Comparative Qualitative and Quantitative Determination of Alkaloids in Narcotic and Condiment *Papaver somniferum* Cultivars

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In the present study morphinan, tetrahydrobenzylisoquinoline, benzo[*c*]phenanthridine, and phthalideisoquinoline alkaloids were determined qualitatively and quantitatively by HPLC and LC-MS analysis in tissues of the Tasmanian *Papaver somniferum* L. elite cultivar C048-6-14-64. The data were compared with the results from the low-morphine cultivar "Marianne". In the elite cultivar, 91.2% of the latex alkaloids consist of the three pharmaceutically most valuable alkaloids: morphine, codeine, and thebaine. In the root system, the major alkaloids are sanguinarine/10-hydroxysanguinarine and dihydrosanguinarine/10-hydroxydihydrosanguinarine. In the stems and leaves of C048-6-14-64, the same alkaloids were measured as in the latex. In the stems, a gradient in relative total alkaloid content from the top downward toward the roots was observed. The concentration of morphine was decreasing toward the roots, whereas an increasing gradient from the upper to the lower stem parts was detected for codeine. The relative total alkaloid concentration in leaves remained constant; no gradient was observed. The cultivar "Marianne" displayed a shifted pattern of alkaloid accumulation and reduced levels of total alkaloid. In the condiment cultivar, 80.5% of the alkaloids of the latex consisted of the two phthalideisoquinoline alkaloids narcotoline and noscapine. Only 18.8% of the relative total alkaloid content were morphinan alkaloids. In contrast to the narcotic cultivar, in which the benzo[*c*]phenanthridines in roots dominated over the morphinan and tetrahydrobenzylisoquinoline alkaloids, the concentration of benzo[*c*]phenanthridines in "Marianne" was similar to that of morphinan and tetrahydrobenzylisoquinoline alkaloids. These data suggest a differential alkaloid regulation in each cultivar of *P. somniferum.*

Papaver somniferum L. is considered to be one of the oldest cultivated medicinal plants of Europe. Opium poppy originated in the eastern Mediterranean, and the ancient Sumerians used its seed for food. The "sleep-inducing" property and the medicinal value of the latex have also been known and used throughout human history.[1,2] *P. somniferum* contains more than 80 tetrahydrobenzylisoquinoline-derived alkaloids. These include the pharmaceutically important analgesic and narcotic drug morphine (**1**), the cough suppressant codeine (**2**), the muscle relaxant papaverine, the antitumoric agent noscapine[3] (**3**), and the antimicrobial sanguinarine (**4**). In Figure 1, a schematic of the biosynthetic pathway of the tetrahydrobenzylisoquinoline, morphinan, benzo[*c*]phenanthridine, and phthalideisoquinoline alkaloids in opium poppy is shown. The culture of this plant is worldwide strictly regulated by the International Narcotic Control Board (INCB) as opium per se, and its derivatives are addictive and potentially toxic and have been abused for centuries.

Genetic engineering has become a particularly interesting method for manipulating metabolic productivity and unraveling regulatory aspects of alkaloid biosynthesis.[4] To investigate long-term changes in alkaloid biosynthesis, regeneration and transformation protocols of the desired plant species are necessary. The transformation of opium poppy was first reported for cell suspension cultures.[5] Meanwhile, transformation systems via somatic embryogenesis[6] and shoot organogenesis[7] are available. However, changes in alkaloid biosynthesis in transgenic plants of the industrial opium poppy elite cultivar C048-6-14-64 transformed with berberine bridge enzyme cDNA from *P. somniferum* L.[8,9] have been reported only recently. The current investigation is a qualitative and quantitative study of alkaloids in all tissues of the narcotic opium poppy cultivar C048-6-14-64 used for metabolic engineering[8,9] and "Marianne", a commonly used low-morphine condiment cultivar. This is the first detailed comparative analysis of alkaloids in tissues and latex of a *P. somniferum* narcotic and a condiment cultivar.

Results and Discussion

The aim of this study was a comparison between the two *P. somniferum* L. cultivars C048-6-14-64, an elite narcotic cultivar from Australia, and the low-morphine condiment cultivar "Marianne". One difference that could be recognized easily in the greenhouse was the difference in the total number of leaves. From the 29 plants examined, 10 plants of the narcotic cultivar had 14 or 15 leaves. In contrast, the condiment cultivar "Marianne" produces 30% more leaves. Neither cultivar showed a relation between the leaf number and alkaloid concentration (data not shown).

The qualitative and quantitative determination of morphinan, tetrahydrobenzylisoquinoline, benzo[*c*]phenanthridine, and phthalideisoquinoline alkaloids was done by HPLC and LC-MS analysis. Figure 2 shows the average results of alkaloids accumulated in 29 plants of the narcotic cultivar (Figure 2A,C,E) and 19 plants of the condiment cultivar "Marianne" (Figure 2B,D,F) in the latex (Figure 2A,B) and roots (Figure 2C,D,E,F). In the latex of the cultivar C048-6-14-64 (Figure 2A), the four major alkaloids were morphine (209 ± 63 μg/100 μg protein) (**1**), codeine (63 ± 25 μg/100 μg protein) (**2**), thebaine (294 ± 123 μg/100 μg protein) (**5**), and oripavine (30 ± 16 μg/100 μg protein) (**6**). These measurements support the fact that C048-6-14-64 is a narcotic cultivar that was bred for a high

