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Poppy alkaloid profiling by electrospray tandem mass spectrometry and electrospray FT-ICR mass spectrometry after [*ring*-$^{13}C_6$]-tyramine feeding

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Abstract

Papaver alkaloids play a major role in medicine and pharmacy. In this study, [*ring*-$^{13}C_6$]-tyramine as a biogenetic precursor of these alkaloids was fed to *Papaver somniferum* seedlings. The alkaloid pattern was elucidated both by direct infusion high-resolution ESI-FT-ICR mass spectrometry and liquid chromatography/electrospray tandem mass spectrometry. Thus, based on this procedure, the structure of about 20 alkaloids displaying an incorporation of the labeled tyramine could be elucidated. These alkaloids belong to different classes, e.g. morphinan, benzylisoquinoline, protoberberine, benzo[*c*]phenanthridine, phthalide isoquinoline and protopine. The valuable information gained from the alkaloid profile demonstrates that the combination of these two spectrometric methods represents a powerful tool for evaluating biochemical pathways and facilitates the study of the flux of distant precursors into these natural products.

Keywords: *Papaver somniferum*; Electrospray; MS/MS; Poppy alkaloids; ^{13}C-Labelling; FT-ICR mass spectrometry

1. Introduction

The isoquinoline alkaloids represent a manifold class of compounds within the plant kingdom. Morphine, a complex natural product with wide legal and illegal use as an analgesic, is produced exclusively in the opium poppy *Papaver somniferum* L. together with other pharmaceutically desired tetrahydrobenzylisoquinoline-derived alkaloids, such as codeine, noscapine, papaverine and thebaine (Duke, 1985). The tetrahydrobenzylisoquinoline skeleton in plants is biosynthetically formed by condensation of dopamine and 4-hydroxyphenylacetaldehyde, both derived from (*S*)-tyrosine, to form the first alkaloid in the pathway, the trioxygenated intermediate (*S*)-norcoclaurine. (*S*)-Norcoclaurine is transformed to (*S*)-reticuline by methyltransfer and hydroxylation. (*S*)-Reticuline is a central intermediate in the biosynthetic pathway of *Papaver* alkaloids. It can undergo various intramolecular coupling reactions that lead to a plethora of alkaloid types, such as the morphinan, protoberberine, benzo[*c*]phenanthridine, phthalideisoquinoline and protopine, which are found mainly in species of the Papaveraceae, Monimiaceae, Ranunculaceae, Berberidaceae and Menispermaceae. (*S*)-Reticuline is clearly one of the most versatile molecules in plant secondary metabolism.

Abbreviations: ESI, electrospray ionization; CID, collision induced dissociation; LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; FT-ICR-MS, Fourier transform ion cyclotron resonance mass spectrometry; SRM, selected reaction monitoring.

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While the biosynthetic pathway leading to the pentacyclic morphinan alkaloids has been largely elucidated (see Kutchan, 1998), the flux of carbon derived from the primary metabolite (*S*)-tyrosine into the multitude of alkaloid types has not been investigated in the opium poppy. As an alkaloid precursor, ^{13}C-labeled (*S*)-tyrosine is diluted during feeding experiments, such that the isotopic enrichment does not always allow for clear determination of the incorporation pattern. In comparison, [1-$^{13}C_6$]-tyramine, which is also a distant, but more specific, precursor to the alkaloids, is well incorporated into the target molecules as determined by ^{13}C NMR spectroscopy (Roberts et al., 1987). However, in order to detect labeled intermediates and end products of established and predicted biosynthetic pathways in low concentrations, mass spectrometry is the favored analytical tool of choice. Mass spectrometry coupled with the use of biosynthetic precursors labeled with stable isotopes allows that the labeled atoms be tracked during the course of biosynthesis. Recently, stable isotopes and tandem mass spectrometry were used for profiling the metabolism of glycerophospholipid species (''metabolipidomics'', Bleijerveld et al., 2006).

Electrospray mass spectrometry represents a very sensitive method for investigating alkaloids because of their good ion efficiencies. During the last decade, electrospray tandem mass spectrometry has been successfully applied in the analysis and characterization of isoquinoline-type alkaloids (Henion et al., 1994; Aebi and Henion, 1996; Bringmann et al., 1998; Fabre et al., 2000; Gioacchini et al., 2000; Raith et al., 2003; Stévigny et al., 2004; Wu and Moyer, 2004; Schmidt et al., 2005; Sturm et al., 2006; Wickens et al., 2006). On the other hand, the Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) represents a mass spectrometric method yielding an excellent resolving power, mass accuracy and sensitivity. During the last years, there have been first efforts to apply this method in analytical problems on metabolomics (Aharoni et al., 2002; Brown et al., 2005).

