups (3412 cm^{-1}), aromatic rings (1515 , 1597 cm^{-1}) and a five-membered lactone ring (1745 cm^{-1}) conjugated with

double bond (1644 cm^{-1}). HR-MS confirmed the composition $\text{C}_{27}\text{H}_{32}\text{O}_{11}$ with $[\text{M} + 1]^+$ 533 and main fragments at m/z 370 $[\text{M} - \text{Glc}]^+$ (for lignan aglycone) and m/z 151 (for dimethoxybenzyl fragment). The ^1H NMR spectrum confirmed the presence of three aromatic methoxy groups (singlets at δ 3.884, 3.777 and 3.773), β -D-glucose moiety (anomeric H-1 as doublet at δ 4.99 with $J(1,2) = 7.3\text{ Hz}$) and one vinyl proton (doublet at δ 7.49 with allylic coupling $J = 2.0\text{ Hz}$). Complete analysis of ^1H and ^{13}C NMR spectra (Table I) and the optical rotation $[\alpha]_{\text{D}}^{20} -7.6$ indicated that the structure could be identical with the hemislin B glucoside²⁶. Even the consideration that compounds **1** and **2** may have a close biogenetic relation, documented by the α -positions of the tertiary OH group in **1** and the double bond in **2** (possibly formed by a simple dehydration of **1**), indicated structural relation considering *R* configuration at C-8'. However, the identity has been rejected by hydrolysis of **2** providing aglycone **4** with a distinct positive optical rotation (see below), evidencing enantiomeric structure **2** for our new lignan glucoside carthamoside.



1, R = D-Glc

3, R = H



2, R = D-Glc

4, R = H

Tracheloside (**1**) and carthamoside (**2**) were hydrolysed to obtain the respective aglycones. From tracheloside (**1**) was obtained aglycone identical with trachelogenin²⁵ (**3**) proven also by the optical rotation $[\alpha]_{\text{D}}^{20} -31.6$ (in contrary to +29.6 for enantiomeric (+)-wikstromol²⁷). From carthamoside (**2**) was obtained aglycone carthamogenin^{22,28} (**4**) (with $[\alpha]_{\text{D}}^{20} +62.1$) identical with the (+)-7,8-didehydroarctigenin²⁹ ($[\alpha]_{\text{D}}^{20} +16.8$). The positive optical rotation of carthamogenin (**4**) reflects the (7*E*,8'*S*) configuration, which is supported also by positive optical rotations $[\alpha]_{\text{D}}^{20} +86$ of gadain³⁰ and +65.7 of another dibenzylbutyrolactone analogue from *Peperonia duclouxii*³¹ (both with 8'*S* configuration). The so far known carthamoside isomers with the 8'*R* configuration have negative optical rotations ($[\alpha]_{\text{D}}^{20} -25$, ref.³² and -16.4 , ref.³³), similarly as other (7*E*,8'*R*) analogues³⁴. The presence of two

OH groups in **3** and one OH group in **4** together with their positions in the molecule were confirmed by 1D- and 2D-ROESY spectra after in situ trichloroacetyl isocyanate acylation (TAI method)³⁵. Their structures were confirmed by complete analysis of their ¹H and ¹³C NMR spectra (Table II) and by optical resolution as above referred. The obtained results fall well in the relation between optical rotations and configurations at C-8 and C-8' in dibenzylbutyrolactone lignans as presented earlier³⁶. The revealed enantiomeric diversity of major lignan constituents in the same plant species as observed in this case, well illustrates a considerable diversity in lignan biosynthesis³⁷.

The aglycones **3** and **4** were used as reference compounds for searching these two lignan lactones also in the native extract. In this way, both lignans, trachelogenin (**3**) and carthamogenin (**4**) were detected, isolated and structurally identified in low polarity fractions of the extract. Both lignans **3** and **4** together with their glycosides **1** and **2** were included in our current bioactivity investigation, and some results were already published^{22,23,38}. Unfortunately, the C-8' configurations of carthamoside and carthamogenin were in the paper²² improperly presented.