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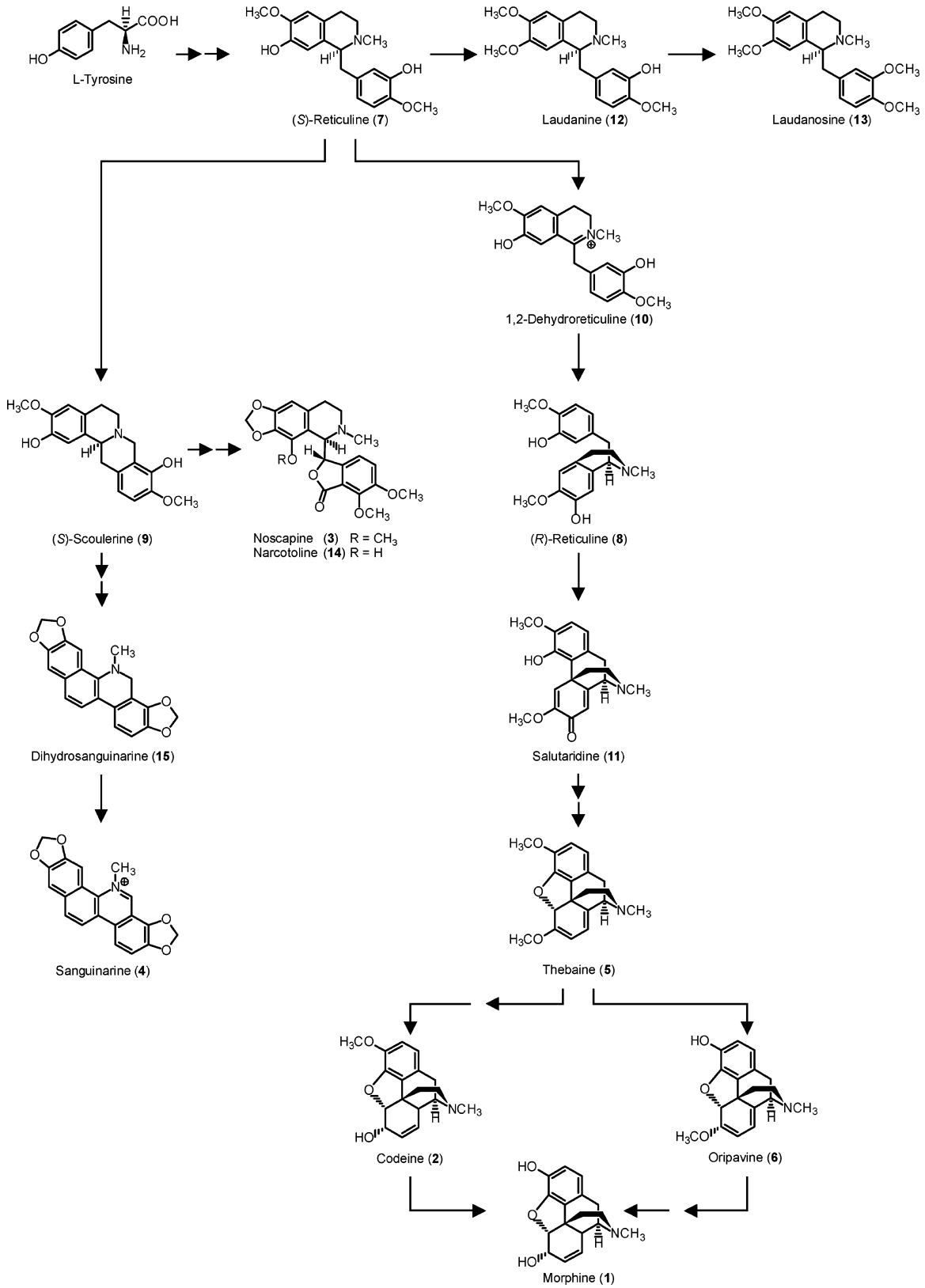

Figure 1. Schematic of the biosynthetic pathway leading from L-tyrosine to the benzylisoquinoline, morphinan, benzo[*c*]phenanthridine, and phthalideisoquinoline alkaloids in *Papaver somniferum* L. Selected intermediates are shown.

concentration of morphinan alkaloids; 91.2% of the latex alkaloids consist of the three pharmaceutically most valuable alkaloids: morphine, codeine, and thebaine. Codeinone reductase (COR), which catalyzes the penultimate step of morphine biosynthesis, the reduction of codeinone to codeine, was recently detected by both 2-D gel electrophoresis[10] and immunolocalization[11] in latex. Oripavine is a natural opium alkaloid that was isolated initially from a Tasmanian poppy variety.[12] By means of radioactive application experiments, an alternative pathway leading from thebaine to morphine was postulated (Figure 1). Thus oripavine arises through 3-*O*-demethylation of thebaine. After enol-ether cleavage to morphinone, reduction to morphine takes place through action of COR.[13] The abun-

