New Taxonomically Significant Sesquiterpenoids from *Leontodon autumnalis*

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The methanolic extract of subaerial parts of *Leontodon autumnalis* afforded four new and two known sesquiterpenoids of the guaiane type. The known compounds were identified by means of ^{1}H and ^{13}C NMR spectroscopy as crepidiaside A (**1**) and B (**2**). The structures of the new compounds were determined by extensive 1D and 2D NMR experiments as 15-glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1α,5α,6*β*,7α,-10αH-12,6-olide (**3**); 15-glucopyranosyloxy-2-oxo-guai-3-en-1α,5α,6*β*,7α,10α,11*β*H-12,6-olide (**4**); 15-hydroxy-2-oxo-guai-3-en-1α,5α,6*β*,7α,10α,11*β*H-12,6-olide (**5**); and 15-glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1*β*,5α,6*β*,7α,10αH-12,6-olide (**6**), respectively. HPLC−DAD and HPLC−MS analyses of crude extracts of subaerial parts of 25 different taxa of the genus *Leontodon* revealed that compounds **1** and **2** occur in all investigated members of the section Oporinia (*L. autumnalis*, *L. croceus*, *L. helveticus*, *L. montaniformis*, *L. montanus*, *L. pyrenaicus*, and *L. rilaensis*) and in *L. duboisii* from the section Kalbfussia. Compounds **1**−**6** are detectable neither in other investigated taxa of Kalbfussia (*L. cichoraceus*, *L. muelleri*, and *L. palisae*) nor in any members of the subgenus Leontodon. Compounds **3**−**5** occur in high amounts only in *L. croceus* and *L. pyrenaicus* and in samples of *L. autumnalis* from northwestern Europe. In other members of the section Oporinia, in *L. duboisii* as well as in samples of *L. autumnalis* from the Pyrenees, the Alps, the Carpathians, and southern Central Europe, these substances occur only in trace amounts; in *L. montanus* and its closest relatives, compounds **3**−**5** are not detectable at all. Compound **6** is only detectable in samples of *L. autumnalis*, *L. helveticus*, *L. pyrenaicus*, *L. rilaensis*, and *L. duboisii*.

The genus *Leontodon* comprises around 50 species, and its natural distribution area covers Europe, northern Africa, and western Asia.[1] The infrageneric system of the genus is, like in many other genera of the tribe Lactuceae so far, based on a limited number of morphological characteristics.[2] *Leontodon* comprises two subgenera, Oporinia and Leontodon, and five sections. The subgenus Oporinia is divided into the sections Oporinia and Kalbfussia; the subgenus Leontodon includes the sections Asterothrix, Leontodon, and Thrincia.[2] *L. autumnalis* is a small herb belonging to the section Oporinia and distributed all over Europe to western Siberia.[1,2]

Sesquiterpenoids have proven to be reliable taxonomic markers in the Asteraceae.[3] The occurrence of guaiane-type compounds has so far been reported from two species of the genus *Leontodon*, *L. autumnalis* and *L. hispidus*.[4−6] From flowerheads of *L. autumnalis* two guaian-12,6-olides, 8-deoxylactucin and jacquinelin, have been reported.[4] An investigation of *L. hispidus*, a species belonging to the section Leontodon, revealed the presence of hypocretenolides (guaian-12,5-olides).[5,6] In continuation of our chemotaxonomic and pharmacologic investigation of the genus we reinvestigated the sesquiterpenoid spectrum of *L. autumnalis*.[5−8] Repeated Si gel column chromatography and subsequent semipreparative HPLC of methanol extracts of subaerial parts of *L. autumnalis* yielded six additional sesquiterpenoids of the guaian-12,6-olide type (**1**−**6**).