From the lignan glycosides **1** and **2** containing polar fraction, an additional compound **5** was obtained. Its IR spectrum indicated the presence of aromatic ring (1513 and 1594 cm⁻¹), C=C double bond (1653 cm⁻¹), C-O bond (1273, 1207, 1183, 1124 and 1029 cm⁻¹) and OH group (3385 cm⁻¹). The ¹H NMR spectrum confirmed the presence of 20 protons, including one aromatic methoxy group (δ 3.88) and four labile exchangeable protons (two OH and two NH). ¹³C NMR showed 20 carbons: 17 sp²-carbon atoms (nine of the -CH= type, seven of the >C= type and one C=O), two methylene and one methoxy group. HR-MS confirmed the composition C₂₀H₃₀N₂O₄ with [M + 1]⁺ 353 and main fragments at *m/z* 177 (for the feruloyl moiety) and *m/z* 159 (for the hydroxy(ethyl)indolyl fragment). Complete analysis of ¹H and ¹³C NMR spectra (Table III) proved the identity of compound **5** with *N*-(*E*)-feruloylserotonin.

In order to investigate biological activities of these compounds we scaled up separations by a modified procedure²⁰ using compound **5** as an internal standard. The *N*-(*E*)-feruloylserotonin-containing fraction obtained by column chromatography was analysed and purified by HPLC in several solvent systems, resulting in one single-peak product. The NMR spectra, however, showed that the obtained sample was a mixture of four isomeric compounds, one major and three minors. The previously described HPLC conditions (reviewed in ref.²⁰) and also those used in our experiments²⁰, were found insufficient for separation of this family of compounds. A majority

TABLE I
 ^1H and ^{13}C NMR data of compounds **1** and **2** in CD_3OD

Position	1		2	
	$\delta(\text{C})$	$\delta(\text{H})(J_{\text{H,H}})$	$\delta(\text{C})$	$\delta(\text{H})(J_{\text{H,H}})$
1	133.34	—	128.29	—
2	115.92	6.79 d (2.0)	117.40	7.23 d (2.1)
3	147.17	—	150.40	—
4	150.53	—	149.68	—
5	117.78	7.08 d (8.3)	113.44	6.82 d (7.7)
6	124.15	6.69 dd (8.3;2.0)	125.17	7.22 dd (7.7;2.1)
7	41.81	3.16 d (13.6) 2.90 d (13.6)	138.40	7.49 d (2.0)
8	77.30	—	130.04	—
9	180.44	—	174.94	—
1'	131.70	—	131.93	—
2'	113.93	~6.72 m	114.17	6.73 d (2.1)
3'	149.16	—	149.44	—
4'	150.59	—	150.80	—
5'	113.22	6.87 d (7.8)	115.02	7.23 d (8.0)
6'	122.26	~6.72 m	122.05	6.74 dd (7.7;2.1)
7'	32.16	2.80 dd (13.7;5.1) 2.52 dd (13.7;9.5)	38.36	2.94 dd (14.2;5.3) 2.76 dd (14.2;8.6)
8'	44.59	2.44 m	40.42	4.06 m (8.6;6.8;5.3;2.0;1.9)
9'	71.80	~3.99 m	71.70	4.36 dd (9.2;6.8) 4.30 dd (9.2;1.9)
OCH_3	56.78	3.802 s	56.87	3.884 s
	56.56	3.800 s	56.52	3.777 s
	56.54	3.797 s	56.40	3.773 s
Glc				
1	102.83	4.86 d (7.6)	102.25	4.99 d (7.3)
2	74.86	3.48 dd (9.6;7.6)	74.94	3.53 dd (9.2;7.3)
3	77.80	3.45 t (9.6;9.6)	77.85	3.49 dd (9.2;8.7)
4	71.28	~3.38 m	71.28	3.40 dd (9.6;8.7)
5	78.17	~3.38 m	78.31	3.47 ddd (9.6;5.6;1.8)
6	64.22	3.85 dd (12.1;1.8) 3.68 dd (12.1;5.4)	62.49	3.90 dd (12.1;1.8) 3.70 dd (12.1;5.6)