We have carried out an investigation using [*ring*-$^{13}C_6$]-tyramine as a biogenetic precursor of these alkaloids for feeding to *P. somniferum* seedlings. Feeding of [*ring*-$^{13}C_6$]-tyramine to germinating seeds of *P. somniferum* for a period of nine days in the light resulted in a high incorporation rate of label into secondary metabolites (up to ca. 50%). Using high-resolution electrospray (ESI) FT-ICR mass spectrometry and liquid chromatography/electrospray tandem mass spectrometry the course of the labeled precursor could be followed very effectively. The so obtained mass spectrometric data enabled the identification not only of all major alkaloids of interest, but also of minor and new, overlooked compounds occurring in trace amounts. Moreover, the investigation of the fragmentation behavior of labeled and unlabeled compounds yield a detailed insight in the nature of key ions. It is shown that the mass spectrometric methods used represent powerful metabolite profiling tools for evaluating the biochemical pathway leading from tyramine to benzylisoquinoline and morphinan alkaloids and other biogenically related compounds.

2. Results and discussion

2.1. High-resolution ESI-FT-ICR mass spectrometry

The alkaloid-containing, basidified fraction resulting from poppy seedling extraction was dissolved in methanol and investigated by direct-infusion ESI-FT-ICR mass spectrometry (Fig. 1a and 1b). The good ion efficiencies of alkaloids and a high isotopic enrichment of the metabolites allowed for an effective detection of a series of $[M+H]^+$ ions in the extract. The prominent *m*/*z* values of even-mass indicating nitrogen-containing compounds in the ESI-FT-ICR mass spectrum reflect a typical *Papaver* alkaloid pattern. Most of the alkaloids appeared in the mass range from *m*/*z* 300 to 400, as shown by the partial positive ion ESI-FT-ICR mass spectrum (Fig. 1b). The incorporation of [*ring*-$^{13}C_6$]-tyramine is indicated by a nominal mass shift of 6 amu. It is obvious that the main alkaloids with this mass shift have $[M+H]^+$ ions at *m*/*z* 312.15965 (thebaine, **18**) with the corresponding [*ring*-$^{13}C_6$]-labeled compound **18c** (*m*/*z* 318.17978), *m*/*z* 328.15466 (334.17490), *m*/*z* 330.17029 (336.19039), *m*/*z* 354.13394 (360.15415) and *m*/*z* 370.16534 (376.18531), respectively. As shown in Table 1, the ESI-FT-ICR mass spectrometric data are of high mass accuracy, the method is suited for detection of alkaloids. In most cases, the mass accuracies were in a range from 0.1 to ca. 1.5 ppm. The incorporation of $^{13}C_6$ is clearly evidenced by the FT-ICR mass spectrometric measurement.

In selected cases, the nominal mass can be resolved into two or three different *m*/*z* values representing unique elemental compositions. For example, *m*/*z* 332, which is occupied by three different masses, corresponds to the $^{13}C_6$-labeled cheilanthifoline (**10c**) at 332.15881, sanguinarine (**13**) at *m*/*z* 332.09237 and an unknown metabolite at *m*/*z* 332.17650, respectively (Table 1). The triplet at *m*/*z* 332 is well separated, the measured resolution of ca. 45.000 enables a good differentiation between potential overlapping ions. Furthermore, an estimation of the $^{13}C_6$-enrichment of the different masses can be accomplished with such measurements. Since different *Papaver* alkaloids can have the same elemental composition (e.g. *N*-methylcoclaurine (**6**) and codeine (**19**)), LC–MS/MS experiments are necessary to identify and characterize the various alkaloids.

2.2. Liquid chromatography/electrospray tandem mass spectrometry

The alkaloid extract from the poppy seedlings was subjected to liquid chromatography/electrospray tandem mass spectrometry (LC-ESI-MS/MS). The collision-induced dissociation (CID) mass spectra of the resolved alkaloids yielded information not only to the type of alkaloids, but