Results and Discussion

The ESIMS of compound **1** showed quasimolecular ion peaks at *m*/*z* 445 $[M + Na]^+$ and 423 $[M + H]^+$ and a major fragment at 261 $[M - 162 + H]^+$. By comparison of ^{1}H and ^{13}C NMR spectra with data given in the literature, compound **1** was identified as crepidiaside A, which is the 15-*O*-*β*-D-glucopyranoside of 8-deoxylactucin.[9] ESIMS data of compound **2** revealed quasimolecular ion peaks at *m*/*z* 447 $[M + Na]^+$ and 425 $[M + H]^+$ and a major fragment at 263 $[M - 162 + H]^+$. ^{1}H and ^{13}C NMR spectra identified this compound as crepidiaside B, which was previously isolated from *Crepidiastrum keiskeanum* Nakai.[9] The ESIMS of compound **3** showed quasimolecular ion peaks at *m*/*z* 447 $[M + Na]^+$ and 425 $[M + H]^+$ and a major fragment at 263 $[M - 162 + H]^+$, thus suggesting that **3** is a dihydroderivative of **1**. This was confirmed by HRFABMS, which showed quasimolecular ion peaks at *m*/*z* 425.1797 $[M + H]^+$ and 447.1520 $[M + Na]^+$ appropriate for a molecular formula of $C_{21}H_{28}O_9$, and by the ^{1}H NMR spectrum (Table 1), which showed signals for one vinylic proton located in the α-position to a carbonyl function (δ_H 6.52 dt, $J_{3,15} = J_{3,5} =$ 2.0 Hz), one olefinic methylene group [δ_H 6.19 (d, $J_{13,7} =$ 3.5 Hz) and δ_H 5.67 (d, $J_{13,7} = 3.5$ Hz)], one oxygen-bearing methylene group (δ_H 4.84 and δ_H 4.76), one oxygen-bearing methine group (δ_H 4.55, dd, $J_{6,5} = 11.0$ Hz, $J_{6,7} = 9.5$ Hz), and one anomeric sugar proton (δ_H 4.43, d $J_{1',2'} = 7.5$ Hz). These signals, as well as additional signals for a glucose moiety (δ_H 3.26−3.89), a tertiary proton (δ_H 2.78), and two pairs of intracyclic methylene protons (δ_H 2.12, δ_H 1.60 and δ_H 2.02, 1.78), closely resemble the corresponding signals of compound **1**. In contrast to compound **1**, the signal for the methyl group C-14 is shifted upfield from δ_H 2.44 to δ_H 0.77 and appears as a doublet ($J_{14,10} = 7.5$) instead of a singlet. Furthermore, two additional signals at δ_H 2.84 (H-1) and δ_H 2.60 (H-10) appear, indicating that compound **3** lacks the 1,10 double bound. This is finally proved by the ^{13}C NMR data (Table 2) and data obtained from HSQC and HMBC experiments. Thus, compound **3** is a 1,10-dihydro derivative of crepidiaside A (**1**). The hydrogenation in positions 1 and 10 gives rise to the possibility of four different diastereoisomers. As $J_{1,5}$ proton coupling con-

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Table 1. ^{1}H NMR Data for Compounds **3**−**6** (500 MHz)[a]

position	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**6**[c]
1	2.84, 1H dd (7.0, 4.0)	2.82, 1H dd (7.5, 4.0)	2.81, 1H dd (7.0, 4.0)	2.17, 1H m[d]	2.19, 1H dd (10.5, 5.5)
2					
3	6.52, 1H dt (2.0, 2.0)	6.49, 1H dt (2.0, 2.0)	6.30, 1H dt (2.0, 2.0)	6.38, 1H br d (1.5)	6.41, 1H br d (1.5)
4					
5	3.42, 1H ddd (11.0, 7.0, 2.0)	3.28, 1H br dd (10.5, 7.5)	3.25, 1H m	3.21, 1H br dd (10.5, 4.0)	3.25, 1H br dd (10.5, 5.5)
6	4.55, 1H dd (11.0, 9.5)	4.53, 1H dd (10.5, 10.5)	4.49, 1H dd (10.5, 9.5)	3.93, 1H dd (10.5, 10.5)	4.05, 1H dd (10.5, 10.5)
7	2.78, 1H m	1.84, 1H dd (10.5, 2.5)	1.83, 1H ddd (11.5, 10.5, 2.5)	2.71, 1H m	2.74, 1H dd (10.5, 2.0)
8	2.12, 1H m	1.87, 1H m	1.86, 1H m	2.02, 1H m	2.02, 1H m
	1.60, 1H m	1.54, 1H m	1.51, 1H m	1.48, 1H m	1.45, 1H m
9	2.02, 1H m	1.96, 1H m	1.94, 1H m	1.89, 1H m	1.85, 1H m
	1.78, 1H m	1.68, 1H m	1.69, 1H m	1.76, 1H m	1.65, 1H m
10	2.60, 1H m	2.57, 1H m	2.56, 1H m	2.17, 1H m[d]	2.09, 1H m
11		2.42, 1H dq (11.5, 7.5)	2.39, 1H dq (11.5, 7.0)		
12					
13	6.19, 1H d (3.5)	1.19, 3H d (7.5)	1.18, 3H d (7.0)	6.08, d (3.5)	6.03, 1H d (3.0)
	5.67, 1H d (3.5)			5.56, d (3.5)	5.63, 1H d (3.0)
14	0.77, 3H d (7.5)	0.77, 3H d (7.5)	0.78, 3H d (7.0)	1.27, 3H d (8.0)	1.23, 3H (8.5)
15	4.84, 1H[d]	4.82, 1H[d]	4.60, 1H br d (19.0)	4.84, 1H[d]	4.62, 2H br s
	4.76, 1H br d (19.0)	4.68, 1H br d (19.0)	4.46, 1H br d (19.0)	4.79, 1H br s	
1′	4.43, 1H d (7.5)	4.41, 1H d (7.5)		4.41, 1H d (8.5)	4.30, 1H d (7.5)
2′	3.26, 1H d (7.5)	3.26, 1H d (7.5)		3.30, 1H m[d,e]	3.14, 1H d (7.5)
3′	3.37, 1H m[d]	3.36, 1H m[d]		3.37, 1H m[d]	3.35, 1H m[d]
4′	3.31, 1H m[d,e]	3.30, 1H m[d,e]		3.25, 1H dd (8.5, 8.5)[e]	3.07, 1H dd (7.5, 7.5)[e]
5′	3.31, 1H m[d,e]	3.29, 1H m[d,e]		3.30, 1H m[d,e]	3.19, 1H m[d,e]
6′	3.89, 1H dd (12.0, 2.0)	3.88, 1H dd (12.0, 2.0)		3.88, 1H br d (11.5)	3.72, 1H br d (11.5)
	3.69, 1H dd (12.0, 5.0)	3.68, 1H dd (12.0, 5.0)		3.68, 1H dd (11.5, 4.0)	3.49, 1H dd (11.5, 4.0)