TABLE II
¹H and ¹³C NMR data of compounds **3** and **4** in CDCl₃

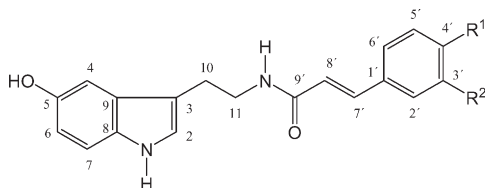
Position	3		3 + TAl ^a	4		4 + TAl ^a
	δ(C)	δ(H)(J _{H,H})	δ(H)(J _{H,H})	δ(C)	δ(H)(J _{H,H})	δ(H)(J _{H,H})
1	125.94	—	—	125.63	—	—
2	112.12	6.72 d (2.0)	7.12 d (1.9)	112.26	7.04 d (2.0)	7.13 d (1.5)
3	145.08	—	—	147.60	—	—
4	147.82	—	—	148.09	—	—
5	114.29	6.84 d (8.0)	7.15 d (8.2)	114.96	6.99 d (8.2)	7.23 d (7.6)
6	123.17	6.63 dd (8.0;2.0)	6.86 dd (8.2;1.9)	123.92	7.22 dd (8.2;2.0)	7.22 dd (7.6;1.5)
7	42.19	3.09 d (13.7) 2.93 d (13.7)	3.39 d (14.2) 3.20 d (14.2)	137.42	7.53 d (1.9)	7.57 d (2.0)
8	76.41	—	—	126.53	—	—
9	178.29	—	—	172.74	—	—
1'	130.94	—	—	130.41	—	—
2'	111.37	6.63 d (2.1)	6.46 d (2.0)	111.49	6.68 d (2.0)	6.64 d (2.1)
3'	146.59	—	—	146.71	—	—
4'	149.10	—	—	149.13	—	—
5'	112.60	6.80 d (8.2)	6.77 d (8.2)	112.68	6.81 d (8.1)	6.79 d (8.2)
6'	120.82	6.68 dd (8.2;2.1)	6.52 dd (8.2;2.0)	120.68	6.75 dd (8.1;2.0)	6.70 dd (8.2;2.1)
7'	31.57	2.96 m ~2.53 m	2.49 dd (13.5;4.6) 2.35 dd (13.5;10.5)	37.26	3.08 dd (14.4;4.2) 2.65 dd (14.4;10.2)	3.03 dd (13.5;4.6) 2.69 dd (13.5;10.0)
8'	43.85	~2.53 m	2.93 m (10.5;4.6;8.2;7.9)	39.68	3.83 m	3.84 m (10.0;5.8;4.6;2.7;2.0)
9'	70.04	~4.03 m	4.29 dd (8.8;8.2) 4.19 dd (8.8;7.9)	69.69	4.28 d (4.2)	4.32 dd (8.8;5.8) 4.29 dd (8.8;2.7)
OCH ₃	55.95	3.860 s	3.860 s	55.97	3.921 s	3.886 s
	55.92	3.800 s	3.851 s	55.92	3.864 s (2)	3.857 s (2)
	55.85	3.797 s	3.846 s	55.88		

The signals of NH protons in OTAC groups: ^a 8.73 s and 8.68 s; ^b 8.74 s.