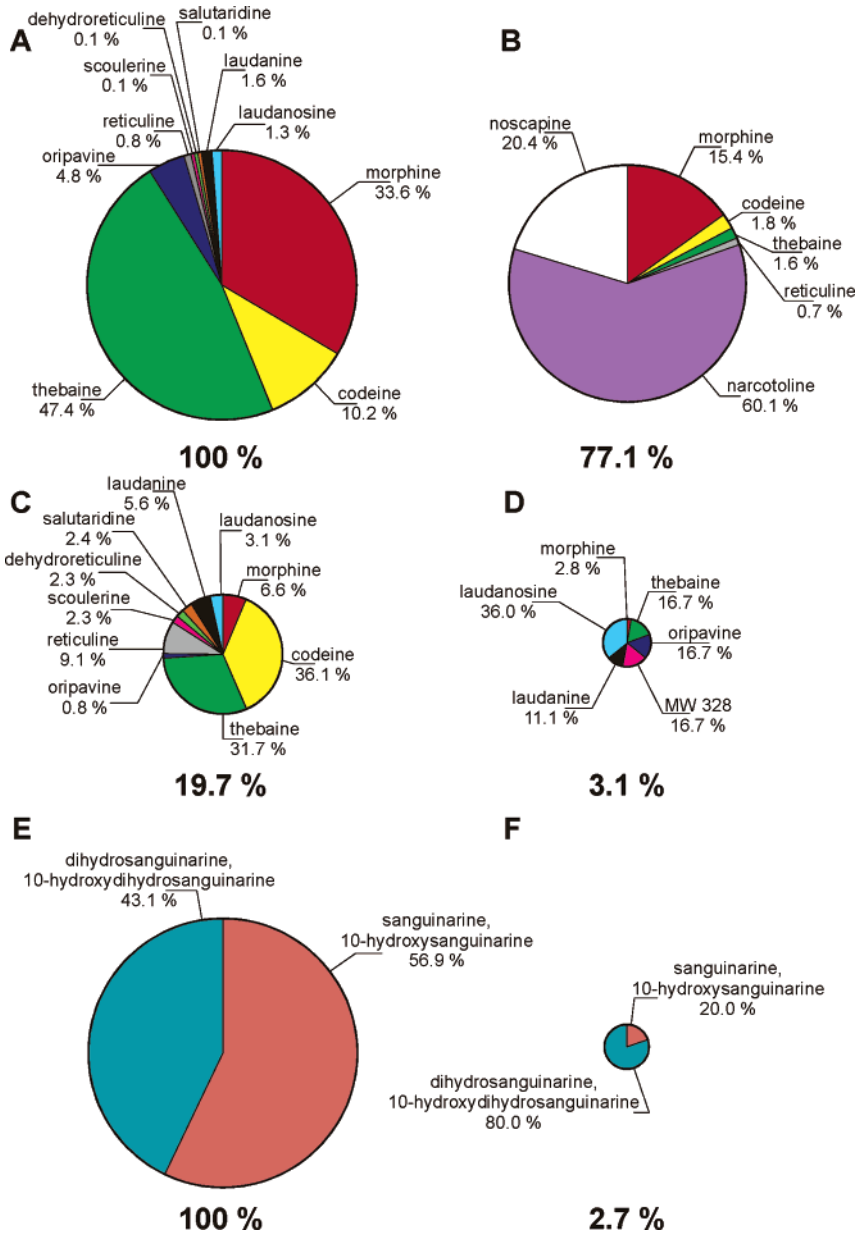

Figure 2. Determination of tetrahydrobenzylisoquinoline, morphinan, and phthalideisoquinoline alkaloids in latex (A, B) and roots (C, D) and benzo[*c*]phenanthridine alkaloids in roots (E, F). Panels A, C, and E present the average alkaloid content of 29 opium poppy plants of the narcotic cultivar C048-6-14-64. Panels B, D, and F present the average alkaloid content of 19 opium poppy plants of the cultivar "Marianne". The alkaloids in latex were calculated as μg alkaloid/100 μg of soluble protein or in roots as μg alkaloid/100 mg root fresh weight. The data are normalized to 100% to produce the individual divisions within pie graphs, representing relative ratios of the alkaloids in latex or roots. The exact concentrations with standard deviation are presented in the Results and Discussion section. Total C048-6-14-64 alkaloids in latex and roots (A, E) were set to 100% to produce the relative diameters of the pie graphs, representing the relative total alkaloid contents of the narcotic cultivar compared to the condiment cultivar "Marianne".

Table 1. Selected Reaction Monitoring (SRM) of the Alkaloids 1,2-Dehydroreticuline (**10**), Salutaridine (**11**), and (S)-Scoulerine (**9**)

compound	RT_{LCMS} (min)[a]	SRM	CE (eV)[b]
dehydroreticuline (ref 27)	6.62	*m/z* 328 → *m/z* 312	−40
salutaridine (ref 26)	6.82	*m/z* 328 → *m/z* 237	−30
scoulerine	8.42	*m/z* 328 → *m/z*178	−30

[a] RT_{LCMS} = retention time (LC-MS). [b]CE = collision energy.

dance of oripavine is evidence that the narcotic cultivar originates from Tasmanian varieties and that both biosynthetic pathways are active in C048-6-14-64. Other biosynthetic intermediates such as reticuline (5 ± 2 μg/100 μg protein) (**7**,**8**), (*S*)-scoulerine (1 ± 1 μg/100 μg protein) (**9**), 1,2-dehydroreticuline (1 ± 1 μg/100 μg protein) (**10**), salutaridine (1 ± 1 μg/100 μg protein) (**11**), laudanine (10 ± 5 μg/100 μg protein) (**12**), and laudanosine (8 ± 5 μg/100 μg protein) (**13**) are present only in small amounts (Figure 2A). In morphine biosynthesis, both enantiomers of reticuline (**7**, **8**) are formed (Figure 1). With the methods used in this study, we were not able to distinguish between enantiomers. For this reason, the reticuline concentration presented herein is likely a mixture of both enantiomers. The alkaloids 1,2-dehydroreticuline (**10**), salutaridine (**11**), and (*S*)-scoulerine (**9**), which all have the same retention time in HPLC chromatograms, were characterized by an $[M]^+$ (**10**) or $[M + H]^+$ ion (**9**, **11**) at *m/z* 328 in their ESI mass spectra. To distinguish and identify these three alkaloids, the LC-ESI-selected reaction monitoring (SRM)[14] method was used, via measuring the base peak ion for each compound (Table 1). The quantitation of **10**, **11**, and **9** was

Table 2. Selected Reaction Monitoring (SRM) Measurements of the Alkaloids in the Roots of *Papaver somniferum* L. (cultivar "Marianne")

compound	RT_{LCMS} (min)[a]	occurrence[b]	SRM	CE (eV)[c]
morphine (ref 26)	2.54	+	*m/z* 286 → *m/z* 165	−38
reticuline (ref 27)	6.80	+	*m/z* 330 → *m/z* 192	−25
narcotoline	8.30	++	*m/z* 400 → *m/z* 206	−25
laudanine	9.22	+	*m/z* 344 → *m/z* 206	−25
noscapine	12.48	++	*m/z* 414 → *m/z* 220	−25
10-hydroxysanguinarine	(14.40)	n.d.	*m/z* 348 → *m/z* 262	−40
sanguinarine	15.50	++	*m/z* 332 → *m/z* 274	−40
10-hydroxydihydrosanguinarine	(16.82)	n.d.	*m/z* 350 → *m/z* 335	−25
dihydrosanguinarine	21.50	++	*m/z* 334 → *m/z* 319	−25