[a] Coupling constants in Hz are given in parentheses. [b] Measured in MeOH-d_4. [c] Measured in DMSO-d_6. [d] This is an overlapping signal. [e] Signals may be interchangeable within each column.

Table 2. ^{13}C NMR Data for Compounds **3**−**6** (125 MHz)

position	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**6**[b]
1	54.5	54.7	55.3	56.5	55.0
2	211.5	211.8	211.6	209.0	
3	130.7	130.5	129.3	130.0	129.4
4	181.4	181.9	186.3	175.4	
5	52.2	52.1	52.1	51.8	49.9
6	83.7	83.3	83.4	87.7	86.1
7	48.1	52.2	52.2	54.3	52.8
8	25.4	26.2	26.2	20.1	19.4
9	35.8	35.5	35.7	36.3	35.6
10	35.3	35.7	36.2	31.7	30.7
11	140.9	42.6	42.9	141.0	
12	171.7	180.4	180.8	171.7	
13	120.8	13.0	13.0	118.1	118.4
14	15.3	15.1	15.1	21.9	22.3
15	69.6	69.5	63.0	69.7	68.0
1′	104.2	104.2		104.2	103.6
2′	75.1	75.1		75.1	74.4
3′	78.1	78.0		78.0[a]	79.1
4′	71.5	71.5		71.5	70.8
5′	78.1	78.1		78.0[a]	77.4
6′	62.7	62.7		62.6	61.7

[a] Measured in MeOH-d_4. [b] Measured in DMSO-d_6, data obtained from a HSQC experiment.

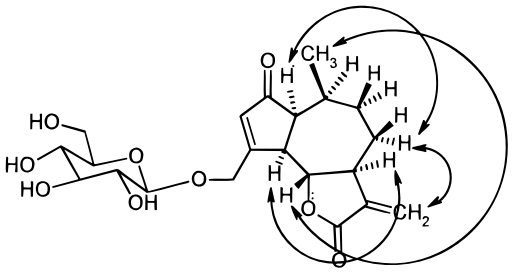


Figure 1. Important NOEs observed for compound **3**.

stants in guaiane derivatives are quite similar irrespective of the orientation of the proton in position 1, we used a NOE experiment to elucidate the relative configurations in positions 1 and 10.[10] The observed NOEs between the protons in positions C-14 and C-6, C-5 and C-7, C-13 and C-8α, and C-8α and C-1 clearly indicate that the proton in position C-1 is α-oriented, whereas the methyl group in position C-10 is in the *β*-orientation (Figure 1). Thus, compound **3** is 15-glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1α,5α,6*β*,7α,10αH-12,6-olide.