TABLE III
 ^1H and ^{13}C NMR data of compounds **5–8** in CD_3OD

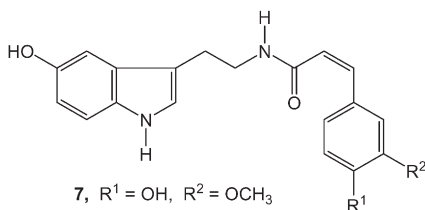
Position	5		6		7	8
	$\delta(\text{C})$	$\delta(\text{H})(J_{\text{H,H}})$	$\delta(\text{C})$	$\delta(\text{H})(J_{\text{H,H}})$	$\delta(\text{H})(J_{\text{H,H}})$	$\delta(\text{H})(J_{\text{H,H}})$
2	124.32	7.03 bs	124.27	7.03 bs	6.95 bs	6.95 bs
3	112.55	–	112.51	–	–	–
4	103.56	6.96 dd (2.3;0.6)	103.54	6.96 dd (2.4;0.6)	–6.95 dd (2.3;0.6)	6.94 dd (2.4;0.5)
5	151.20	–	151.15	–	–	–
6	111.60	6.66 dd (8.6;2.3)	112.66	6.66 dd (8.6;2.4)	6.66 dd (8.6;2.3)	6.66 dd (8.7;2.4)
7	112.42	7.16 dd (8.6;0.6)	112.40	7.16 dd (8.6;0.8)	7.15 dd (8.6;0.6)	7.15 dd (8.7;0.5)
8	133.19	–	133.14	–	–	–
9	129.49	–	129.45	–	–	–
10	26.46	2.94 bt (7.2)	26.45	2.93 bt (7.2)	2.88 bt (7.2)	2.88 bt (7.2)
11	41.49	3.58 bt (7.2)	41.48	3.57 bt (7.2)	3.51 bt (7.2)	3.52 bt (7.2)
1'	128.39	–	129.55	–	–	–
2'	112.69	7.12 d (2.0)	112.51	7.04 d (2.1)	7.35 d (2.0)	7.04 d (2.2)
3'	149.33	–	150.86	–	–	–
4'	149.86	–	148.03	–	–	–
5'	116.48	6.79 d (8.2)	114.55	6.91 d (8.3)	6.72 d (8.3)	6.75 d (8.4)
6'	123.25	7.03 dd (8.2;2.0)	122.05	7.00 dd (8.3;2.1)	6.93 dd (8.3;2.0)	6.90 dd (8.4;2.2)
7'	142.00	7.74 d (15.6)	141.73	7.41 d (15.7)	6.61 d (12.7)	6.58 d (12.6)
8'	118.95	6.42 d (15.6)	119.51	6.40 d (15.7)	5.84 d (12.7)	5.85 d (12.6)
9'	169.29	–	169.12	–	–	–
OCH_3	56.40	3.88 s	56.37	3.88 s	3.80 s	3.83 s

of negative effects of separation discussed in that paper was removed by using a column packed with Separon SGX Phenyl phase. Four compounds were detected in this chromatogram, which were, after separation, identified by NMR spectroscopy (Table III) as *N*-(*E*)-feruloylserotonin (**5**), *N*-(*E*)-isoferuloylserotonin (**6**), *N*-(*Z*)-feruloylserotonin (**7**), and *N*-(*Z*)-isoferuloylserotonin (**8**). The isomers were eluted from the phenyl phase column in two groups. In the first group were present *Z* isomers (feruloyl followed by the isoferuloyl isomer), in the second group were *E* isomers (again feruloyl followed by the isoferuloyl isomer) with the predominant *N*-(*E*)-isoferuloylserotonin (**6**).



5, $R^1 = \text{OH}$, $R^2 = \text{OCH}_3$

6, $R^1 = \text{OCH}_3$, $R^2 = \text{OH}$



7, $R^1 = \text{OH}$, $R^2 = \text{OCH}_3$

8, $R^1 = \text{OCH}_3$, $R^2 = \text{OH}$

The content of *Z* isomers (*cis*-cumaroyl-, feruloyl- or isoferuloylserotonins) in plants is usually much lower than the content of *E* isomers (*trans*-analogues). Therefore, it is possible that *E* isomers as minor components were not detected in earlier analyses, and thus not reported in the respective papers (as surveyed in refs^{20,39,40}). The identification of *Z* and *E* isomers of *N*-(*p*-cumaroyl)serotonins, present in *Amorphophallus konjac* in almost equivalent ratio⁴¹, as well as our results, support indirectly such assumption. Chromatographic analysis of *N*-feruloylserotonin isomers **5–8** has shown that simple column chromatography, as well as common reverse or normal phase HPLC separations allowed only isolation of a mixture of isomers²⁰. Final separation of individual isomers required special conditions, in this case use of a column filled with Phenyl phase.