[a] RT_{LCMS} = retention time (LC-MS). [b] + = traces; ++ = clearly detected; n.d. = not detected. [c]CE = collision energy.

based on calibration curves. (*S*)-Scoulerine was originally isolated from opium.[15] This alkaloid is the catalytic product of action of the berberine bridge enzyme (BBE) on the branchpoint intermediate (*S*)-reticuline. This reaction is the first committed step in the pathway leading to the benzo[*c*]phenanthridine alkaloids (Figure 1). BBE transcripts have been detected in roots, stems, leaves,[16,17] and flower buds.[17] Western blot analysis of BBE confirmed the presence in stems.[11] For this reason (*S*)-scoulerine should be present in all aerial parts of the opium poppy plants, including the latex. Laudanine (7-*O*-methylreticuline) is the reaction product from a previously published enzyme of tetrahydrobenzylisoquinoline alkaloid biosynthesis, (*R,S*)-reticuline 7-*O*-methyltransferase (7-OMT). 7-OMT transcripts were detected predominantly in buds and stems and, to a much lesser degree, in leaves of *P. somniferum*.[18] This finding correlates with latex as the site of laudanine accumulation.

The condiment cultivar "Marianne" displays a different set of alkaloids in latex and shows a reduced relative total alkaloid content (Figure 2B). In contrast to the Tasmanian narcotic cultivar, 80.5% of the alkaloids in latex are the phthalideisoquinoline alkaloids narcotoline (288 ± 119 μg/100 μg protein) (**14**) and noscapine (98 ± 46 μg/100 μg protein) (**3**). The biosynthesis of noscapine or narcotine and its demethylated derivative narcotoline is not yet elucidated. However, application experiments in the late 1960s demonstrated that (*S*)-scoulerine is a precursor of phthalideisoquinoline alkaloids.[19,20] The narcotic cultivar was bred to possess a high concentration of morphinan alkaloids only. For this reason, the phthalideisoquinoline alkaloids were removed by conventional breeding and are, therefore, below detection limits in C048-6-14-64. Other intermediates that could be detected in the latex of "Marianne" were morphine (74 ± 27 μg/100 μg protein) (**1**), codeine (9 ± 4 μg/100 μg protein) (**2**), thebaine (8 ± 5 μg/100 μg protein) (**5**), and reticuline (3 ± 1 μg/100 μg protein) (**7**, **8**). In 1989, Williams and Ellis examined the age and tissue distribution of some major alkaloids in the cultivar "Marianne".[21] They observed a time- and tissue-specific accumulation of morphine, codeine, noscapine, and narcotoline in aerial and root tissues. However, alkaloids in the latex were not reported.[21] In the cultivar "Marianne", only 18.8% of the relative total alkaloid content consists of morphinan alkaloids. Although a large number of plants from each cultivar were analyzed, the standard errors were still high because of the variation in the relative total alkaloid content of latex in each cultivar. The relative total alkaloid concentration varies in C048-6-14-64 between 287 and 1047 μg alkaloid/100 μg soluble protein and in "Marianne" between 276 and 1086 μg alkaloid/100 μg soluble protein. Although the relative total alkaloid concentration from plant to plant is variable and displays a Gaussian distribution, the relative ratio of alkaloids is constant in "Marianne" and in the narcotic cultivar (data not shown). The data confirm that both *P. somniferum* cultivars are stable high (C048-6-14-64) or low ("Marianne") morphine inbred lines.

The morphinan, tetrahydrobenzylisoquinoline, and phthalideisoquinoline alkaloid concentrations were also determined in roots (Figure 2C,D). In contrast to latex, the total alkaloid concentration is reduced in the roots of both cultivars. In the narcotic cultivar, morphine (1.2 ± 0.5 μg/100 mg fresh weight (FW)) (**1**), codeine (6.7 ± 3.5 μg/100 mg FW) (**2**), thebaine (5.9 ± 3.8 μg/100 mg FW) (**5**), oripavine (0.2 ± 0.1 μg/100 mg FW) (**6**), reticuline (1.7 ± 0.8 μg/100 mg FW) (**7**,**8**), (*S*)-scoulerine (0.4 ± 0.5 μg/100 mg FW) (**9**), dehydroreticuline (0.4 ± 0.5 μg/100 mg FW) (**10**), salutaridine (0.5 ± 0.5 μg/100 mg FW) (**11**), laudanine (1.0 ± 0.6 μg/100 mg FW) (**12**), and laudanosine (0.6 ± 0.3 μg/100 mg FW) (**13**) were detected. In the roots of C048-6-14-64, the same alkaloids were detected as in the latex, but the total alkaloid content (19.7%) and the relative ratio of the alkaloids were altered. The relative concentrations of morphine, thebaine, and oripavine were reduced. All other alkaloids showed higher concentrations. Interestingly, the major morphinan alkaloid in roots was codeine (Figure 2C). The cultivar "Marianne" displayed a different profile in the roots (Figure 2D). Compared to the narcotic cultivar, the total alkaloid content was even more reduced (3.1%) and the relative ratio of alkaloids was different: morphine (0.01 ± 0.02 μg/100 mg FW) (**1**), thebaine (0.06 ± 0.08 μg/100 mg FW) (**5**), oripavine (0.06 ± 0.06 μg/100 mg FW) (**6**), alkaloids molecular weight 328 (0.06 ± 0.05 μg/100 mg FW) (**10**, **11**, **9**), laudanine (0.04 ± 0.07 μg/100 mg FW) (**12**), and laudanosine (0.13 ± 0.19 μg/100 mg FW) (**13**). In contrast to latex, codeine, reticuline, narcotoline, and noscapine were not detected. On the other hand, alkaloids that were not abundant in the latex were detected: oripavine, alkaloids with a molecular ion at 328 ((*S*)-scoulerine, dehydroreticuline, and salutaridine), laudanine, and laudanosine.