The ESIMS spectrum of compound **4** showed quasimolecular ion peaks at *m/z* 449 $[M + Na]^+$ and 427 $[M + H]^+$ and a major fragment at 265 $[M - 162 + H]^+$. The molecular formula of $C_{21}H_{30}O_9$ was established by HR-FABMS featuring signals at *m/z* 427.1846 $[M + H]^+$ and 449.1688 $[M + Na]^+$. ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), HSQC, HMBC, and NOE data indicated that compound **4** is the 11,13-dihydro derivative of compound **3**. α-Orientation of the methyl group at position C-11 was proved by a NOE experiment. This experiment also verified that relative configurations in positions C-1 and C-10 of substance **4** are the same as in compound **3**. Accordingly, compound **4** is 15-glucopyranosyloxy-2-oxo-guaia-3-en-1α,5α,6*β*,7α,-10α,11*β*H-12,6-olide.

The positive ESIMS of compound **5** was uninformative; negative ESIMS showed a quasimolecular ion peak at *m/z* 263 $[M - H]^-$. HREIMS gave a signal at *m/z* 264.1371 $[M]^+$, establishing the molecular formula as $C_{15}H_{20}O_4$. ^{1}H and ^{13}C NMR spectra of compound **5** were almost superimposable on the corresponding signals of the sesquiterpene moiety of compound **4** (Tables 1 and 2), with the only exceptions of signals for C-15 and C-4 and the signals for the protons in position C-15, which were shifted upfield (C-15, 6.5 ppm; H-15a and H-15b, 0.22 ppm) and downfield (C-4, 4.4 ppm), respectively (Tables 1 and 2). These shifts and the absence of the signals for the glucopyranosyl moiety identified compound **5** as the corresponding aglycon of compound **4**. Relative configurations in positions C-1, C-10, and C-11 of compound **5** were identical with the configurations of compound **4**, which were verified by a NOE experiment. Thus, compound **5** is 15-hydroxy-2-oxo-guai-3-en-1α,5α,6*β*,7α,10α,11*β*H-12,6-olide.

The ESIMS of compound **6** was identical with that of compound **3** and showed quasimolecular ion peaks at *m/z* 447 $[M + Na]^+$ and 425 $[M + H]^+$ and a major fragment at 263 $[M - 162 + H]^+$, suggesting that substance **6** represents a diastereoisomer of **3**. The molecular formula of $C_{21}H_{28}O_9$

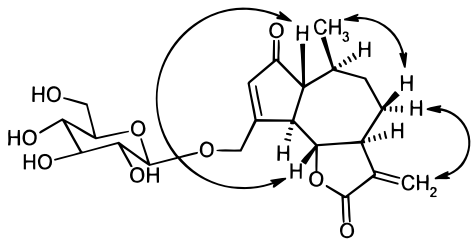
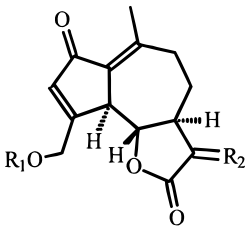


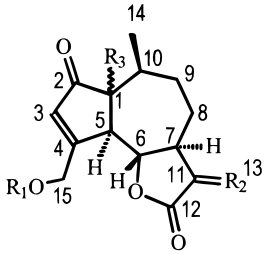
Figure 2. Important NOEs observed for compound **6**.

was established by HRFABMS, which showed a quasimolecular ion peak at *m/z* 425.1852 $[M + H]^+$, and by ^1H NMR, ^{13}C NMR, HSQC, and HMBC experiments, which confirmed this assumption (Tables 1 and 2). To establish the relative configuration by NOE experiment, the solvent had to be changed from MeOH-d_4 to DMSO-d_6, because in MeOH-d_4, the signals for the protons attached to C-1 and C-10 had identical δ_H values of 2.17 (Table 1). In DMSO-d_6 the signals were sufficiently separated ($\delta_{H-1} = 2.19$ and $\delta_{H-10} = 2.09$), and the observed NOE between H-1 and H-6, H-14 and H-8β, and H-13 and H-8α indicated that the proton in position C-1 and the methyl group in position C-10 are both β-oriented (Figure 2). Consequently, compound **6** is 15-glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1β,5α,6β,7α,10αH-12,6-olide.



1 R_1 = β-D-Glc, R_2 = CH_2

2 R_1 = β-D-Glc, R_2 = α CH_3, β H



3 R_1 = β-D-Glc, R_2 = CH_2, R_3 = α H

4 R_1 = β-D-Glc, R_2 = α CH_3, β H, R_3 = α H

5 R_1 = H, R_2 = α CH_3, β H, R_3 = α H

6 R_1 = β-D-Glc, R_2 = CH_2, R_3 = β H

Compounds **3**−**6** represent new natural compounds. A 1,10-dihydro derivative of jacquinelin has been prepared by means of palladium-catalyzed hydrogenation of jacquinelin, but its relative configuration was not established.[11] Such 1,10-dihydrojacquinelin might either be identical with compound **5** or might represent a diastereoisomer of this substance.