Biological activities of the isolated and identified *Leuzea* lignans and feruloylserotonin conjugates were assessed in various test models available to our research partners. The insect feeding preferences were tested against selected insect stored-product pests²³ and compared with a series of other structurally related natural or chemically modified lignans. Activities of the *Leuzea* lignan glucosides **1** and **2** varied from strong antifeedant (for *Tribolium confusum*) up to weak attractant (for *Sitophilus granarius*) extremes, in contrast to the other, mostly strong feeding deterrent lignans²³. The effect on the ecdysteroid receptor assessed in the specific *Drosophila melanogaster* B_{II} cell bioassay for ecdysteroid agonist and antagonist activities^{9,22} was similar to other tested lignans: carthamoside (**2**) showed a low agonist activity, tracheloside (**1**) remained inactive²². Tracheloside (**1**) was included in a series of selected lignans tested for their immunomodulatory properties³⁸. Its activity was remarkably weak compared with the structurally related lignans possessing non-polar methoxy or methylenedioxy substituents¹⁸. Analgesic and also anxiolytic effects of the natural crystalline *N*-feruloylserotonin fraction containing isomers **5–8** has been tested on nociception and anxiety in Wistar rats⁴². The *N*-feruloylserotonin fraction had selective effects especially in the high-pain threshold rats. It decreased anxiety by increasing exploratory activity, and reduced stress-induced analgesia after acute stress⁴³. Our investigation indicates that *L. carthamoides* may come to be a new rich natural source of serotonin derivatives for various bioactive prepreations.

Our reported and also unpublished preliminary results indicate that *Leuzea carthamoides* contains, besides bioactive ecdysteroids^{1–3}, also other valuable secondary metabolites with a potency to be effective in assumed therapeutic exploitation.

EXPERIMENTAL

Infrared spectra (in cm⁻¹) were recorded on a Bruker IPS-88 instrument using KBr pellets. Optical rotations were measured at 20 °C on a Rudolph research analytical Autopol IV polarimeter in methanol. NMR spectra (δ , ppm; *J*, Hz) were measured on a Varian Unity-500 spectrometer (¹H at 500 MHz, ¹³C at 125.7 MHz) in CD₃OD and/or CDCl₃. ¹H NMR chemical shifts were referenced to TMS (in CDCl₃) and/or residual solvent peak (δ 3.31 in CD₃OD). ¹³C NMR chemical shifts were referenced to the solvent signal (δ (CDCl₃) 77.0 and δ (CD₃OD) 49.00). Homonuclear 2D-COSY and 2D-ROESY spectra were used for structure assignment of protons. ¹³C APT spectra and heterocorrelated 2D-HMQC or 2D-HMBC spectra were combined to assign all carbon signals. Mass spectra were recorded on a ZAB-EQ spectrometer with fast atom bombardment (FAB) ionisation using a glycerol-thioglycerol mixture as a matrix.

Plant Material

Seeds of *Leuzea carthamoides* (Willd.) DC., syn. *Rhaponticum carthamoides* (Willd.) Iljin (Asteraceae) were collected at the horticulture station Velký Osek (Czech Republic) from cultivated four-years-old plants.

Extraction and Isolation

Seeds (700 g) of *Leuzea carthamoides* were first processed by CO₂ supercritical fluid extraction in order to exploit the obtained seed oil as a side product intended for special application. The defatted residue was extracted with MeOH (yielding 137 g of extract) to recover characteristic *Leuzea* phytoecdysteroids³. Two portions of this extract were separated in two different ways: by column chromatography on silica gel (as described below), or by low-pressure liquid chromatography on Sephadex LH-20 (as described earlier²⁰).

Column chromatography separation of the MeOH extract (10 g) was performed using silica gel (450 g, ICN SiliTech, particle size 32–63 nm, activated at 120 °C for 15 h). For elution was used the gradient solvent system CHCl₃–MeOH 20:1 (1400 ml), 18:1 (900 ml), 16:1 (900 ml), 14:1 (1000 ml), 12:1 (1850 ml), 10:1 (1400 ml), 8:1 (700 ml), 6:1 (900 ml), 4:1 (450 ml), 2:1 (600 ml), followed by mixing the distilled eluent from previous fractions with MeOH in ratio 4:1 (600 ml), 3:1 (350 ml), 2:1 (600 ml), 1:1 (400 ml) and MeOH (400 ml). The chromatographic fractions were after a TLC monitoring (using Kieselgel GF₂₅₄ and CHCl₃–MeOH 20:1) combined into 24 collective fractions.