It is well known that benzo[*c*]phenanthridines are the major alkaloids in the root system of opium poppy.[17] The concentration of this class of alkaloids in each cultivar (Figure 2E,F) was compared to the morphinan and tetrahydrobenzylisoquinoline alkaloids detected in the root (Figure 2C,D). In root extracts of the narcotic cultivar, the major alkaloids were the benzo[*c*]phenanthridines sanguinarine/10-hydroxysanguinarine (53 ± 54 μg/100 mg FW) (**4**) and dihydrosanguinarine/10-hydroxydihydrosanguinarine (40 ± 23 μg/100 mg FW) (**15**) (Figure 2E). With the HPLC conditions used in this study, sanguinarine and 10-hydroxysanguinarine or dihydrosanguinarine and 10-hydroxydihydrosanguinarine could not be distinguished. For this reason, the alkaloid concentration presented herein is a mixture of either sanguinarine and 10-hydroxysanguinarine or dihydrosanguinarine and 10-hydroxydihyd-

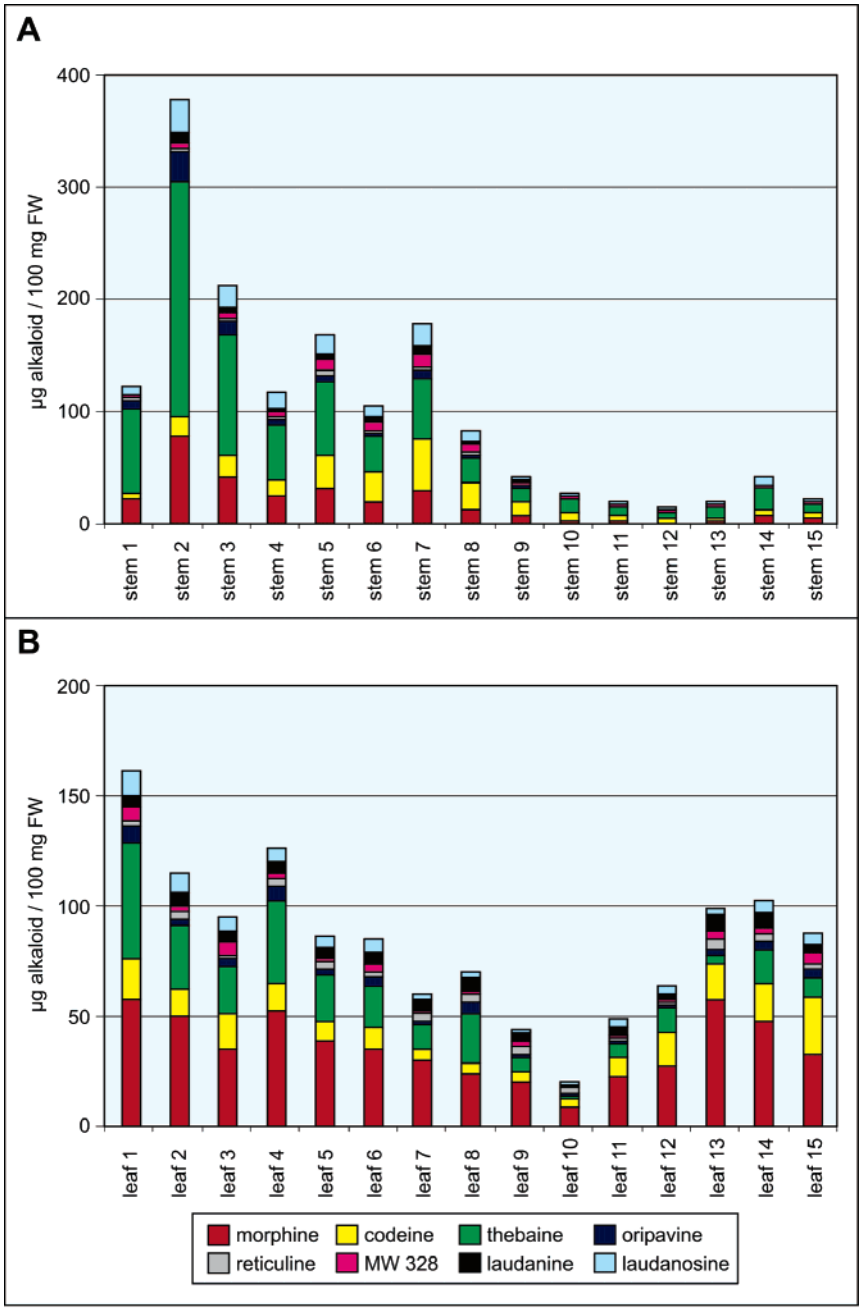

Figure 3. Determination of benzylisoquinoline and morphinan alkaloids in stem sections (A) and in leaves (B) of the narcotic cultivar C048-6-14-64. The data represent the alkaloid concentration of one characteristic plant out of the 29 plants measured. The alkaloids in leaves and stem sections were calculated as μg alkaloid/100 mg fresh weight. In these measurements, the alkaloids 1,2-dehydroreticuline (**10**), (*S*)-scoulerine (**9**), and salutaridine (**11**) were not distinguished by selected reaction monitoring (SRM).