To identify compounds **1**−**6** in crude methanolic extracts of subaerial parts of different *Leontodon* taxa an analytical HPLC system was established (Figure 3). Retention times, on-line diode array detection (DAD) UV spectra, and on-line mass spectra were assessed as criteria for the identity

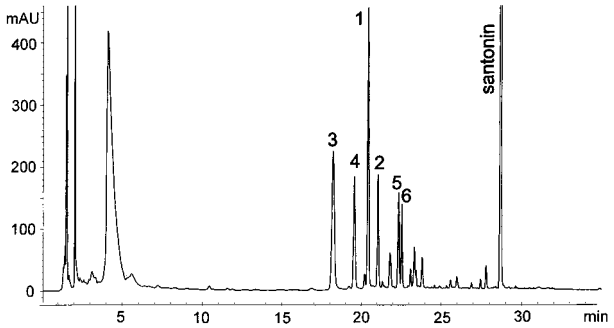


Figure 3. HPLC of a crude extract of *L. autumnalis* (CZ-L143).[a] [a]Measured at 245 nm, flow rate 1.00 mL, column: Zorbax Rx-C_{18}, 4.6 × 150 mm (3.5 μm particles), linear gradient: H_2O-CH_3CN 88:12 to 85:15 in 15 min, in 10 min to 65:35%, and to 40:60 after another 5 min, stop time 35 min.

of the observed peaks with the isolated substances. Compound **5** was identified only on the basis of retention times and DAD UV spectra inasmuch as on-line ESIMS of this compound were uninformative. On-line ESIMS of the other substances showed the same quasimolecular ion peaks and major fragments as given in the structure elucidation section above. On-line UV absorption maxima (λ_{max}) were measured as 257 nm (**1**, **2**), 225 nm (**3**), 232 nm (**4**, **5**), and 220 nm (**6**), respectively.

Full scientific names and collection sites of the investigated *Leontodon* taxa are available as supplementary data. Results of HPLC screening for compounds **1**−**6** in different *Leontodon* taxa are shown in Table 3. Lactucin-type compounds such as **1**−**6** seem to be restricted to the members of the section Oporinia and *L. duboisii* and are absent from all other *Leontodon* taxa investigated. In detail, compounds **1** and **2** were detected in all investigated samples of *L. autumnalis*, *L. croceus*, *L. helveticus*, *L. montaniformis*, *L. montanus* subsp. *melanotrichus*, *L. montanus* subsp. *montanus*, *L. pyrenaicus*, *L. rilaensis*, and *L. duboisii*. In the sample of *L. montaniformis* and in the samples of both subspecies of *L. montanus* none of the compounds **3**−**6** could be detected. In the samples of *L. helveticus* compound **3** occurred as a trace compound (<1% of total amount of **1**−**6**) and in the samples of *L. duboisii* and *L. rilaensis*, as well as in a number of samples of *L. autumnalis*, compound **3** occurred also only as a minor constituent (<10% of total amount of **1**−**6**). In a number of samples of *L. autumnalis*, in the sample of *L. pyrenaicus*, and especially in the samples of *L. croceus*, compound **3** was one of the main sesquiterpenoids. Similar to **3**, compounds **4** and **5** were minor or trace constituents in extracts of *L. duboisii*, *L. helveticus*, and *L. rilaensis*, as well as in a number of samples of *L. autumnalis*, but contributed more than 10% of the total sesquiterpenoid content of the samples of *L. croceus*, *L. pyrenaicus*, and some of the *L. autumnalis* samples. Finally, compound **6** was absent from the *L. croceus* extracts and occurred as a minor compound in extracts of *L. helveticus*, *L. pyrenaicus*, *L. rilaensis*, and some of the *L. autumnalis* samples. In the sample of *L. duboisii*, as well as in a number of the *L. autumnalis* samples, it represented more than 10% of the whole sesquiterpenoid content. These results indicate that sesquiterpenoids of the lactucin-type are, within the genus *Leontodon*, characteristic for members of the section Oporinia.