Solid part from fraction 15 was filtered, washed with CHCl₃ and purified on a silica gel column (20 g) with 5% MeOH in CHCl₃ providing compound **1** (250 mg). Fractions 16–18 (1.1 g) were further separated on silica gel column (50 g) with a mobile phase containing 2% MeOH in CHCl₃ (400 ml), and 5% MeOH in CHCl₃ (900 ml) giving according to TLC two single compounds **1** (65 mg) and **2** (170 mg).

Fractions 6–10 (1.4 g) were further separated on a silica gel column (60 g) with a mobile phase containing 2% MeOH in CHCl₃ (1000 ml), and 5% MeOH in CHCl₃ (900 ml) giving according to TLC one large *N*-feruloylserotonin-containing fraction (420 mg). This fraction was further analysed and subsequently also separated by HPLC (using Knauer-modular HPLC system). For separation were used semipreparative columns (250 × 8 mm) packed with Separon SGX C-18 (7 μm) and Separon SGX Phenyl (7 μm), respectively, produced by Tessek Praha. MeOH–water mobile phase was used under various chromatographic conditions, as reported in detail in our previous paper²⁰. Four constituents of this fraction were isolated: **5** (4.1 mg), **6** (14.6 mg), **7** (2.6 mg) and **8** (2.5 mg). Compounds **5** and **6** were isolated in purity over 92%, which did not change during solvent evaporation and NMR spectroscopy. Purity of compounds **7** and **8** changed rather rapidly during the processing. The final purity reached only 65%, due to common *Z/E* isomerisation of their feruloyl/isoferuloyl moiety.

Tracheloside (**1**)

Compound **1** was obtained as amorphous solid from fraction 15 by repeated column chromatography using 5% MeOH in CHCl₃ for elution. $[\alpha]_D^{20}$ –50.2 (c 0.159 in MeOH). IR (KBr): 1515, 1591, 1606 (aromatic); 1765 (lactone); 3532, 3431 (OH). FAB-MS, *m/z* (rel.%): 573 (20) [M + Na], 551 (8) [M + 1], 489 (12), 475 (8), 389 (10), 388 (10) [M – Glc], 185 (26), 151 (59) [dimethoxybenzyl], 137 (58) [hydroxy(methoxy)benzyl], 93 (100). HR-MS, *m/z*: 551.210173 [M + H]⁺, for C₂₇H₃₅O₁₂ required 551.212852. For ¹H and ¹³C NMR data, see Table I.

Carthamoside (2)

Compound **2** was obtained as amorphous solid from fractions 16–18 by repeated column chromatography using 5% MeOH in CHCl_3 for elution. $[\alpha]_{\text{D}}^{20} -7.6$ (c 0.629 in MeOH). IR (KBr): 1515, 1597 (aromatic); 1644 (double bond); 1745 (lactone); 3412 (OH). FAB-MS, m/z (rel.%): 555 (6) $[\text{M} + \text{Na}]$, 533 (4) $[\text{M} + 1]$, 371 (18) $[\text{M} - \text{Glc} + 1]$, 181 (26), 151 (100) [dimethoxybenzyl], 137 (17), 115 (68), 110 (48), 91 (84). HR-MS, m/z : 533.207006 $[\text{M} + \text{H}]^+$, for $\text{C}_{27}\text{H}_{33}\text{O}_{11}$ required 533.202287. For ^1H and ^{13}C NMR data, see Table I.