rosanguinarine. The concentration of benzo[*c*]phenanthridine alkaloids (100%) in roots (Figure 2E) is about 5-fold higher than the concentration of the morphinan and tetrahydrobenzylisoquinoline alkaloids (19.7%) (Figure 2C). In the cultivar "Marianne", 80% of the benzo[*c*]phenanthridine alkaloids is dihydrosanguinarine (2.0 $\pm$ 0.9 μg/100 mg FW) (**15**). Only 20% of the relative total alkaloid content is sanguinarine (0.05 $\pm$ 0.06 μg/100 mg FW) (**4**) (Figure 2F). In contrast to the narcotic cultivar, in which the benzo[*c*]phenanthridines in roots (100%) dominate over the morphinan and tetrahydrobenzylisoquinoline alkaloids (19.7%), the concentration of benzo[*c*]phenanthridines in "Marianne" (2.7%) is similar to morphinan and tetrahydrobenzylisoquinoline alkaloids (3.1%). The localization of benzo[*c*]phenanthridines is a matter of controversy. Some groups isolated sanguinarine from aerial parts of the plant, e.g., stems and leaves, but not from capsules or seeds,[22] whereas others found sanguinarine predominantly in the roots of opium poppy.[23] The aerial parts of each cultivar were analyzed for the occurrence of benzo[*c*]phenanthridine alkaloids. In neither C048-6-14-64 nor "Marianne" was 10-hydroxysanguinarine or 10-hydroxydihydrosanguinarine detected in aerial plant parts (data not shown). In the leaves and stems of the narcotic cultivar, sanguinarine and dihydrosanguinarine occurred only in traces as determined by LC-MS. Benzo[*c*]phenanthridines were not detectable in the latex. These data are in agreement with the previous results of Hakim and co-workers[22] carried out by paper chromatography and paper electrophoresis. In contrast, in the leaves of "Marianne" traces of dihydrosanguinarine, but not sanguinarine, were measured (data not shown). Because the alkaloid concentration in the root system of

Marianne is per se very low, only traces of dihydrosanguinarine and sanguinarine were detected by SRM (Table 2).

These data suggest that the biosynthesis of the benzo[*c*]phenanthridines, namely, enzymatic steps downstream of BBE, should occur predominantly in the root system of opium poppy. BBE, and in turn the product of its enzymatic reaction, must be localized in the aerial parts of the plant. Consistent with this, (*S*)-scoulerine was detected in leaves, stems, roots, and latex. The presence of BBE transcripts,[16,17] as well as Western blot analysis[11] and immunocytolocalization studies,[24] supports this finding. Bock and co-workers demonstrated by electron microscopic examination of young roots and shoots that BBE is localized in idioblasts, which are not connected to the laticifer system. Their data support a spatial separation of benzo[*c*]phenanthridine and morphine biosynthesis *in planta*.[24] Additionally, since, noscapine and narcotoline and their precursor (*S*)-scoulerine were found in aerial plant parts, the biosynthesis leading to the phthalideisoquinolines must occur in above ground parts of opium poppy.[19,20]

Finally, alkaloids in stem sections (Figure 3A) and in leaves (Figure 3B) of the elite cultivar C048-6-14-64 were determined. In these measurements, the alkaloids 1,2-dehydroreticuline, (*S*)-scoulerine, and salutaridine were not distinguished by selected reaction monitoring (SRM). The data represent the alkaloid concentration of all stem sections and of all leaves of one characteristic plant out of six plants measured. Each of these six plants possessed 15 leaves. The numbering of the leaves and stem sections started from the upper parts of the plants leading downward toward the roots. Stem sections were collected between internodes. Although the total alkaloid content of each single leaf and stem section varied from plant to plant, the ratio of the alkaloids within the leaves and stems was not variable. Figures 3A and 3B demonstrate the general alkaloid pattern in leaves and roots. The tendency was identical in all six plants measured. For this reason, a detailed alkaloid concentration of the plant chosen for Figure 3 is not presented. In the stem sections, a gradient in the total alkaloid content from the upper parts toward the roots was observed. The highest concentration of alkaloids was measured in stem section 2 (Figure 3A). This finding may be due to the fact that a part of the latex from stem section 1 was removed by latex collection from the capsule. The laticifer system of opium poppy has a high turgor, potentially transferring latex from stem section 1 into the incised capsule. The alkaloid pattern in stems is identical with the pattern in latex. The main alkaloids in stems were also morphine, codeine, and thebaine (75−80%). The morphine concentration is reduced toward the roots, whereas the codeine concentration increases from the upper to the lower plant parts. The concentration of thebaine remained constant throughout all stem sections.

The distribution of total alkaloid concentration in leaves forms a bimodal curve (Figure 3B). The highest concentration of alkaloids could be observed in the upper leaves of *P. somniferum*. The alkaloid concentration decreases toward the roots. Normally the last four to five leaves of each plant were necrotic and contained a higher relative concentration of alkaloids. In the plant chosen for Figure 3, the total alkaloid concentration decreases from leaf 1 to leaf 10. Leaves 11−15 were necrotic. In these leaves, a higher concentration of alkaloids was again observed. This finding was confirmed by similar analyses with five additional plants. The alkaloids in the leaves display the same pattern as in latex and stems; 75−80% of the alkaloids in leaves were morphine, codeine, and thebaine. In contrast to stem sections, where an increasing gradient from the upper to the lower parts was observed for codeine and a decreasing concentration of morphine toward the roots, the alkaloid concentration in leaves remained constant; no gradients were observed.

In the current study, a comparative overall measurement of alkaloids in tissues of an opium poppy narcotic cultivar and the low-morphine variety "Marianne" is described. From the results, it becomes clear that although both cultivars are *P. somniferum* species, they display a very different pattern of alkaloids. These findings suggest a different gene regulation in alkaloid biosynthesis and would potentially explain different findings in gene expression and/or alkaloid content.

Experimental Section

General Experimental Procedures. The protein concentration in latex was determined with Bradford[25] reagent (Bio-Rad) according to the manufacture's protocol.