Second, it becomes obvious that there are at least three different patterns of distribution of these compounds within the section Oporinia. Extracts of one group only contain crepidiasides A (**1**) and B (**2**); this group consists of the taxa of the series Mediani (*L. montaniformis*, *L. montanus*

Table 3. Occurrence of Sesquiterpenoids **1**−**6** in Subaerial Parts of Different *Leontodon* Taxa[a]

taxon	sample	total amount of compounds **1**−**6** (ppm ± SD)[b]	relative amounts of compounds **1**−**6** (% of total amount)					
			1	**2**	**3**	**4**	**5**	**6**
subgenus Oporinia								
sectio Oporinia								
L. autumnalis	CZ-L130	4561 ± 269	71.4	11.4	1.5	0.5	0.9	14.3
	CZ-L140	4927 ± 29	74.6	12.7	2.2	0.7	0.4	9.5
	CZ-L143	2568 ± 105	30.1	12.1	25.9	14.6	10.2	7.1
	CZ-L149	3359 ± 30	75.3	15.9	1.6	0.4	0.1	6.8
	CZ-L183	3466 ± 47	65.5	16.4	3.3	1.9	1.0	11.9
	CZ-L192	2679 ± 68	68.6	16.6	1.9	1.6	1.3	9.9
	CZ-L209	3873 ± 118	50.7	19.0	16.6	4.2	4.7	4.8
	CZ-L211	2221 ± 46	14.2	6.5	37.3	16.3	13.0	12.7
	CZ-L212	5828 ± 265	66.0	8.6	14.8	2.9	4.2	3.5
	CZ-L213	2913 ± 222	16.2	5.6	38.1	10.0	9.2	20.9
	CZ-L214	4344 ± 306	49.5	7.2	25.9	6.7	2.5	8.2
L. croceus	CZ-L069	2493 ± 84	4.2	1.9	69.3	10.1	14.6	0.0
	CZ-L184	3317 ± 95	1.8	3.5	64.0	16.6	14.1	0.0
	CZ-L186	3789 ± 493	3.8	2.8	68.2	15.3	9.9	0.0
L. helveticus	CZ-L122	2642 ± 90	69.1	26.1	0.2	0.2	1.3	3.1
	CZ-L216	4476 ± 99	64.6	28.5	0.2	1.1	4.8	0.9
	CZ-L217	3499 ± 236	49.6	42.5	0.4	1.3	4.9	1.2
L. montaniformis	CZ-L128	4055 ± 321	81.0	19.0	0.0	0.0	0.0	0.0
L. montanus subsp. *melanotrichus*	CZ-L123	5065 ± 14	70.5	29.5	0.0	0.0	0.0	0.0
L. montanus subsp. *montanus*	CZ-L150	3160 ± 27	70.0	30.0	0.0	0.0	0.0	0.0
	CZ-L189	3626 ± 28	58.0	42.0	0.0	0.0	0.0	0.0
L. pyrenaicus	CZ-L197	537 ± 9	10.6	11.5	42.9	21.9	11.1	2.0
L. rilaensis	CZ-L187	4804 ± 364	42.9	34.6	9.3	5.5	1.6	6.1
sectio Kalbfussia								
L. cichoraceus	CZ-L176	not detectable						
L. duboisii	CZ-L195	1755 ± 60	66.6	15.8	2.3	1.1	0.9	13.4
L. muelleri	CZ-L177	not detectable						
L. palisae	CZ-L089	not detectable						
subgenus Leontodon	all samples	not detectable						

[a] Results of HPLC/DAD/MS investigations. [b] Estimated by ratio of peak areas to area of santonin as internal standard.

subsp. *melanotrichus*, and *L. montanus* subsp. *montanus*).[2] Another group is defined by high amounts of crepidiasides A and B and low amounts of compounds **3**−**6** (below 20% of the total sesquiterpenoid amount); this group encompasses samples of *L. autumnalis*, *L. helveticus*, *L. rilaensis*, and *L. duboisii*. The third group is characterized by high relative amounts of compounds **3**−**6** (more than 40% of the total sesquiterpenoid amount) and comprises samples of *L. autumnalis*, *L. croceus*, and *L. pyrenaicus*. It is evident that there are at least two different chemotypes of *L. autumnalis*, one sharing the features of the second group, the other comprising the characteristics of the third group. The collection site data suggest that these chemotypes show different geographical distribution patterns. The samples of western Germany (CZ-L143, CZ-L211, CZ-L213, and CZ-L214) show high amounts of compounds **3**−**6**, whereas samples of *L. autumnalis* from the Alps (CZ-L130, CZ-L140, and CZ-L149), the Pyrenees (CZ-L192), and the Carpathians (CZ-L183) contain only trace amounts of these substances. In the southern Rhineland (CZ-L209 and CZ-L212), intermediate forms between these two chemotypes exist. The plants from both chemotypes are not able to be differentiated by morphological means and there seems to be no correlation between these chemically defined taxa and the subspecies of *L. autumnalis*, which are recognized by some authors.[12]