Trachelogenin (3)

Compound **1** (50 mg) was refluxed in a mixture of MeOH (5 ml) and 10% H_2SO_4 (10 ml) for 60 min, then 30 ml H_2O was added and the solvent partly evaporated in order to remove MeOH. After cooling the mixture was extracted with AcOEt, dried with anhydrous MgSO_4 and evaporated. The residue was purified on a short silica gel column with 10% AcOEt in CHCl_3 yielding aglycone **3** (30 mg). The obtained compound was used as a standard for TLC monitoring of seed extract chromatography fractions. Compound **3** (5 mg) was isolated from fractions 3–4 by NP-HPLC using a semipreparative SGX column (250×8 mm) with mobile phase 10% MeOH in hexane (isocratic conditions). $[\alpha]_{\text{D}}^{20} -31.6$ (c 0.111 in MeOH). IR (KBr): 1516, 1603 (aromatic); 1769 (lactone); 3430 (OH). FAB-MS, m/z (rel.%): 411 (4) $[\text{M} + \text{Na}]$, 389 (12) $[\text{M} + 1]$, 388 (18) $[\text{M}]$, 151 (68) [dimethoxybenzyl], 137 (100) [hydroxy-(methoxy)benzyl], 93 (18). HR-MS, m/z : 388.156096 $[\text{M}]^+$, for $\text{C}_{21}\text{H}_{24}\text{O}_7$ required 388.152203. For ^1H and ^{13}C NMR data, see Table II.

Carthamogenin (4)

Compound **2** (50 mg) was processed in the same way as compound **1** yielding aglycone **4** (35 mg) used as a standard for monitoring fractions 2–4 of the seed extract. Compound **4** (12 mg) was later isolated also from fractions 3–4 by the same HPLC process as described for compound **3**. $[\alpha]_{\text{D}}^{20} +62.1$ (c 0.307 in MeOH). IR (KBr): 1515 (aromatic); 1644 (double bond); 1745 (lactone); 3422 (OH). EI-MS, m/z (rel.%): 370 (18) $[\text{M}]^+$, 219 (24), 151 (100) [dimethoxybenzyl]. HR-MS, m/z : 370.140908 $[\text{M}]^+$, for $\text{C}_{21}\text{H}_{22}\text{O}_6$ required 370.141639. For ^1H and ^{13}C NMR data, see Table II.

***N*-(*E*)-Feruloylserotonin (5)**

Compound **5** (4.1 mg) was isolated in 92% purity. IR (KBr): 1265, 1513, 1648 (amide); 3380 (NH, OH). EI-MS, m/z (rel.%): 352 (43) $[\text{M}]^+$, 177 (81) $[\text{C}_{10}\text{H}_9\text{O}_3]$, 159 (69) $[\text{C}_{10}\text{H}_9\text{NO}]$, 146 (10) $[\text{C}_9\text{H}_8\text{NO}]$, 133 (70), 116 (10), 113 (18), 106 (41), 89 (100). HR-MS, m/z : 352.138500 $[\text{M}]^+$, for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_4$ required 352.142307. For ^1H and ^{13}C NMR data, see Table III.

***N*-(*E*)-Isoferuloylserotonin (6)**

Compound **6** (14.6 mg) was isolated in purity over 95%. IR (KBr): 1267, 1510, 1653 (amide); 3395 (NH, OH). EI-MS, m/z (rel.%): 352 (7) $[\text{M}]^+$, 177 (14), 160 (19), 147 (15), 146 (51), 117 (5), 91 (7). HR-MS, m/z : 352.138659 $[\text{M}]^+$, for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_4$ required 352.142307. For ^1H and ^{13}C NMR data, see Table III.

N-(*Z*)-Feruloylserotonin (**7**)

Compound **7** (2.6 mg) was isolated in 68% purity. Relevant ^1H NMR data (Table III) were extracted from the obtained spectra.

N-(*Z*)-Isoferuloylserotonin (**8**)

Compound **8** (2.5 mg) was isolated in 65% purity. Relevant ^1H NMR data (Table III) were extracted from the obtained spectra.

The research was supported by the Grant Agency of the Czech Republic (Grants No. 203/04/0298 and No. 203/01/0183) and in part from the research project Z40550506. We thank Dr J. Kohoutová for MS spectra, Dr S. Vašíčková for IR spectra and Ms M. Kojecká for technical assistance.

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