Plant Material. The genotypes of *P. somniferum* L. used were the narcotic cultivar C048-6-14-64 and the cultivar "Marianne", both obtained from Tasmanian Alkaloids Pty Ltd, Westbury, Australia. The plants were grown in a greenhouse under a high-pressure sodium lighting system (Philips, SON-T AGRO 400 W) at 20−24 °C with a 18−20 °C temperature shift at night, a relative humidity of 60%, and a 16 h photoperiod.

Harvest of Latex, Stems, Leaves, and Roots. Two days after the petals dropped, latex was collected from the opium poppy plants by incising the capsule longitudinally with a scalpel. The exuded latex was collected with a pipet and was stored in 200 μL of collection buffer (100 mM potassium phosphate pH 7.2, 500 mM D-mannitol, 20 mM ascorbic acid)[10] and frozen in liquid nitrogen. The samples were stored at −80 °C prior to analysis. Subsequently leaves, stems, and roots were cut with a scalpel, and after determination of the fresh weight the tissues were frozen in liquid nitrogen and stored at −80 °C prior to analysis. The numbering of the leaves and stems started from the upper parts of the plants leading toward the roots. Stem sections were collected between internodes.

Extraction and Isolation of Alkaloids. Latex samples were thawed on ice and centrifuged for 30 min at 12 000 rpm and 4 °C. The supernatant was used to determine the protein concentration. An aliquot of the supernatant was mixed with an internal standard (dihydrocodeine), diluted with 70% (v/v) EtOH, and after a second centrifugation (30 min, 12 000 rpm, 4 °C) analyzed by HPLC or LC-MS.

The pellet was also mixed with dihydrocodeine and treated for 6 min in an ultrasound waterbath at 4 °C. After sonication, the latex vesicles were centrifuged (30 min, 12 000 rpm, 4 °C) and an aliquot of the supernatant was analyzed by HPLC or LC-MS.

Leaf, stem, or root samples were ground to a powder with a mortar and pestle in liquid nitrogen. The plant material was extracted at room temperature with 1−20 mL of 70% (v/v) EtOH and mixed with an internal standard (dihydrocodeine). After filtration with a Baker cartridge (Bakerbond 7328-06, T. Baker), the solution was concentrated under vacuum. The aqueous phase was made alkaline with $NaHCO_3$ and subsequently extracted five times with 1 mL of EtOAc. The extract was concentrated under vacuum, and the alkaloids were dissolved in 300 μL of 70% (v/v) EtOH and analyzed by HPLC or LC-MS.

HPLC Analysis of Alkaloids. Latex, leaf, and stem samples were analyzed by HPLC on a liquid chromatography system (Agilent Technologies, Waldbronn, Germany) using a reversed-phase column (LiChrospher 60 RP-select B, 4 × 250 mm, 5 μm, UV detection at 210, 282, and 440 nm). The alkaloids were separated at a flow rate of 1 mL min^{-1} using the gradient H_2O−MeCN (0−25 min 0−46% MeCN, 25−26 min 46−100% MeCN, 26−33 min 100% MeCN) containing 0.01% (v/v) phosphoric acid. Peaks were routinely identified

from their UV spectra and by comparison of their retention times to those of authentic standards. Subsequently the identity of the peaks was confirmed by LC-MS.

Root samples were analyzed by HPLC using the equipment as described above with a modified gradient as follows: 0−25 min 0−60% MeCN, 25−26 min 100% MeCN, 26−33 min 100% MeCN, and UV detection at 282 nm.

Analysis by LC/ESI-TOFMS. ESIMS measurements and LC separations were carried out on a Mariner TOF mass spectrometer 5232 (Applied Biosystems, Lincoln, NE) equipped with a Turbulon spray source (PE-Sciex, Concord, ON, Canada) using an LC1100 series system of Agilent (Waldbronn, Germany), adapted to flow rates at 0.2 mL min^{-1}. Samples were injected (2 μL) on a Superspher 60 RP-select B column (125 × 2 mm, 5 μm). The following LC conditions were used: solvent A is MeCN−H_2O (2:98) and solvent B is MeCN−H_2O (98:2), with 0.2% HCO_2H in both solvents. The gradient increased from 0% to 46% B in 25min, to 90% in 1 min, and held at 90% for 7 min; post time was 5 min. For the separation of benzo[*c*]phenanthridines, the gradient was started from 0% to 60% B in 25 min, held at 60% for 5 min, and then followed in the same manner as described above.

The TOF mass spectrometer was operated in the positive ion mode, nebulizer gas (N_2) flow was 0.5 L min^{-1}, curtain gas (N_2) flow was 1.5 L min^{-1}, and heater gas (N_2) flow was 7 L min^{-1}. The spray tip potential of the ion source was 5.5 kV, heater temperature 320 °C, nozzle potential 200 V, quadrupole temperature 140 °C, and detector voltage 1.56 kV. The other settings varied depending on tuning. A resolution of 5000 was used in the mass calibration. The monoisotopic masses of the protonated molecular ions $[M + H]^+$ were calculated using the data explorer software of the TOF instrument.