Because both chemotypes of *L. autumnalis* inhabit a wide range of different altitudinal zones and grow in a number of different habitats as such roadsides, meadows, and ruderal areas, it seems highly unlikely that factors other than taxonomic ones account for the observed differences in the spectrum of sesquiterpenoids. However, at the moment we cannot exclude the possibility that the plants synthesize these substances as phytoallexins in response to microorganisms or other stresses, which on their part might be restricted to certain distribution areas. In a further study, plants of different origins will be cultivated under the same conditions to investigate this problem.

In conclusion, these results indicate that substances **1**−**6** are highly reliable taxonomic markers for and within the section Oporinia, and the results are congruent with the assumption that the section Oporinia, as defined by Widder[2] on a morphological foundation, might be indeed a monophyletical lineage. However, Widder[2] includes *L. duboisii* in the section Kalbfussia, despite possessing erect buds instead of nodding buds.[2,13] Erect buds are one of the main characteristics of the section Oporinia.[2] As shown above, *L. duboisii* shares the phytochemical characteristics of the section Oporinia and with its erect buds it features also some of the morphological characteristics of this section. On this basis, it should be included in the section Oporinia. Some of its closest allies for example, *L. cantabricus* Widder,[13] also share this morphological feature. Therefore, a phytochemical investigation for compounds **1**−**6** in these species would be an interesting task and could eventually support the transfer of these taxa into the section Oporinia.

However, the question whether the whole genus *Leontodon* is a monophyletical entity or just an artificial assemblage of taxa, remains open. Chemical data available up to now indicate that chemotaxonomic relations from different *Leontodon* sections to other genera of the Lactuceae are closer than relations between members of different sections of *Leontodon*. For example, hypocretenolides isolated from *L. hispidus* also occur in *Crepis aurea* (L.) Cass., *Hedypnois cretica* (L.) Dum.Courset, and *Hypochoeris cretensis* Benth.[5,14−16] Furthermore, 8-deoxylactucin, jacquinelin, and crepidiasides A and B occur not only in members of the *Leontodon* section Oporinia but also in

the genera *Cichorium*,[17] *Crepidiastrum*,[9] *Lactuca*,[18−24] *Mycelis*,[25] *Picris*,[26,27] *Sonchus*,[10,28] and *Youngia*.[29]

As phytochemical data alone are not sufficient to establish a system that is in congruence with phylogenesis, further investigations of the relations of the different sections of *Leontodon* to other genera of the Lactuceae by modern molecular techniques, such as RFLP and RAPDs analyses of cpDNA are necessary to verify the implications of our phytochemical findings.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian V-500 spectrometer at 500 and 125 MHz, respectively. HPLC analyses were performed using a Hewlett-Packard HP-1090 liquid chromatograph employed with a DAD detector. HPLC parameters: flow rate 1.00 mL/min, solvent A: H_2O, solvent B: CH_3CN; linear gradient from 12% B to 15% B in 15 min, in 10 min to 35% B and to 60% B in 5 min, stop time 35 min; detection wavelength: 245 nm; injection volume: 10 μL; column: Zorbax Rx-C_{18}, 4.6 mm × 150 mm, particle size 3.5 μm. LC-MS analyses were performed with a Finnigan MAT SSQ 7000 mass spectrometer by ESI in the positive mode, employing a CID value of 0, a corona amperage of 5 μA, a sheath gas pressure of 65 psi, and a vaporizer temperature of 400 °C. For HPLC−MS analyses the HPLC flow was split with a split ratio of 1:4. HRMS analyses were performed on a Finnigan MAT 95 mass spectrometer by FAB and EI ionization, respectively.

Si gel chromatography was carried out with Merck G-60 (230−400 mesh) material. Semipreparative HPLC was performed using a Gilson 302 LC system equipped with a Knaur UV/vis filter-photometer set to a wavelength of 205 nm and a Merck 10 × 250 mm LiChrospher RP_{18} (10 μm material) column under isocratic conditions with mixtures of water and CH_3CN containing 20% (**3**−**4**), 22.5% (**1**−**2**), and 25% (**5**−**6**) CH_3CN, respectively. Extraction procedures for HPLC analyses: Air-dried plant material was ground with a Moulinex type 980 coffee-mill, 1.00 g of plant material was mixed with 1.00 mL of a stock solution containing 1.00 mg/mL of santonin as internal standard and extracted three times with 25 mL of MeOH for 7.5 min with an IKA-25 Ultraturrax apparatus. Extracts were combined and brought to dryness in vacuo. The residue was dissolved in 2.50 mL of MeOH, filtered, and used for HPLC analysis. For quantification, each analysis was run in triplicate.

Extraction and Isolation. The air-dried plant material was ground and extracted four times with 500 mL of MeOH for 10 min with an IKA-25 Ultraturrax apparatus. Extracts were combined and dried in vacuo. Compounds **1**, **2**, and **6** were isolated from collection CZ-L163 (34.9 g of plant material, yielding 8.43 g of crude methanolic extract), and compounds **3**, **4**, and **5** were isolated from collection CZ-L179 (44.4 g of plant material, yielding 13.3 g of crude methanolic extract). The crude extracts were repeatedly chromatographed on Si gel with gradients of CH_2Cl_2−MeOH, CH_2Cl_2−Me_2CO, and EtOAc−Me_2CO, respectively. Fractions containing identical compounds were unified after TLC analysis using EtOAc−MeCH−Me_2CO 3:1:1 as solvent and spraying with vanillin−sulfuric acid and subsequent heating as detection method. The final step of purification was performed by semipreparative HPLC as given above, yielding 2.5 mg of **1**, 3.0 mg of **2**, 3.4 mg of **3**, 3.3 mg of **4**, 2.0 mg of **5**, and 1.9 mg of **6**, respectively.

15-Glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1α,5α,6*β*,7α,10αH-12,6-olide (3): colorless crystals; mp 180−190 °C (dec); FT-IR (microspectrometry) ν_{max}^{ZnSe} cm^{-1} 3350, 2921, 2858, 1754, 1681, 1582, 1420, 1265, 1080, 1045, 995; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 447 $[M + Na]^+$ (100), 442 $[M + NH_4]^+$ (38), 425 $[M + H]^+$ (14), 263 [M − glucose + H]$^+$ (36); HRFABMS *m/z* 425.1797 $[M + H]^+$ (calcd for $C_{21}H_{29}O_9$, 425.1812), 447.1520 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

15-Glucopyranosyloxy-2-oxo-guai-3-en-1α,5α,6*β*,7α,10α,-11*β*H-12,6-olide (4): colorless crystals; mp 180−190 °C (dec); FT-IR (microspectrometry) ν_{max}^{ZnSe} cm^{-1} 3350, 2925, 2856, 1766, 1688, 1618, 1421, 1165, 1079, 996; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 449 $[M + Na]^+$ (100), 444 $[M + NH_4]^+$ (22), 427 $[M + H]^+$ (4), 265 [M − glucose + H]$^+$ (16); HRFABMS *m/z* 427.1846 $[M + H]^+$ (calcd for $C_{21}H_{31}O_9$, 427.1968), 449.1684 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_9Na$, 449.1787).

15-Hydroxy-2-oxo-guai-3-en-1α,5α,6*β*,7α,10α,11*β*H-12,6-olide (5): colorless crystals; mp 141−145 °C; FT-IR (microspectrometry) ν_{max}^{ZnSe} cm^{-1} 3350, 2923, 2855, 1775, 1693, 1616, 1460, 1418, 1383, 1119, 1050, 994; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 263 [M − H]$^-$ (100), 527 [2M − H]$^-$ (8); HREIMS *m/z* 264.1371 $[M]^+$ (calcd for $C_{15}H_{20}O_4$, 264.1362).

15-Glucopyranosyloxy-2-oxo-guaia-3,11(13)-dien-1*β*,5α,6*β*,7α,10αH-12,6-olide (6): colorless crystals; mp 168−172 °C; FT-IR (microspectrometry) ν_{max}^{ZnSe} cm^{-1} 3350, 2923, 2875, 1766, 1701, 1594, 1411, 1272, 1127, 1078, 989; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 447 $[M + Na]^+$ (100), 442 $[M + NH_4]^+$ (38), 425 $[M + H]^+$ (6), 263 [M − glucose + H]$^+$ (20); HRFABMS *m/z* 425.1852 $[M + H]^+$ (calcd for $C_{21}H_{29}O_9$, 425.1812).

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Supporting Information Available: A table with full scientific names and collection sites of all investigated taxa is available free of charge via the Internet at http://pubs.acs.org.

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