Retention times and HRMS data were as follows: morphine: 7.8 min; *m/z* 286.1465 $[M + H]^+$, calcd for $C_{17}H_{20}NO_3$, 286.1437; dihydrocodeine: 10.7 min; *m/z* 302.1723 $[M + H]^+$, calcd for $C_{18}H_{24}NO_3$, 302.1751; codeine: 11.0 min; *m/z* 300.1610 $[M + H]^+$, calcd for $C_{18}H_{22}NO_3$, 300.1594; oripavine: 12.4 min; *m/z* 298.1453 $[M + H]^+$, calcd for $C_{18}H_{20}NO_3$, 298.1438; reticuline: 14.1 min; *m/z* 330.1719 $[M + H]^+$, calcd for $C_{19}H_{24}NO_4$, 330.1700; scoulerine, salutaridine, dehydroreticuline: 14.8 min; *m/z* 328.1531 $[M + H]^+$, calcd for $C_{19}H_{22}NO_4$, 328.1543; laudanine: 15.7 min; *m/z* 344.1872 $[M + H]^+$, calcd for $C_{20}H_{26}NO_4$, 344.1856; thebaine: 17.0 min; *m/z* 312.1579 $[M + H]^+$, calcd for $C_{19}H_{22}NO_3$, 312.1594; laudanosine: 17.5 min; *m/z* 358.2022 $[M + H]^+$, calcd for $C_{21}H_{28}NO_4$, 358.2013; narcotoline: 15.4 min; *m/z* 400.1416 $[M + H]^+$, calcd for $C_{21}H_{22}NO_7$, 400.1391; noscapine: 20.6 min, *m/z* 414.1514 $[M + H]^+$, calcd for $C_{22}H_{24}NO_7$, 414.1547); sanguinarine: 28.5 min; *m/z* 332.0977 $[M + H]^+$, calcd for $C_{20}H_{14}NO_4$, 332.0917; 10-hydroxysanguinarine: 26.4 min, *m/z* 348.0839 $[M + H]^+$, calcd for $C_{20}H_{14}NO_5$, 348.0866, dihydrosanguinarine: 28.7 min; 334.1095 $[M + H]^+$, calcd for $C_{20}H_{16}NO_4$, 334.1074; 10-hydroxydihydrosanguinarine: 27.1 min; *m/z* 350.1038, $[M + H]^+$, calcd for $C_{20}H_{16}NO_5$, 350.1023.

The positive ion electrospray (ES) mass spectra were obtained with a Finnigan MAT TSQ 7000 instrument (electrospray voltage 4.5 kV; heated capillary temperature 220 °C; sheath gas, nitrogen) coupled with a Surveyor MicroLC system equipped with an RP18-column (5 μm, 1 × 100 mm, Ultrasep). For the determination of the alkaloids salutaridine, 1,2-dehydroreticuline, and scoulerine (Table 1), selected reaction monitoring (SRM) was used with an HPLC gradient system starting from H_2O−MeCN, 85:15 (each containing 0.2% HOAc) to 10:90 within 15 min, followed by a 15 min isocratic period and a flow rate of 70 μL min^{-1}; collision gas, argon; collision pressure, 1.8 × 10^{-3} Torr.

40 eV CIDMS of dehydroreticuline (27): *m/z* 328 ($[M]^+$, 11), 313 (13), 312 ($[M - CH_3 - H]^+$, 100), 296 (6), 284 (41), 267 (7), 204 (5), 190 (10), 176 (8).

30 eV CIDMS of salutaridine (26): *m/z* 328 ($[M + H]^+$, 37), 297 (11), 285 (23), 282 (26), 270 (34), 265 (35), 255 (22), 239 (41), 237 (100), 233 (18), 211 (45), 207 (39), 205 (18), 183 (15), 58 (50), 44 (14).

25 eV CIDMS of scoulerine: *m/z* 328 ($[M + H]^+$, 40), 296 (3), 178 (100), 176 (3), 151 (15).

The CID mass spectra of the authentic alkaloids noscapine, sanguinarine, 10-hydroxysanguinarine, dihydrosanguinarine, and 10-hydroxydihydrosanguinarine were recorded using the same HPLC gradient system with a flow rate of 50 μL min^{-1}.

The ESI-CID mass spectra were measured using the energies given below.

25 eV CID mass spectrum of noscapine: *m/z* 414 ($[M + H]^+$, 25), 365 (6), 353 (24), 350 (3), 220 (100, isoquinoline moiety), 179 (5).

40 eV CID mass spectrum of sanguinarine: *m/z* 332 ($[M]^+$, 49), 330 (10), 317 ($[M - Me]^+$, 74), 316 (11), 304 ($[M + H - CO]^+$, 25), 302 ($[M - CH_2O]^+$, 13), 289 (10), 274 ($[M - CH_2O - CO]^+$, 100), 261 (15), 259 (10), 247 (18), 246 ($[M - CH_2O - 2CO]^+$, 47), 244 (33), 233 (11), 218 (33), 216 (21).

40 eV CID mass spectrum of 10-hydroxysanguinarine: *m/z* 348 ($[M]^+$, 34), 346 (11), 333 ($[M - Me]^+$, 79), 332 (37), 320 ($[M - CO]^+$, 79), 318 ($[M - CH_2O]^+$, 26), 305 (34), 304 (47), 290 ($[M - CH_2O - CO]^+$, 70), 277 (35), 262 ($[M - CH_2O - 2CO]^+$, 100), 247 (65), 232 (39), 221 (29), 204 (27).

25 eV CID mass spectrum of dihydrosanguinarine: *m/z* 334 ($[M + H]^+$, 27), 319 ($[M + H - Me]^+$, 100), 318 (34), 304 ($[M + H - CH_2O]^+$, 18), 276 ($[M + H - CH_2O - CO]^+$, 7).

25 eV CIDMS of 10-hydroxydihydrosanguinarine: *m/z* 350 ($[M + H]^+$, 33), 335 ($[M + H - Me]^+$, 100), 334 (19), 320 ($[M + H - CH_2O]^+$, 18), 319 (16), 292 ($[M + H - CH_2O - CO]^+$, 6), 291 (4).

The SRM measurements for the determination of noscapine, sanguinarine, 10-hydroxysanguinarine, dihydrosanguinarine, and 10-hydroxydihydrosanguinarine in roots of *P. somniferum* (cultivar "Marianne") were recorded using the reactions leading to the corresponding base peak ion (Table 2). The tentative occurrence of narcotoline was deduced from the prominent signal in the SRM *m/z* 400 $[M + H]^+ \rightarrow$ *m/z* 206 (isoquinoline moiety) similar to noscapine. A brief description of the SRM method is given by Niessen.[14]

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